GENETICS AND ECOLOGY OF THE INVASIVE MARINE ALGA, *SARGASSUM MUTICUM* WITHIN WALES: A MULTIDISCIPLINARY APPROACH TO A MANAGEMENT PROBLEM

PAUL HALLAS

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Faculty of HESAS

Abstract

The introduction of non-native species represents a key component of global environmental change and is now recognized as the second biggest threat to global biodiversity after habitat destruction. Aspects of both the ecology and genetics of the invasive algae, *Sargassum muticum* from the Welsh coastline were investigated with a view to generating information necessary for potential future management strategies for this non-native alga. Based on the DNA sequencing markers investigated, both the invasive and native populations of *S. muticum* display low variability. Analysis of the *Trn*W_I mitochondrial intergenic spacer regions revealed the occurrence of a previously unrecorded haplotype from the native range. Although sequence data could not pinpoint precise source locations, distribution of both the *Trn*W_I and *cox* 3 haplotypes suggested regions of central Japan as a likely source area. Amplified fragment length polymorphisms (AFLPs) were also trialed as an additional method for examining the invasion genetics of the alga. However, contrary to previous studies of macroalgae, the development of AFLPs for *S. muticum* in this investigation was hindered by a lack of reproducibility attributable to the quality of the DNA. Hence, it is suggested that in future studies, more stringent DNA purification procedures may help resolve the problems of reproducibility. Results from the population ecology investigations demonstrated variability in plant morphology dependent on environmental conditions. Growth and reproductive phenology of the alga within Wales appears similar to other introduced populations within the UK, although there was variability5 in the extent of fertility between contrasting habitats. Comparisons of the associated epibiota of *S. muticum* and structurally similar native seaweed hosts from two contrasting locations in Wales were also examined. Contrasts in community structure and species composition were most evident between sample locations indicating a significant impact of environmental conditions on epibiota assemblages. With regards to the associated epifauna, the native alga, *Cystoseira* spp. at Rhosneigr supported a community very similar to that of the invader, a result attributed to the overall similar morphological complexity of the two macroalgae. In contrast, at Tal y Foel, the native alga *Fucus vesiculosus* supported a different and less diverse assemblage of associated epifauna compared to *S. muticum*. The ecological and molecular data are discussed in light of the current knowledgebase of *S. muticum* and in the context of the management of non-native marine species within Wales.

Dedication and Acknowledgements

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Author’s Declaration

I declare that the work in this thesis was carried out in accordance with the requirements of the University’s Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate’s own work. Work done in collaboration with, or with the assistance of others, is indicated as such. Any views expressed in the thesis are those of the author.

SIGNED .... DATE ....

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ABBREVIATIONS AND DEFINITIONS

AFLP	amplified fragment length polymorphism
AIC	akaike information criterion
ANOSIM	analysis of similarities
ANOVA	analyses of variance
ATP	adenosine triphosphate
BI	bayesian inference
BSA	bovine serum albumin
CCW	Countryside Council for Wales
CEU	cohesive end unit
CLUSTER	cluster analysis
CTAB	cetyl trimethyl ammonium bromide
DNA	deoxyribonucleic acid
dNTPs	deoxynucleotide triphosphates
EDTA	ethylenediaminetetraacetic acid
GPS	global positioning system
HCL	hydrochloric acid
HWE	Hardy-Weinberg equilibrium
ITS	internal transcribed spacer
JNCC	Joint Nature Conservation Council
MDS	multidimensional scaling
ML	maximum likelihood
MP	maximum parsimony
mtDNA	mitochondrial deoxyribonucleic acid
NJ	neighbour joining
PCR	polymerase chain reaction
RAPD	random amplified polymorphic deoxyribonucleic acid
rDNA	ribosmonal/recombinant deoxyribonucleic acid
RFU	relative fluorescent unit
RNase	ribonuclease A
SAC	Special Area of Conservation
SIMPER	similarity percentages

SSSI Site of Special Scientific Interest

TBE tris/borate/EDTA

TE tris/EDTA

UV ultraviolet

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CHAPTER ONE GENERAL INTRODUCTION

1.1 BIOLOGICAL INVASIONS

Biological invasions refer to the spread and establishment of species into areas outside of their native geographical range (Shigesada and Kawasaki, 1997). The movement of foreign species into new regions is not a novel concept as the spatial distribution of many species fluctuates naturally over varying timescales as a result of environmental change and biological interactions. However, the relative frequency of these range shifts has increased considerably over the last century largely due to anthropogenic activities (Everett, 2000, Mack et al., 2000). The increase in worldwide trade, transport and tourism has provided opportunities for species to extend beyond their natural range. Before proceeding with the following discussion it should be noted that the concept of "non-native" species is an arbitrary one, as it is humans who have effectively designated the artificial boundaries of species distributions. In the context of biological invasions it seems ironical that it is now humans who are largely responsible for breaking down the distinction of the boundaries of species. The majority of translocated organisms usually fail to establish viable populations in their new environment (Sakai et al., 2001). However, some successfully introduced species possess attributes that allow rapid expansion and persistence of their populations, often to the detriment of native biodiversity (Williamson, 1996). It is these organisms that have coined the term "invasive species".

Invasive species are recognised as one of the most significant threats to ecosystem stability and function (Ricciardi and Rasmussen, 1998, Mack et al., 2000), and have been ranked alongside habitat destruction as the second biggest cause of global biodiversity loss (Enserink, 1999, Allendorf and Lundquist, 2003). The impacts of exotic species introductions can be significant at a range of scales from the displacement and extinction of native species, right through to the alteration of ecosystem structure and function (Vitousek et al., 1987). Biological invasions are now positioned alongside global warming as a major element of global environmental change (Vitousek et al., 1997, Occhipinti-Ambrogi, 2007).

The introductions of foreign species can also incur a considerable impact on economy. The annual costs associated with non-indigenous species in the USA alone were estimated at US$137 billion (Kolar and Lodge, 2001). While the vast majority of invasive species are reported in terms of their detrimental environmental and socio-economic effects, some introductions have had positive outcomes (Ricciardi et al., 1997). In the initial phases of becoming naturalised an introduced species may contribute to the structural and/or functional diversity of an ecosystem. For example, the introduction of the American slipper limpet, *Crepidula fornicata*, into the French bay of Marennes-Oleron led to increases in endemic species richness and abundance (de Montaudouni & Sauriau 1999). However, preliminary ecological enhancement can quickly give way to negative effects following the rapid population expansion of invasive species which can subsequently occur (Sakai et al. 2001).

Introductions of non-native species, intentional and unintentional have become a common occurrence especially in coastal marine areas (Carlton, 2000). This is largely attributable to the increase in shipping and maricultural activities over the last century. For instance shipping between the USA and the UK may have caused the introduction of over 20% of the foreign species in British waters (Eno et al., 1997). Ballast water discharges in particular have been linked to the invasion of numerous non-native marine species (Williams et al., 1988, Ruiz et al., 2000) with notable examples including the zebra mussel *Driessena polymorpha* (Martel et al., 2001) and the comb jelly *Mnemiopsis leidyi* (Occhipinti-Ambrogi and Savini, 2003). Understanding the pathways of species introductions is pivotal in identifying susceptible locale and therefore being able to implement appropriate management strategies.

Of the species that become successfully established, introduced marine macroalgae constitute a significant ecological threat to native biodiversity given their potential role as ecosystem engineers (Wallentinus and Nyberg, 2007). Notable examples include *Caulerpa taxifolia* (M. Vahl) C. Agardh, *Undaria pinnatifida* (Harvey) Suringar and *Sargassum muticum* (Yendo) Fensholt. Among the 113 species of macroalgae that have been introduced into European waters, the brown alga, *Sargassum muticum* is ranked within the eight most invasive (Nyberg and Wallentinus, 2005).

1.2 STUDY SPECIES: *SARGASSUM MUTICUM*

1.2.1 Distribution of *S. muticum*: Overview of global distribution and current distribution within Wales

Sargassum muticum (Yendo) Fensholt is a large, brown fucoid alga indigenous to the north-west Pacific coastal waters of Japan, China, Korea and Russia (Critchley et al., 1990b). However, in the last 60-70 years, the geographical distribution of this alga has spread dramatically (see Fig. 1.1). The first record of its introduction was in 1944 when it was discovered in an oyster growing region of British Columbia, Canada (Scagel, 1956). Since then it has become established in numerous locations along the Pacific coast of North America and currently ranges from Alaska down to Mexico (Lindstrom, 1977, Nicholson et al., 1981, Aguilar-Rosas and Aguilar-Rosas, 1985, Nunez-Lopez and Valdez, 1998).

It was first recorded in European waters in 1973, where 30 attached plants were found on the shore at Bembridge, Isle of Wight (Farnham et al., 1973). However, the initial site of entry into Europe is still subject to controversy and it is thought that *S. muticum* was indirectly introduced during the 1960s associated with the importation of Pacific oysters (*Crassostrea gigas*) from British Columbia or Japan to France for aquaculture (Druehl, 1973, Farnham and Jones, 1974, Ribera and Boudouresque, 1995), and that drifting plants from the Atlantic French coast were responsible for the UK introduction (Critchley et al., 1983, Eno et al., 1997). The further discovery of established populations of *S. muticum* within the Solent region generated considerable concern about the potential deleterious effects of its introduction and spread on native communities (Critchley et al., 1983, Critchley et al., 1986). Following discussions, an eradication programme was initiated trialling various methods of removal including hand-gathering, mechanical clearance, herbicides and biological control (Gray and Jones, 1977, Critchley et al., 1986). However, despite substantial efforts no effective technique for its permanent removal was evident and regrowth of the alga was rapid. The clearance attempts may have slowed the rate of expansion of *S. muticum* along the south coast but the alga has continued to spread around the British Isles. Since that time *S. muticum* has now established populations along the entire south coast of the UK to the Isles of Scilly (Critchley et al., 1983), the east coast of Kent (Eno et al., 1997) and

along the north Cornish coast to Lundy (Eno et al., 1997). Several locations along the Welsh coast have also been colonised, and in 2004 the first Scottish population of the alga was discovered in Loch Ryan on the southwest coast. Following these initial discoveries, routine monitoring of the Scottish coastline has revealed attached stands of the alga on the east coast of the Isle of Cumbrae, on the mainland shore at Hunterston Power Station, on the east coast of the Isle of Arran, and on the southeast coast of the Mull of Kintyre (Harries et al., 2007a). Drift material has also been recorded from several locations including the Clyde Marina, Loch Fyne, Corrie on the Isle of Arran, and further north in the Firth of Lorn and Oban during 2007 (Davison, 2009). Given a distance of up to 100km between the nearest population of on the southern end of the Mull of Kintyre and the most northerly drift material recorded, existence of other populations not as yet identified between these two regions has been highlighted (Harries et al., 2007a). At present the established populations found on the Isle of Cumbrae currently represents the northern UK limit of *S. muticum*.

The first appearance of the alga in Northern Ireland was in 1995 growing within oyster beds in Strangford Lough (Boaden, 1995) and in 2001 it was discovered in the Republic of Ireland in Cashel Bay (Loughnane and Stengel, 2002). A recently published report has now shown that the alga has spread extensively around Ireland and has colonised areas on both the west and southeast coasts (Kraan, 2007). The current distribution of *S. muticum* within the British Isles is shown in Fig. 1.2A. Elsewhere in Europe this species is known along the Atlantic coasts of Portugal (Eno et al., 1997), Spain (Fernandez et al., 1990), France (Critchley et al., 1983), and North Sea coast of The Netherlands (Prud'homme Van Reine and Nienhuis, 1982, Critchley et al., 1983, Critchley and Dijkema, 1984, Critchley et al., 1990a) to Scandinavia (Karlsson and Loo, 1999, Stæhr et al., 2000) as well as a several isolated populations in the Mediterranean (Rueness, 1989, Curiel et al., 1998, Boudouresque and Verlaque, 2002).

The first records of *S. muticum* from Wales came from reports of drift material noted off the Pembrokeshire coast in 1983 (Eno et al., 1997) and subsequently in 1997 (Davison, 1999). The first attached plants were recorded in 1998 where 12 well established plants were found in West Angle Bay, Pembrokeshire by a Countryside Council for Wales (CCW) survey team. All the plants were subsequently removed. In 2000, a new established population was found further north along the coast in a pool at

Broad Haven (Morrell, 2007). In North Wales the first record of *S. muticum* was in 2001 from the Menai Strait (Gabrielle Wyn pers. comm. 2007). At present, the distribution of this alga within Wales extends from Port Talbot in the south to Beaumaris in the North (CCW, 2007, Grant, 2007; see Fig. 1.2B and Table 1.1). The source of the introduction to Wales remains unknown although it has been suggested that drift from Lundy Island, where it first appeared in 1993, provided the inoculants to Pembrokeshire (Eno et al., 1997). Furthermore it is not known if its spread into North Wales has occurred naturally via drifting plants from Pembrokeshire or whether there have been direct introductions associated with shellfish culture in the area. Another possible scenario is that the introduction to North Wales may have occurred through drifting material from Strangford Lough, Northern Ireland where *S. muticum* has been established since 1995 (Davison, 1999).

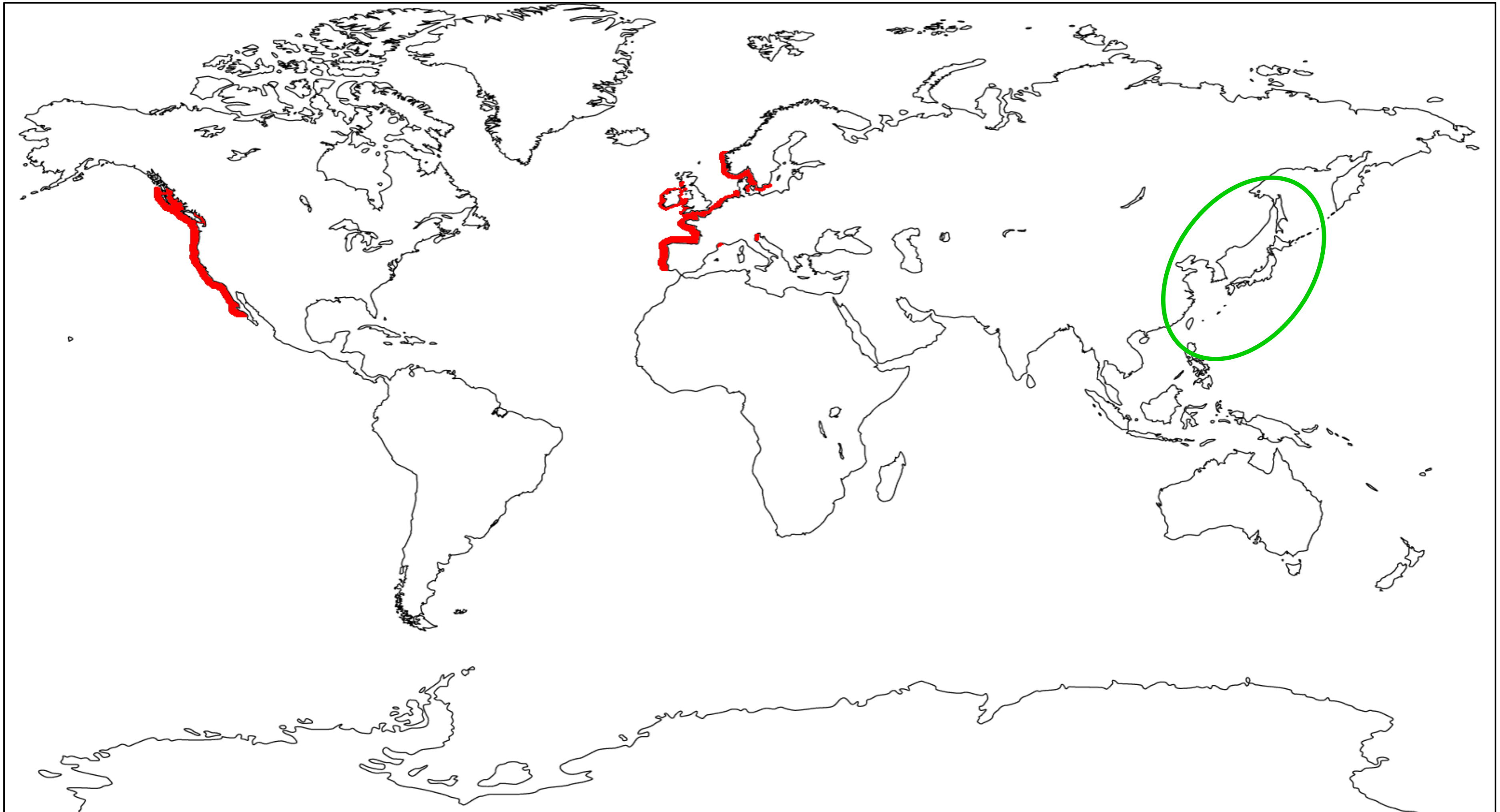


Fig. 1.2 Current global distribution of *S. muticum*. Red shading indicates introduced range in both the north-eastern Pacific and north-eastern Atlantic. Green circle indicates region of native range.

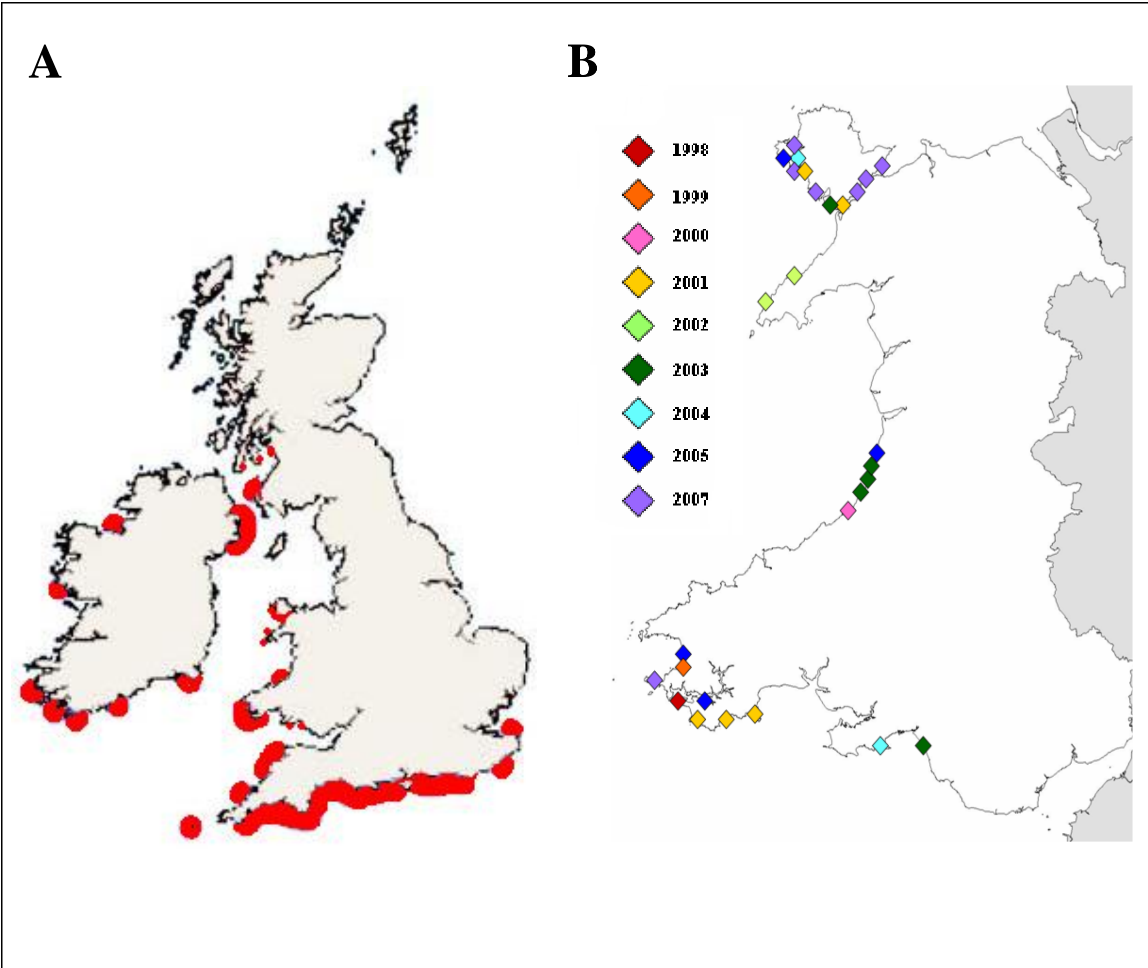


Fig. 1.2 **(A)** Current recorded distribution of attached *S. muticum* in UK and Ireland (Davison, 2009) and **(B)** present distribution of established populations of *S. muticum* around the coast of Wales with dates of first record (CCW, 2007, Grant, 2007).

Table. 1.1 Chronology of Welsh *S. muticum* records including both attached and drift plants from 1998-2009 (adapted from CCW *S. muticum* records spread sheet table).

Record	Date	Location	Grid Reference	Description	Source
1	June 1998	West Angle Bay, Pembrokeshire	SM850034	On bedrock at the north end of West Angle Bay beach.	CCW Phase 1 biotope LR.FK.Sar
2	April 1999	Shag Rock, St Brides Bay	SM859142	Shallow LR.SwSed pools with *Sargassum muticum* inside this hashed area within ELR.BPat.Cht. Abundance of *Sargassum muticum* in pools varies between Occasional and Common.	CCW Phase 1 Target Note
3	July 1999	Shag Rock, St Brides Bay	SM859142	Large deep LR.FK.Sar pool with *Sargassum muticum* (12m x 10m). Too deep to take species list	CCW Phase 1 biotope LR.FK.Sar
4	April 2000	Pen y Gloyn, Cardigan bay	SN448625	The original record for *Sargassum* in Cardigan Bay still remains unknown, however the understanding was that it was on the rocky platform to the south of Aberaeron around SN448625, but this has never been confirmed.	Anecdotal, Julian Woodman
5	April 2001	Star Cliff, Caldey Island	SS126970	Large pool at back of shore surrounded by ELR.BPat.Cht.	CCW Phase 1 biotope LR.FK.Sar
6	May 2001	Mermaid Inn, Menai Strait	SH473643	Large sand-bottomed deep pool (excised channel). LR.SwSed rockpool. *Sargassum muticum* and *Elminius modestus* frequent.	CCW Phase 1 Target Note
7	June 2001	West of Pen y Holt Bay, South Pembrokeshire	SR897953	Found at Pen-y-Holt Bay.	CCW Phase 1 biotope LR.FK.Sar
8	June 2001	Freshwater East, South Pembrokeshire	SS021980	*Sargassum muticum* washed up on the shore.	CCW Phase 1 Target Note
9	July 2001	Rhosneigr, West Anglesey	SH314728	Only two plants. Almost certainly rockpools.	Anecdotal, Ivor Rees
10	July 2001	Priory Bay, Caldey Island	SS134969	Atypical form of this biotopes. Pools are shallow in mid-eulittoral zone dominated by green algae but too species rich to be LR.G. Found in <1% of SLR.Fspi and MLR.FvesB.	CCW Phase 1 biotope LR.FK.Sar
11	June 2002	Porth Dinllaen, North Lleyn	SH278420	*Sargassum* found north side of the Llyn Peninsula earlier this year - probably in rockpools.	Anecdotal, Nova Mieszkowska
12	June 2002	Porth Oer, North Lleyn	SH165298	*Sargassum* found north side of the Llyn Peninsula earlier this year - probably in rockpools.	Anecdotal, Nova Mieszkowska
13	Sept 2002	MS30 South of Caernarfon	SH457618	Occurs on the site.	Extracted from Marine Recorder Surveys
14	May 2003	Carreg Ti-Pw to Aberarth, Cardigan Bay	SN507671	Occurs on the site.	CCW Phase 1 Site species list

15	May 2003	Newborough, West Anglesey	SH398634	6 attached plants on the Newborough / Llanddwyn shore. This was without searching so I suspect there may be more. They were on the lower parts of the cobbles.	Anecdotal, Ivor Rees
16	May 2003	Morfa Bychan to Pantyrallad,Cardigan Bay	SN560760	Occurs on the site.	CCW Phase 1 Site species list
17	August 2003	Pantyrallad to Carreg Ti-Pw, Cardigan Bay	SN544720	Occurs on the site.	CCW Phase 1 Site species list
18	Sept 2003	Port Talbot Harbour, Glamorgan	SS753880	Occurs on the site.	CCW Phase 1 Site species list
19	April 2004	Newborough, West Anglesey	SH392633	Occurs on mussels beds near the island.	Anecdotal, Ivor Rees
20	April 2004	Pwlldu Bay, Gower	SS574868	Large LR.SwSed pool with *Sargassum muticum* (R) and *Polysiphonia elongata*. LR.SwSed make up 2% of ELR.BPat.Cht.	CCW Phase 1 Target Note
21	May 2004	Inland Sea, Anglesey	SH279783	Large plants within the channel and smaller plants on mobile cobbles on the east shore	Anecdotal, Monica Jones
22	Sept 2004	Cei Bach, Cardigan Bay	SN406600	Occurs on the site	2004 IECS Cardigan Bay SAC LR.Rkp.SwSed pools survey
23	Sept 2004	Craig Ddu, Aberaeron	SN436615	Occurs on the site	2004 IECS Cardigan Bay SAC *Sabellaria alveolata* reefs survey
24	March 2005	Pwll Crochan Flats, Milford Haven	SM922036	Big plants attached to stones all along the lower shore. Grid reference and easting and northing coordinates are nominal location on Pwll Crochan Flats assigned by James Dargie (7/11/2007); not to be taken as the precise location of record!	Anecdotal, John Moore
25	April 2005	Newgale Sands, Pembrokeshire	SM854194	Occurring within large tidal pool.	Anecdotal, Aethne Cooke
26	June 2005	Trearddur Bay, West Anglesey	SH252790	Large rockpool with 1 plant approx. 1m in length.	Anecdotal, Monica Jones
27	July 2005	Morfa Bychan, Ceredigion	SN566778	*Sargassum* explosion. *Sargassum* was found within LR.SwSed pools and within LR.Cor pools on the bedrock platform on both mid and upper shore.	Anecdotal, Kathryn Birch & Anne Bunker
28	Sept 2005	Porth Dinllaen, North Lleyn	SH279419	3 plants occurring sublittorally on mixed cobbles between 52 56.628N 004 33.714W and 52 56.809N 004 33.746W	Anecdotal Seasearch, Lucy Kay

29	Sept 2005	Foryd Estuary, Menai Strait	SH450612	Big plants attached to stones at very low water next to No. 10 buoy.	Anecdotal, Sion Roberts
30	May 2007	Port Dinorwic Marina, Menai Strait	SH524678	Single plant seen from boat in shallow water. Attached to bottom.	Anecdotal, Bill Sanderson
31	May 2007	Cei Bach, Cardigan Bay	SN406600	Occurs on the site	2007 ASML/CCW Cardigan Bay SAC intertidal monitoring survey - Rockpools
32	March 2007	Pwlldu Bay, Gower	SS574868	Present in six midshore rockpools, up to 40 individuals in largest pool. Plants 70cm length, attached to bedrock and to boulders in deeper pools.	Anecdotal, Paul Hallas
33	June 2007	Martins Haven, Pembrokeshire	SM760092	Plant seen from boat in shallow water. Attached to bottom.	Anecdotal, Bill Sanderson
34	June 2007	Broad Haven, Pembrokeshire	SM861140	*Sargassum muticum* washed up on the shore.	Anecdotal, Bill Sanderson
35	June 2007	Porth Ysgaden, North Lleyn	SH219374	Single, relatively small plant growing sublittorally attached to a stone in shallow water within the bay at Porth Ysgaden (probably not far seaward of LAT)	Anecdotal, Lucy Kay
36	June 2007	Tal y Foel (Mermaid Inn) (Menai Strait)	SH474645	Very abundant, found in a band at low water. Large individuals.	Anecdotal, Laura Grant
37	June 2007	Porth Cwyfan, Anglesey	SH336682	Found abundantly in large shallow pools at low water springs to the south side of the church	Anecdotal, Laura Grant
38	June 2007	Beaumaris (Menai Strait)	SH608759	15 individuals from 27cm to 373cm found in a narrow band at low water springs. Attached to pebbles.	Anecdotal, Laura Grant
39	July 2007	Llanidan (Menai Strait)	SH499667	Large individuals in a band at low water of spring tides.	Anecdotal, Laura Grant
40	July 2007	Moel-y-don (Menai Strait)	SH519678	Large individuals in a band at low water of spring tides.	Anecdotal, Laura Grant
41	July 2007	Rhosneigr, Anglesey	SH314726	Very abundant at this site. Found in many rockpools and attached to pebbles in the rock protected lagoons.	Anecdotal, Laura Grant
42	July 2007	Porth Aels, Aberffraw, Anglesey	SH338675	Large rockpool with several large Sargassum plants	Anecdotal, Monica Jones
43	July 2007	Church Island (Menai Strait)	SH549717	Abundant in the lagoon separated from the main channel of the Menai Strait	Anecdotal, Laura Grant
44	July 2007	West Dale, Pembrokeshire	SM798058	no attached, drift fragments on the strandline	Anecdotal, David Jones
45	July 2007	Dale, Pembrokeshire	SM811059	attached/bedrock/stones/strandline	Anecdotal, David Jones
46	August 2007	Lydstep Haven, Pembrokeshire	SS093977	2 plants found in shallow rockpool plus drift specimen.	Anecdotal, David Jones

47	August 2007	Trearddur Bay, Anglesey	SH255780	Very abundant at this site. Found in 8 rockpools (although probably more) and in the lagoon at low water of spring tide.	Anecdotal, Laura Grant
48	August 2007	Borthwen, Anglesey	SH272748	Found in lower shore rockpools and on pebbles on sand on the lower shore.	Anecdotal, Laura Grant
49	August 2007	Church Island (Menai Strait)	SH552717	Two cobbles with very large *Sargassum* plants on the low shore, looked as though they have hopped along the Menai Strait.	Anecdotal, Paul Brazier
50	August 2007	Watwick, Pembrokeshire	SM817039	3 small plants in shallow rockpools plus one sublittoral specimen.	Anecdotal, David Jones
51	August 2007	Sandy Haven, Pembrokeshire	SM858070	attached/bedrock/strandline	Anecdotal, David Jones
52	August 2007	Traeth Penrhos, Holy Island	SH261818	Two individuals found in a sandy pool attached to rocks on the mid to low shore	Anecdotal, Laura Grant
53	August 2007	Lindsway Bay, Pembrokeshire	SM843065	2 small attached plants in shallow rockpool near high water mark, plus drift specimens.	Anecdotal, David Jones
54	August 2007	Giltar point, Pembrokeshire	SS125983	7 plants found in rockpools, plus 3 drift specimens.	Anecdotal, David Jones
55	August 2007	Llanddwyn Island, Anglesey	SH392632	Found attached to a large rock in a sandy pool. 2 individuals.	Anecdotal, Laura Grant
56	August 2007	Newborough, Anglesey	SH401629	Found attached to a large rock in a sandy pool. 2 individuals.	Anecdotal, Laura Grant
57	August 2007	Newgale, Pembrokeshire	SM855196	Dominant species in large areas of deep tidal pool creating surface canopy.	Anecdotal, David Jones
58	March 2008	Bracelet Bay, Gower	SS629870	occurs on the site	Anecdotal Judith Oakley
59	May 2008	Porth Lleidiog, Foryd, Menai Strait	SH458618	30% cover by *Sargassum muticum* on the lower shore, amongst cobbles with *Fucus serratus*. Covers an area of 10's of metres.	Anecdotal, Paul Brazier
60	October 2008	Pembroke Ferry, Pembrokeshire	SM974047	Single small plant attached to stone buried within sediment.	Anecdotal, Paul Hallas
61	2009	Worms Head Causeway, Gower	SS4087	Present in a number of rockpools	Anecdotal, Judith Oakley

Within the alga's native range its distribution is far less well resolved than compared with its introduced distribution (Cheang et al., 2010c). This can be attributed in part to the morphological plasticity of many species of *Sargassum* leading to taxonomic confusion in identification (Critchley, 1983d). The distribution of the alga around the Japanese islands appears to be governed by the Kuroshio and Tsushima warm water currents (Critchley, 1983d). According to Zhao et al. (2008) *S. muticum* is also a common component of the algal flora along the north coast of China with recorded stands of the alga from the Shandong peninsula (see Fig. 1 from Zhao et al. 2008). The increasing uses of complementary molecular based approaches in taxonomic investigations will undoubtedly provide useful information for the clarification of *Sargassum* species distribution limits (Cheang et al., 2010c).

1.2.2 Habitats of *S. muticum*

Within the native distribution of *Sargassum muticum* the alga can be found in relatively sheltered localities, growing on hard substrata in the upper sublittoral (Davison, 2009). When present with other *Sargassum* species, *S. muticum* typically occurs lower down on the shoreline (Critchley, 1981c, Davison, 2009). In its introduced range, the alga typically occurs just below the low water mark within the sublittoral fringe, although it can also be found in areas of standing water within the intertidal zone such as eulittoral pools, channels and lagoons areas. Its extension into areas within the high intertidal is restricted by the alga's low tolerance to desiccation (Norton, 1977b). In contrast, its incursion into the subtidal is determined largely by light intensity (Norton, 1977a) with most subtidal populations occurring between 6-10m depth, although plants down to 24m have been recorded off the coast of California (Nicholson et al., 1981). Sheltered areas with hard-bottom substrata are preferential for colonisation but the alga may also attach to pebbles, stones and shells located in soft-bottom substrates (Strong et al., 2006). Within certain sheltered areas it forms dense monospecific stands and can become the dominant macroalgae (Jephson and Gray, 1977, Norton, 1977a, Critchley, 1983a). For example in the San Juan islands densities as high as 126 individuals m^{-2} have been recorded (Britton-Simmons, 2004). It has also been reported from more exposed shores where it is largely restricted to tidal pools (Espinoza, 1990, Andrew and Viejo, 1998a). However, it rarely attains large numbers in these areas due to the extensive covers of turf and foliose algal species which prevent widespread settlement

of *S. muticum* germlings (Andrew and Viejo, 1998b). Artificial structures such as floating pontoons, jetties and moorings also provide suitable attachment sites for colonisation *S. muticum* (Fletcher and Fletcher, 1975, Knoepfflerpeguy et al., 1985, Kraan, 2007) and observations of its epiphytic growth on other large macroalgae has also been reported (Withers et al., 1975, Critchley, 1983a).

S. muticum exhibits a broad tolerance to a wide range of abiotic conditions. Salinity tolerances range from 5-34‰, (Norton, 1977a, Steen, 2004), with optimal growth reported at 34‰ (Eno et al., 1997). Mature plants have greater tolerance to reduced salinities compared with germlings (Norton, 1977a) and these contrasting tolerance limits of the different life stages of *S. muticum* have been demonstrated as a key feature of its ability to successfully colonise brackish water environments (Steen, 2004). It is also tolerant of a wide range in temperature from 5°C up to a 30°C (Norton, 1977b, Hales and Fletcher, 1989) with optimal growth occurring at 25°C (Eno et al., 1997). The preference for higher water temperatures in this species will likely be a key factor in its continued range expansion especially in the face of climatic change. It is also tolerant of polluted areas with high levels of nitrate being favourable for growth (Critchley et al., 1990a).

Within Wales, preliminary reports confirm the general trends of habitat preference by *S. muticum* with most established populations occurring within relatively sheltered localities, either in pools, lagoons or attached to artificial substrata (CCW, 2007, Grant, 2007, Jones, 2008). Information regarding the different habitats colonised by *S. muticum* will therefore be crucial for predicting the future spread of the alga within Wales and other introduced regions.

1.2.3 Biology of *S. muticum*

Sargassum muticum (Yendo) Fensholt, is a large brown fucoid alga. First described from Japan by Yendo (1907) as *Sargassum kjellmanianum* forma *muticus* due to differences from typical *S. kjellmanianum*, *S. muticum* was finally recognised as a separate species by Fensholt (1955) on the basis of differences in reproductive structure (Critchley, 1983d). *Sargassum muticum* possesses monoecious reproductive structures in contrast to the morphologically similar species, *S. kjellmanianum* and *S. miyabei* which are typically dioecious plants (Yoshida, 1978, Critchley, 1983d, Critchley et al.,

1990b). The alga is attached to the substrate by a perennial discoid holdfast (up to 5cm in diameter) which gives rise to a single perennial main axis. Each year several main branches (primary laterals) arise from the main stem which in turn give rise to secondary laterals and higher order branches giving the fronds a feathery appearance (Fig. 1.3). In addition a number of basal leaf-like laminae also arise annually from the main axis. A detailed description of the morphology of *S. muticum* taken from European populations is given by Critchley (1983c).

In its native habitat *S. muticum* is one of the smaller *Sargassum* species attaining lengths between 75-150cm (Yendo, 1907, Rueness, 1989) and comprises a relatively minor component of the native macroalgal flora (Norton, 1977b, Critchley, 1983d). However, in its introduced range plants can attain much larger sizes with records of plants up to 12m reported from the Brittany coast of France (Critchley et al., 1990b). In the UK plants approaching 6m have been recorded although lengths from 1 – 4m are usually attained (Davison, 1999). The number of primary laterals that develop from year to year increases with age providing a useful approximate measure of plant age (Critchley, 1981a). The laterals also bear several leaf-like laminae ranging in size with larger leaves developing on the primary laterals. This alga is easily distinguished from most other fucoids, certainly within the UK, due to the presence of numerous small, stalked air vesicles which arise from the axils of laminae on the laterals. These air-bladders provide positive buoyancy causing the fronds of the alga to stand upright in the water column. During the reproductive period stalked, cigar-shaped reproductive receptacles develop in the axils of the laminae on the higher order laterals. These receptacles are monoecious containing both the male and female gametes but within different cavities known as conceptacles.

Phenotypic plasticity occurs in *S. muticum* in relation to environmental variables such as exposure, temperature and light intensity (Critchley, 1983b, Andrew and Viejo, 1998b). Morphological variation in response to environmental factors is well documented in marine algae (Norton et al., 1981, Russell, 1986). In addition, density-dependent effects have also been shown to cause changes in the morphology of *S. muticum*, with high plant densities resulting in taller and thinner growth forms (Andrew and Viejo, 1998a, Andrew and Viejo, 1998b, Arenas et al., 2002). The effects of density on plant morphology have also been documented in other marine macroalgae (Reed,

1990, Holbrook et al., 1991, Sjotun and Fredriksen, 1995). Furthermore, the occurrence of plasticity within other *Sargassum* species, as discussed above has led to taxonomic confusions with the *Sargassum* genus (Critchley, 1983b, Critchley, 1983c).

Fig.1.3 Typical morphology of *S. muticum* displayed as a pressed herbarium specimen (adapted from SWAT, Coastal Biodiversity Survey webpage (2010).

S. muticum has a pseudo-perennial life history. During autumn in the UK, new primary laterals arise from the perennial main axis and over late winter and spring plants undergo a period of rapid vegetative growth with the development of the higher order laterals (Critchley, 1983c). From around August, following the reproductive period, the fronds become senescent and begin to die back leaving only the perennial holdfast and main axis (Norton, 1977b, Gorham and Lewey, 1984) (see Fig. 1.4). However, variability within the growth cycle of *S. muticum* has been reported depending on the area where it has been introduced and appears related to water temperature, light intensity and day length (Lewey and Farnham, 1981, Nicholson et al., 1981, Deysher, 1984, Espinoza, 1990). For example within its native range in Japan the dormancy period where the plant dies back occurs between mid-summer to late autumn (Deysher, 1984). However, in the more southern locations on the Pacific coast of North America where it has been introduced, reductions in the duration the dormancy period occur (Jephson and Gray, 1977). In some areas the plants are able to persist throughout the whole year due to the higher water temperature (Jephson and Gray, 1977, Espinoza, 1990). Within the UK senescence usually begins around August and by mid-winter they are more or less completely decomposed (Gorham and Lewey, 1984, Hales and Fletcher, 1989). However within the Solent region on the south coast of the UK a lack of dormancy has been observed with the initiation of new growth even before the previous seasons fronds have died back completely (Farnham et al., 1981, Davison, 1999).

Typical growth rates average between 1-2 cm per day for the primary laterals (Lewey, 1976, Nicholson et al., 1981, Arenas et al., 1995), and have been shown to be influenced by factors such as temperature and plant age (Nicholson et al., 1981). Within North Wales, a preliminary investigation documented growth rates between 1-5 mm per day (Grant, 2007) considerably less than records from the south coast of the UK. However, the measurements were only recorded over a one month period towards the end of the reproductive season when previous studies have shown vegetative growth to be at a minimum (Norton, 1977b, Gorham and Lewey, 1984). Further studies looking at the seasonality of growth of *S. muticum* from locations spanning the whole of the Welsh coast are therefore required before more detailed comparisons can be concluded.

Fig. 1.4 Winter condition of *S. muticum* present in rockpools at Broad Haven, Pembrokeshire (10th October 2010). Note plants have died back to holdfast and basal stipe and leaves.

Sexual reproduction in *S. muticum* is characterised by the development of receptacles on the high order laterals of the thallus which contain both the oogonia and antheridia. The alga is monoecious and self-fertile in contrast to most British algae (Critchley, 1983c). Eggs are released onto the surface of the receptacles approximately every 13 days where they are fertilised by the motile male antherozoids (Fletcher and Fletcher, 1975, Fletcher, 1980, Engelen et al., 2008, Monteiro et al., 2009). Fertilised zygotes remain attached to the surface of the receptacle for 2-3 days where they continue to develop into small multicellular germlings complete with developing rhizoids before being released into the water column (Fletcher, 1980). The release of well-developed germlings enhances survival under adverse environmental conditions and aids rapid sinking and settlement (Fletcher, 1980, Davison, 2009). A generalised life cycle of *S. muticum* is shown in Fig. 1.5. Fecundity in *S. muticum* is high with reports of up to 500,000 eggs potentially being released from a small plant in its first growth season (Norton and Deysher, 1989). The fecundity of the alga also increases with age due to the increase in the number of fronds that develop which is implicated in the alga's rapid expansion and consolidation following many introductions (Critchley, 1983a).

Similar to growth, the reproductive period of *S. muticum* appears more seasonal at higher latitudes and a link with seawater temperature has been suggested (Deysher, 1984). However a study by Espinoza (1990) showed that the peak of reproduction in population from Baja, California interestingly coincided with a period of cold water up welling providing contradictory evidence as to the influence of temperature on reproductive period. Within the UK the maximum fertility typically occurs during the summer months (Fletcher and Fletcher, 1975). Lewey and Farnham (1981) reported that plant with receptacles could be found all year round within the population at Bembridge, Isle of Wight.

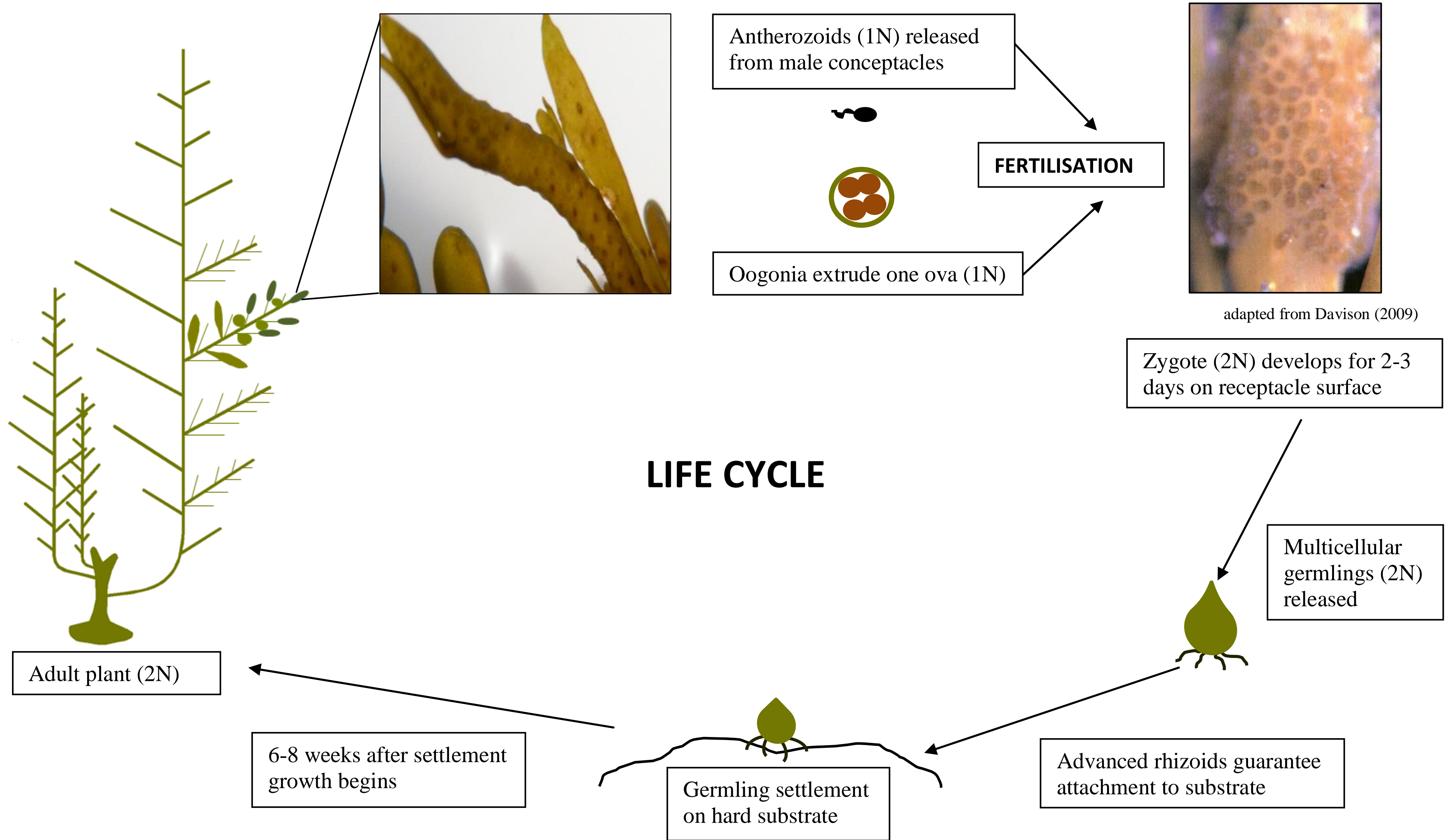


Fig. 1.5 Typical life cycle of *S. muticum.*

1.2.4 Dispersal mechanisms

A variety of dispersal mechanisms exist for *S. muticum* that have contributed to its effective spread around new coastlines. The direct release of germlings, which have a short planktonic duration (approximately 48hrs) contributes to effective dispersal distances on the order of 2-3m from the parental plants, resulting in local consolidation of *S. muticum* stands (Norton, 1980, Deysher and Norton, 1982). However, dispersal distances will be influenced by the environmental conditions at the time of release and drifting germlings have been recorded up to 1.3km from the nearest fertile plant (Deysher and Norton, 1982). Furthermore, modelling of germling dispersal under different hydrographical regimes has demonstrated that distances approaching 2km could be expected under storm conditions of 50cm/s currents and 3m high waves (Gaylord et al., 2002).

A rather unusual method by which *S. muticum* can spread is through a mechanism termed “saltation” or “stone walking”. This occurs when plants attached to loose substrata such as oyster shells or small stones, become larger and more positively buoyant resulting in the attachment substrata being lifted off the bottom resulting in the plant being carried along in a series of short distance leaps by local currents (Critchley, 1981b, Nicholson et al., 1981). It is likely that this dispersal mechanism contributes to intermediate distances of spread in *S. muticum* but will clearly be dependent on the type of habitat invaded (Strong et al., 2006). A study looking at another invasive macroalgae, *Undaria pinnatifida* estimated rates of between 10 and 100 m per year could be attributed to dispersal by “stone walking” (Sliwa et al., 2006).

A third natural dispersal mechanism contributing to the effective spread of *S. muticum* is that of drift material. *Sargassum muticum* regularly sheds its annual grown lateral fronds which can then drift according to wind and water currents. Fronds may also become released as a result of physical damage and grazing by herbivores (Fletcher and Fletcher, 1975). Once fragments have been shed they cannot reattach to the substrate but can still remain viable for up to 3 months expanding the potential range of the alga’s distribution (Farnham et al., 1981). For example tagged specimens of *S. muticum* have been shown to drift up to 900 km travelling around 18 km per day (Segawa et al., 1959). The length of time spent as drift material is also dependent upon

the developmental stage of the material when released. Abscised fertile laterals tend to decay more rapidly with buoyancy lost within 14 days of release (Davison, 1999, Harries et al., 2007a). In contrast abscised vegetative fragments can persist as drift material for considerably longer increasing potential dispersal distances (Harries et al., 2007a). Furthermore these fragments have the potential to self-fertilise en route transporting viable germlings allowing the colonisation of new distant locations (Fletcher and Fletcher, 1975, Farnham et al., 1981, Deysher and Norton, 1982). Drifting of abscised fertile laterals is considered to have been largely responsible for the spread of the alga along 3000 km of the Pacific coast of North America which included a single jump of 1100 km along the southern Californian coastline (Norton, 1978, Norton, 1981a). This dispersal mechanism is largely regarded as the main probable strategy for the alga's spread within several of its introduced regions including NE Pacific, NW Atlantic and the Mediterranean. Further evidence supporting natural drift of fragments as the main mechanism for the alga's expansion and spread comes from the fact that many records of new populations have often been preceded by reports of drift material within the local vicinity (Deysher and Norton, 1982, Critchley et al., 1983, Rueness, 1989, Davison, 2009). For example, before the discovery of the first Welsh population in West Angle Bay, Pembrokeshire, in 1998, drift material had already been observed in the early 1990s of the coast of Pembrokeshire (Davison, 1999).

Finally, the alga may be dispersed over potentially large distances as a result of anthropogenic activities such as shellfish imports (Critchley and Dijkema, 1984, Rueness, 1989, Boaden, 1995) and shipping (Critchley and Morrell, 1982, Godwin, 2003). Specifically, the importations of the Pacific oyster, *Crassostrea gigas*, have been implicated as the likely vector responsible for several worldwide introductions of *S. muticum* (Boaden, 1995, Davison, 1999, Davison, 2009). The establishment of *S. muticum* in the Mediterranean i.e. Thau Lagoon and Venice, both of which are areas of important oyster culture, are clearly associated with the importation of oyster spat into the region (Knoepfflerpeguy et al., 1985, Gargiulo et al., 1992). Furthermore, in a recent experimental study simulating transfers of Pacific oysters from the Thau Lagoon, *S. muticum* was listed among the macroalgal species recorded after culturing (Mineur et al., 2007). A growing body of evidence now exists in support of this vector in the introduction of numerous non-native marine species including several species of marine

macroalgae (Ribera and Boudouresque, 1995, Ribera Siguan, 2003, Schaffelke et al., 2006, Hewitt et al., 2007, Williams and Smith, 2007).

The role of shipping in the spread of *S. muticum* however remains unclear. Dispersal of germlings via ballast is not considered a likely method given the short planktonic duration of germlings and rapid decline in ability for attachment (Davison, 1999). The ability for attachment of germlings is usually lost within 40 days of being exuded from the reproductive receptacles. In contrast, short distance dispersal may be possible via entanglement of drift material on the steering gear of small vessels and the introduction into the Channel Islands has been suggested to have occurred via this method (Critchley and Morrell, 1982). Hull fouling has also been considered unlikely as attached larger plants would be expected to become dislodged in transit. However, Godwin (2003) reported the presence of *S. muticum* attached to the hull of a ship entering Hawaii arriving from California where it was known to be established. Reports of establishment near moorings and pontoons also highlight the potential for localised boat traffic in the dispersal of *S. muticum* (Kraan, 2007). In addition, results of a recent study examining the potential of recreational boating in the spread of non-native marine species recorded *Sargassum muticum* within hull fouling communities studied (Clarke Murray et al., 2011).

1.2.5 Rates of dispersal in *S. muticum*

Recorded rates of dispersal in *Sargassum muticum* are often high compared with many other non-native algae (Lyons and Scheibling, 2009, Mineur et al., 2010). However rates do show variation depending upon the introduced region and may be dependent on coastal topography, substrate availability and prevailing wind and surface currents (Deysher and Norton, 1982, Harries et al., 2007a, Lyons and Scheibling, 2009). Wind rather than water currents is thought to be the main factor driving *S. muticum* drift around coastlines (Deysher and Norton, 1982). This could be due to the fact that the air vesicles keep the plants buoyant so drifting fragments are likely to remain in the surface layers of the water which are influenced more strongly by wind driven currents. Estimates taken off the Californian coast have shown that wind driven dispersal rates of *Sargassum* can be up to 50 times higher than that by water currents (Deysher and Norton, 1982). Within a ten year period *S. muticum* had established populations along

several hundred kilometres of the south coast of England and along the north coast of France (Critchley et al., 1983). A similarly rapid colonisation has also occurred on the west coast of Sweden with hundreds of kilometres of coastline becoming populated over a ten year period (Karlsson and Loo, 1999). Average dispersal rate along the south coast of the UK has been estimated at 30 km per year (Farnham et al., 1981). Records from France indicated that the area of colonised coastline increased by 144 km per year (Strong, 2003). Data compiled from six years of monitoring surveys in Strangford Lough, NI revealed an average dispersal rate of around 5 km per year (Roberts et al., 2004). Within Limfjorden, Denmark Stæhr *et al.* (2000) reported an average dispersal rate of 15 – 17 km per year with *Sargassum* becoming the most dominant macroalga by 1997. Along the Irish coastline a rate of spread of 54 km per year has been estimated with 2 – 3 km per year calculated from one bay (Kraan, 2007). Along the west coast of the UK dispersal rates have been estimated at around 44 km per year since the alga's discovery on the north coast of Cornwall in in the 1990s (Harries et al., 2007a). Based upon the year of first observation a rate of approximately 9 km per year has been suggested for the spread of the alga around Anglesey, North Wales (Grant, 2007). The southward spread of *Sargassum* along the Pacific coast of North America was particularly rapid with an average rate of around 60 – 100 km per year (Farnham et al., 1981, Norton, 1981a, Lyons and Scheibling, 2009). As previously mentioned, rates of dispersal in *S. muticum* are variable depending on the introduced region. For example in a recent review (Lyons and Scheibling, 2009) of expansion rates in invasive macroalgae, the three main introduced regions of *S. muticum* showed significant differences in dispersal rate. Within the Mediterranean, the expansion of *Sargassum* was considerably faster (302 km per year) compared to the NE Atlantic (234 km per year) and NE Pacific (89 km per year) regions (Lyons and Scheibling, 2009). However, it should be noted that recorded dispersal rates of non-indigenous marine species including *Sargassum* are estimations largely based upon the year of first sighting which may not mirror the exact date of arrival of the species at the site especially in case of cryptogenic species.

1.2.6 Ecological impacts of *S. muticum*

New introductions and the continued spread of *S. muticum* have generated considerable fears as to the alga's potential impact on native biodiversity. As habitat

forming species, introductions of marine macroalgae may have important consequences for the structure and function of invaded ecosystems (Pedersen et al., 2005, Schaffelke et al., 2006, Strong et al., 2006, Wallentinus and Nyberg, 2007). According to a UK Biodiversity Action Plan (MarLIN 2007) *S. muticum* is among the top seven non-native species that pose a potential threat to the biodiversity of the UK. These concerns have sparked a large number of studies investigating the effects of the alga's introduction on native biodiversity (Critchley et al., 1990b, Davison, 2009).

Reductions in abundance and growth of indigenous algae through competition with *S. muticum* have been demonstrated by several studies (Ambrose and Nelson, 1982, De Wreede, 1983, Stæhr et al., 2000, Britton-Simmons, 2004, Harries et al., 2007b). For example White and Shurin (2011) demonstrated that increasing densities of *S. muticum* resulted in species richness reductions from native macroalgal communities in Canada, largely as a consequence of light competition. Further indirect effects on native communities have also been documented. For example the decrease in native sea urchin numbers in areas of *S. muticum* from the San Juan Islands was due to the replacement of their native food resources (Britton-Simmons, 2004). However, a number of studies have documented no significant impacts on native algal assemblages by the presence of *S. muticum* (Viejo, 1997, Wilson, 2001, Olabarria et al., 2009) which may be attributed to its pseudo-perennial life cycle (Sanchez and Fernandez, 2005). In some cases an indirect positive effect has resulted as demonstrated by Sanchez et al. (2005) who showed that the reduction in abundance of the dominant native algae by competition with *S. muticum* subsequently lead to increases in several other native algae via competition release. However as most experimental investigations into the impact of *S. muticum* have been conducted over relatively short time scales, the ultimate effects on the community may not have been reached given the temporal scales of succession in the systems studied (Sanchez et al., 2005). It is clear that the ecological impact of *S. muticum* on indigenous algae appears to vary depending on the area of introduction.

One area that has been of significant concern has been the impact of *S. muticum* on native seagrass beds (*Zostera* spp.) which serve as important nursery areas for fish and marine invertebrates and which are currently protected by a UK Habitat Action Plan (Tyler-Walters, 2007). However, to date there are no records of *Zostera* species being directly out-competed by *S. muticum* (Davison, 2009). The common trend that has

emerged is that *S. muticum* is able to benefit from disturbance events within beds of *Z. marina* resulting in the reduction seagrass bed regeneration (Farnham et al., 1981, den Hartog, 1997). For example, at Bembridge a decline in the levels of *Z. marina* and a subsequent increase in *S. muticu*m only occurred following a frost die-back episode of the seagrass (Davison, 1999). In fact, recent research has demonstrated that the colonisation of soft sediments by *S. muticum* can be enhanced in *Z. marina* beds (Tweedley et al., 2008). In addition a further study has shown that native clams within seagrass beds habitats can also facilitate the colonisation by *S. muticum* through the provision of attachment substrate (White and Orr, 2011). Finally, the original fears concerning seagrass replacement by *S. muticum* following natural dieback may be reduced some degree by the recent finding of Polte and Buschbaum (2008). In their study, the increase in abundance of the native pipefish, *Entelurus aequoreus* was directly attributable to the introduction of *S. muticum* into areas of the Wadden Sea originally occupied by seagrass beds.

Given the potential role of *S. muticum* as an ecosystem engineer, impacts of the alga may also be evident within other local marine habitats. For example, a study by Strong et al. (2006) highlighted significant differences within the infauna from under and outside canopies of *S. muticum*, with a greater abundance of opportunistic r-selected species under than outside the canopy. Further community differences were also apparent that were attributable to the duration of population colonisation.

As many larger species of macroalgae can support diverse communities of epibiota, fears were expressed that replacement of indigenous alga by *S. muticum* would result in further losses of native biodiversity. However, the majority of studies to date indicate that *S. muticum* can support a considerable diverse range of epibionts (Withers et al., 1975, Jephson and Gray, 1977, Aguilar-Rosas and Galindo, 1990, Giver, 1999, Bjaerke and Fredriksen, 2003, Wernberg et al., 2004, Buschbaum et al., 2006, Harries et al., 2007b, Cacabelos et al., 2010, Gestoso et al., 2010) . In some habitats, the complex morphology of *S. muticum* allows the alga to support an increased abundance and diversity of epiphytes than compared with native macroalgae (Giver, 1999, Bjaerke and Fredriksen, 2003, Buschbaum et al., 2006). Studies have also examined the distribution of colonising epibionts with the majority occurring on the older, perennial regions of the plant (Withers et al., 1975, Aguilar-Rosas and Galindo, 1990), although exceptions to

this have been reported (Bjaerke and Fredriksen, 2003). A reflection of a pseudo-perennial life history and the presence of anti-fouling tannin-like substances from higher regions of the laterals have been suggested to explain the increased epiphyte loading on the lower portions of the plant (Withers et al., 1975). Therefore in areas where structurally similar, perennial native alga such as *Halidrys siliquosa* dominate, replacement by *S. muticum* may have negative consequences for the longer lived epibiont species due to the seasonal growth cycle of *S. muticum* (Viejo, 1999). For a comprehensive review of the ecological implications of *S. muticum*, see Davison (2009).

Preliminary observations from *S. muticum* growing in North Wales found a total of 29 species of mobile and attached epibiota and also a degree of variability in species richness between contrasting habitats (Grant, 2007). However, no comparisons were made with native algae so the question still remains as to the effect of *S. muticum* on native epibiota within Wales. Furthermore, epibiota were only sampled once so the seasonality of epiphyte abundance and diversity remains unanswered. As yet a general consensus concerning the ecological impacts of the alga on native marine communities has not been reached and it is evident that the potential impacts will vary depending on the area of introduction.

1.2.7 Impacts of *S. muticum* on the physical environment

In addition to the potential impacts on native species and communities, the presence and development of a *Sargassum* canopy may modify physical aspects of the local environment. Sedimentation rates have been shown to be up to four times higher under a canopy of *Sargassum* than compared with native algal canopies (Strong, 2003). Water movement may also be considerably reduced (up to 85%) under dense canopy formations leading to the build-up of areas of stagnant water (Strong, 2003). The buoyant nature of the fronds of *S. muticum*, which spread out over the water surface can severely restrict light penetration to sub-canopy levels (Britton-Simmons, 2004). For example, a number of studies have shown that under a dense canopy of *Sargassum*, the penetration of photosynthetically active radiation can be reduced by 97% (Critchley et al., 1990a, Strong, 2003). As a consequence of reduced light penetration, presence of a *Sargassum* canopy can also lead to thermal stratification of the water column. During

the summer months Strong (2003) recorded water temperatures inside the *Sargassum* canopy that were nearly double that of the surrounding ambient water. Critchley et al. (1990a) also showed similar trends with water at the sediment surface being appreciably cooler. As a consequence of the temperature elevation within the canopy growth and photosynthetic rates of *S. muticum* are expected to increase although this remains to be experimentally tested (Davison, 2009). Finally, effects on nutrient cycling have been implicated by the presence of the alga. For example in Limfjorden, Denmark the increased dominance of *S. muticum* over a native conspecific resulted in an increased turnover of organic matter and associated nutrients (Pedersen et al., 2005).

1.2.8 Economic impacts of *S. muticum*

As *S. muticum* is known to grow on artificial floating structures and often in dense stands fears have been raised regarding the impact of the alga on aquaculture installations (Fletcher and Fletcher, 1975, Davison, 1999). In particular, the introduction and continued spread of *S. muticum* along the west coast of Scotland has been highlighted for concern owing to the large proportion of UK aquaculture products originating from this region (Harries et al., 2007a). Dense growth on shellfish beds may hinder shellfish growth, and buoyant plants have the potential to lift shellfish off the substrate carrying them away from aquaculture sites (Davison, 1999).

Drift material can cause potential problems for fishing practices through entanglement in lines and nets. For example in the Solent region *Sargassum muticum* has been reported to produce large floating mats which foul commercial fishing nets and lead to a reduction in the recreational usage of inshore waters (Gray and Jones, 1977, Critchley et al., 1986). Similar problems have also been reported from Lake Grevelingen in the Netherlands (Critchley et al., 1987). The alga has also been known to become caught up in the propellers of small boats, particularly those with out-board motors (Gray and Jones, 1977). Drift material may also affect water-based recreational activities as well as trapping marine debris which if cast ashore can incur considerable costs for removal and clean up (Davison, 2009).

1.2.9 Control and management strategies for *S. muticum*

The initial discovery of *Sargassum muticum* in the UK (at Bembridge on the Isle of Wight) sparked fears as to the potential negative ecological impacts of the alga on native marine ecosystems. Therefore a decision was taken to make attempts to try and eradicate populations of the alga from areas of Solent. However despite an intensive clearance programme trialling numerous removal methods, the complete eradication of the alga proved unsuccessful (Gray and Jones, 1977, Critchley et al., 1986). A variety of removal methods were investigated for the control of *S. muticum* including handpicking, mechanical control, herbicides and biological control agents. Hand-gathering albeit a highly selective method causing minimal ecological damage was very time consuming and labour intensive and considered impractical when dealing with large, well established stands of the alga (Critchley et al., 1986). However, despite problems encountered with this technique during clearance of large areas, the method may be best suited to small, recently established populations (Davison, 1999). The mechanical control devices tested which included trawling, cutting and suction methods were effective in clearing large amounts of the alga, but also resulted in greater unintentional ecological damage and by-catch. Furthermore the perennial bases of the alga were frequently left intact allowing the rapid regeneration of plants which tended to became fertile earlier on in the season (Critchley et al., 1986, Davison, 1999).

The control by herbicides was also discounted early on due to lack of selectivity, difficulties in their application and the large doses required compensating for the dilution factor (Lewey, 1976, Critchley et al., 1986, Davison, 1999). Despite the presence of several herbivorous grazers on *S. muticum* the potential for biological control of the alga in the UK has been shown to be limited (Davison, 1999). In the region of the seaweed's first introduction to the UK, the only effective grazer tested was the sea-hare, *Aplysia punctata*, but these molluscs were relatively scarce within the areas colonised by the alga (Critchley et al., 1986). In fact it is likely that grazing activity may aid in the dispersal and spread of *Sargassum* by increasing fragmentation of fertile or potentially fertile fronds (Critchley et al., 1986, Harries et al., 2007a).

Overall there are currently no effective management or control strategies in place for *S. muticum*. Of all the methods trialled in the past, it has been shown that

populations of the alga quickly become re-established and in some instances can result in denser, more vigorous population growth (Critchley et al., 1986, Davison, 1999). For an eradication programme of *S. muticum* to have the best chance of success it must ensure that the whole plant is removed in order to prevent sexual maturation and any vegetative regeneration (Davison, 2009). Before abandoning the programme of eradication over 480 tonnes of *Sargassum* had been removed from the shores of the Solent region between 1973 and 1976 (Gray and Jones, 1977).

In addition to the clearance efforts on the south coast of the UK attempts have been made to limit the alga's spread in Strangford Lough, Northern Ireland. Following the seaweeds discovery on oyster trestles at Paddy's Point in Strangford Lough in 1995 (Boaden, 1995), the Environment and Heritage Service (EHS) initiated a containment operation to restrict and monitor existing populations of the alga and try to prevent its further expansion within the Lough (Davison, 1999). Despite the removal (via hand-gathering) of over 25 tonnes of the algal material over a four year period since its initial observation, the area of shoreline colonised by *S. muticum* increased reaching a peak of 47.88 km in 2000 (Roberts et al., 2004, Davison, 2009). A subsequent monitoring study by Strong (2003) revealed that the fertile period of plants from the Strangford Lough lasted around 4 months beginning in August peaking in October, dates which coincided with the original containment operation period. Therefore it is likely that when the clearance operations took place, plants were being harvested during the onset of the reproductive period which could have contributed to its further spread within the region. However, as there is no data on the reproductive status of the harvested plants during the periods of the eradication programme the previous suggestion is largely speculative. Although, it is unlikely that the reproductive phenology of the plants will have shifted dramatically between the time period of harvesting and the commencement of Strong's (2003) monitoring study. Similar to the situation in the 1970s on the south coast of the UK, it appears that operations to eradicate and/or control the spread of *S. muticum* have limited success. However, on the island of Lundy in the Bristol Channel, where the alga first appeared in 1999 (Reach, 2001) small scale manual clearance from rockpools has had some measure of success (Manson, 2009). In general, most eradication attempts that have been made concerning the introduction of *S. muticum* have failed in containing the spread of this alga throughout NW Europe and along the Pacific coast of N. America

into Mexico. Therefore future efforts should be directed more to the identification and monitoring of regions at risk from potential future colonisation by *Sargassum* (Davison, 2009). To date, no non-native marine species have been successfully eradicated from UK waters (Eno et al., 1997).

1.2.10 Examples of successful eradication programs of introduced macroalgae

The eradication of introduced marine macroalgae has rarely been successful (Strong, 2003, Anderson, 2007). However, a few programs dealing with relatively localised populations that have had some measurable success have been documented. The discovery of the green alga, *Caulerpa taxifolia* in a Californian lagoon, in 2000 prompted considerable concern given the well documented rapidity of spread of the alga in the Mediterranean (Jousson et al., 2000). After only 17 days following its discovery an eradication and containment operation was initiated. Due to the rapid response of clearance efforts and the localised area initially colonised, the eradication program of *C. taxifolia* from California has been a success (Anderson, 2005). Another example of a successful eradication of an invasive macroalga concerns the operation to remove *Undaria pinnatifida* from a sunken trawler off the New Zealand coast (Wotton et al., 2004). Crucial to the success of this programme was the early detection of the alga followed by a rapid response, and a detailed knowledge of the alga's reproductive biology aiding in the strategic removal of sporophytes before the release of zoospores (Wotton et al., 2004). For a comprehensive review on management strategies to control invasive seaweeds see Anderson (2007). Control of marine invaders represents a difficult task. The strategies proposed for eradication and containment often pose an additional credible risk to native species. Therefore it is more pertinent that management strategies and policy focus on factors such as the vectors of species introductions in order to prevent arrival and establishment of high risk invasive species in the first instance.

1.3 THESIS OUTLINE

The abundance of previous research on the invasion biology of *Sargassum muticum* clearly highlights the species as one of the most successful of all reported marine invaders (Critchley et al., 1990b, Davison, 1999, Harries et al., 2007a, Davison, 2009). However disparities regarding the potential impacts of the invader exist and seem dependent on habitat and the geographical region invaded. In this research a combination of experimental field based surveys and laboratory molecular investigations were undertaken to explore aspects of the invasion biology of *S. muticum*. The overall aim of the present study was to contribute to the knowledge of the invasion biology of *S. muticum* with a specific focus on the mechanisms of its invasion into and its impacts in Welsh waters.

To date the source(s) of its introduction into Wales, and potential vectors of spread remain unknown. Furthermore no formal investigations have been undertaken to assess the current extent of the alga's distribution and its potential impacts on native biodiversity. The project aims to investigate the invasion dynamics of *S. muticum*, using a combination of molecular genetics and field based surveys, in order to provide baseline information required for the implementation of future management strategies.

The objectives of chapter three were to describe the biology of *S. muticum* from selected sites around spanning the length of the welsh coastline. As mentioned in the introduction chapter, the phenology, morphology and habitats colonised by *S. muticum* differ greatly between geographic regions and seasons. Hence, a principal aim of chapter three was to investigate the phenology of *S. muticum* over a single growth and reproductive season from a selection of four sites located along the welsh coastline that also provided a representation of the contrasting habitats that have been colonised in Wales. Furthermore, the morphology of *S. muticum* from the different habitats was determined to examine the impact of habitat type on growth form of the alga.

The aims of this study can be represented by the following main points:

- What is the current distribution of *S. muticum* within Wales?
- Using a molecular genetics approach to explore the likely mechanisms responsible for the alga's introduction and subsequent spread in Wales.

- How do the seasonal population dynamics of the alga compare between different habitats within Wales and between other non-native populations on a global scale?
- How has the introduction of the alga impacted native macroalgae in terms of the epibiont assemblages supported by the host basiphyte?

CHAPTER TWO GENETIC VARIABILITY OF *SARGASSUM MUTICUM*

2.1 INTRODUCTION

2.1.1 Invasion genetics

Given the increasing frequency of marine bioinvasions, it is important to be able to identify the pathways of introductions for policy and management purposes, and to predict and subsequently prevent future invasions (Ashton, 2006). However, accurate determination of these invasion attributes are often confounded due to the occurrence of a lag phase between the initial colonization event and the onset of population expansion to detectable levels (Sakai et al., 2001, Hebert and Cristescu, 2002). Furthermore, traditional ecological monitoring techniques are unable to identify source populations, multiple introductions and genetic diversity within populations (Holland, 2000). The reconstruction of invasion histories and pathways of introductions is crucial to the development of a fuller understanding of the causes and impacts of species introductions (Darling et al., 2004).

Genetic markers can provide an indirect approach for addressing mechanisms of invasion such as vectors and modes of dispersal (Holland, 2000, Sakai et al., 2001). By characterising the genetics of introduced populations, it is possible to draw conclusions regarding the potential sources of invaders (Jousson et al., 1998, Downie, 2002, Zardus and Hadfield, 2005), routes of spread (Pollux et al., 2003, Bachelet et al., 2004) and the number of founders involved in the introduction (Ficetola et al., 2008). Identification of source region(s) will also be important for providing insights into the biological traits that have contributed to the success of the invasion (Stadler et al., 2005). Furthermore, knowledge of the genetic variability within introduced populations may offer an indication as to the potential for post invasion evolution, and the ability of the introduced populations to respond to natural selection (Lee, 2002). A firm understanding of these factors will be crucial for proactive management and control strategies of invasive species (Provan et al., 2005, Estoup and Guillemaud, 2010).

Following an introduction of a non-native species, levels of genetic diversity are expected to be reduced, compared to the source population due to founder effects

(Allendorf and Lundquist, 2003) and this has been previously recorded in introduced populations of taxa including macroalgae (Tsutsui et al., 2000, Colautti et al., 2005, Provan et al., 2005, Meimberg et al., 2006). Furthermore, genetic drift is more pronounced in small populations, reducing within population diversity but increasing among population differentiation (Ramstad et al., 2004). Despite the potential for genetic bottlenecks in small founding populations, losses of genetic diversity can be minimised when populations undergo a rapid expansion after introduction (Allendorf and Luikart, 2007). Moreover, human-mediated dispersal vectors may act to increase gene flow between introduced populations and so may act to reduce genetic differentiation to an extent (e.g. Martel et al., 2004b). Alternatively, if the introduction involves a large number of genetically variable individuals or multiple introductions have taken place, little or no change in genetic diversity may be recorded (Holland, 2000). In some cases genetic diversity may even be elevated relative to the putative source populations (Voisin et al., 2005, Zardus and Hadfield, 2005). If founding individuals arise from a number of genetically differentiated source populations, then among-population genetic variation can be transformed into within-population genetic variation which can be augmented relative to the source locations (Kolbe et al., 2004). However, it can be difficult to differentiate between introduction scenarios, whether there has been a single introduction from a highly diversified source population or multiple recurrent introductions from several genetically distinct source populations (Simon-Bouhet et al., 2006). Therefore when using genetic data to evaluate the invasion dynamics, it is necessary to characterise the structure and diversity over the global range of the species, including both native and non-native populations (Grapputo et al., 2005, Voisin et al., 2005, Ashton et al., 2008).

2.1.2 Molecular techniques

A range of molecular markers are available for examining genetic variation both within and among populations. Direct sequencing of nuclear markers, in particular the internal transcribed spacer (ITS) regions of ribosomal DNA has been used in a diverse range of studies of marine algae (Bakker et al., 1992, Kooistra et al., 1992, Leskinen and Pamilo, 1997, Stiger et al., 2000, Coyer et al., 2001, Kogame et al., 2005, Hu et al., 2007, Rohfritsch et al., 2007). Relatively high rates of nucleotide substitution within the ITS region provides sufficient phylogenetic resolution permitting systematic

comparisons at species level and below (Wattier and Maggs, 2001). Furthermore, the development of universal PCR primers within the conserved cistronic regions of rDNA permits a rapid screening time for assessing levels of variability (White et al., 1990). In particular ITS has been a popular marker for investigating the introductions of the invasive strains of the green algae belonging to the *Caulerpa* genus (Jousson et al., 1998, Fama et al., 2000, Schaffelke et al., 2002, Verlaque et al., 2003, Stam et al., 2006). The marker was also trialled in a study by Marston and Villalard-Bohnsack (2002) looking at sourcing the introduction of the red algae, *Grateloupia doryphora*, but no variability was detected (Marston and Villalard-Bohnsack, 2002). However, several recent studies have detected intra-individual variation within the ITS region (Fenton et al., 1998, Harris and Crandall, 2000, Koch et al., 2003, Cheang et al., 2010b) and in some instances the level of intra-individual variability can be comparable to that of the inter-individual level (Fama et al., 2000). For example, cloning and subsequent sequencing of one individual of *Caulerpa racemosa* revealed six ITS variants (Fama et al 2000). It is possible, therefore that intra-individual variation in ITS may mask any small to regional scale phylogeographic patterns (Fama et al. 2000). This means that intragenomic variation needs to be taken in account when analysing and interpreting ITS sequence data. Furthermore, occurrence of multiple copies of ITS makes it necessary to perform cloning of PCR products before sequencing can be undertaken in order to identify ITS “sequence types” (Wörheide et al., 2002, Cheang et al., 2010b).

Mitochondrial DNA markers (mtDNA) have been widely applied in phylogeography studies of animals (Avise, 2000). In particular the cytochrome *c* oxidase subunit I gene has been used to help elucidate the invasion histories of many faunal taxa (Cristescu et al., 2001, Hanfling et al., 2002, Kolbe et al., 2004, Martel et al., 2004a, Zardus and Hadfield, 2005, Lopez-Legentil et al., 2006, Simon-Bouhet et al., 2006). However, the use of mtDNA markers in investigations of invasive macroalgae has been much more limited although a number studies have reported their successful application. For example, the invasion history of the kelp, *Undaria pinnatifida* was revealed using two polymorphic markers targeting intergenic spacer regions within the mtDNA genome (Voisin et al., 2005). A preliminary study looking at the recent introduction of *Sargassum filicinum* to the coast of California successfully used sequence data from the mitochondrial cytochrome *c* oxidase subunit 3 gene to indicate a potential Korean origin for the Californian invasion (Miller et al., 2007).

Plastid markers have also proven useful in phylogeographical studies of plants and algae (Wattier and Maggs, 2001). The most common region used has been the gene coding for the large subunit of ribulose-1,5-bisphosphate carboxylase (*rbc*L). Sequence data from this gene was used in a phylogeographic study of the invasive red algae, *Polysiphonia harveyi* and revealed that multiple cryptic introductions of this species had taken place both in New Zealand and in the North Atlantic (McIvor et al., 2001).

2.1.2.1 Amplified fragment length polymorphism

Amplified fragment length polymorphism (AFLP) analysis, originally patented and described in the early 1990s by Zabeau and Vos (1993) and Vos et al. (1995) is now firmly established as an effective DNA fingerprinting technique useful for revealing genetic diversity and differentiation within and among populations. AFLPs have been the method of choice for many plant studies (Mba and Tohme, 2005, Meudt and Clarke, 2007), although their application to animals, fungi, and bacteria is becoming more popular (Bensch and Akesson, 2005, Mendelson and Shaw, 2005).

The principle behind the technique is based upon the digestion of genomic DNA by two restriction enzymes followed by two rounds of selective PCR on a subset of the fragments. Amplified fragments are then separated via electrophoresis and visualised by either gel staining techniques or automated sequencing (Vos et al., 1995, Meudt and Clarke, 2007). The result is a unique genetic fingerprint for each individual with variation in banding pattern a result of substitutions, insertions or deletions within the DNA (Bensch and Akesson, 2005) (see Fig. 2.1).

As AFLP markers are distributed throughout the genome the technique can generate vast numbers of polymorphisms making it useful for a range of applications including assessing genetic diversity (Amsellem et al., 2000, Ludington et al., 2004), population structure (Donaldson et al., 2000, Kusumo and Druehl, 2000, Elderkin et al., 2004), paternity analyses (Gerber et al., 2000) and hybrid identification (Beismann et al., 1997, Congiu et al., 2001, Salmon et al., 2005, Shasany et al., 2005) and more recently for reconstructing phylogenies (Després et al., 2003, Dasmahapatra et al., 2009).

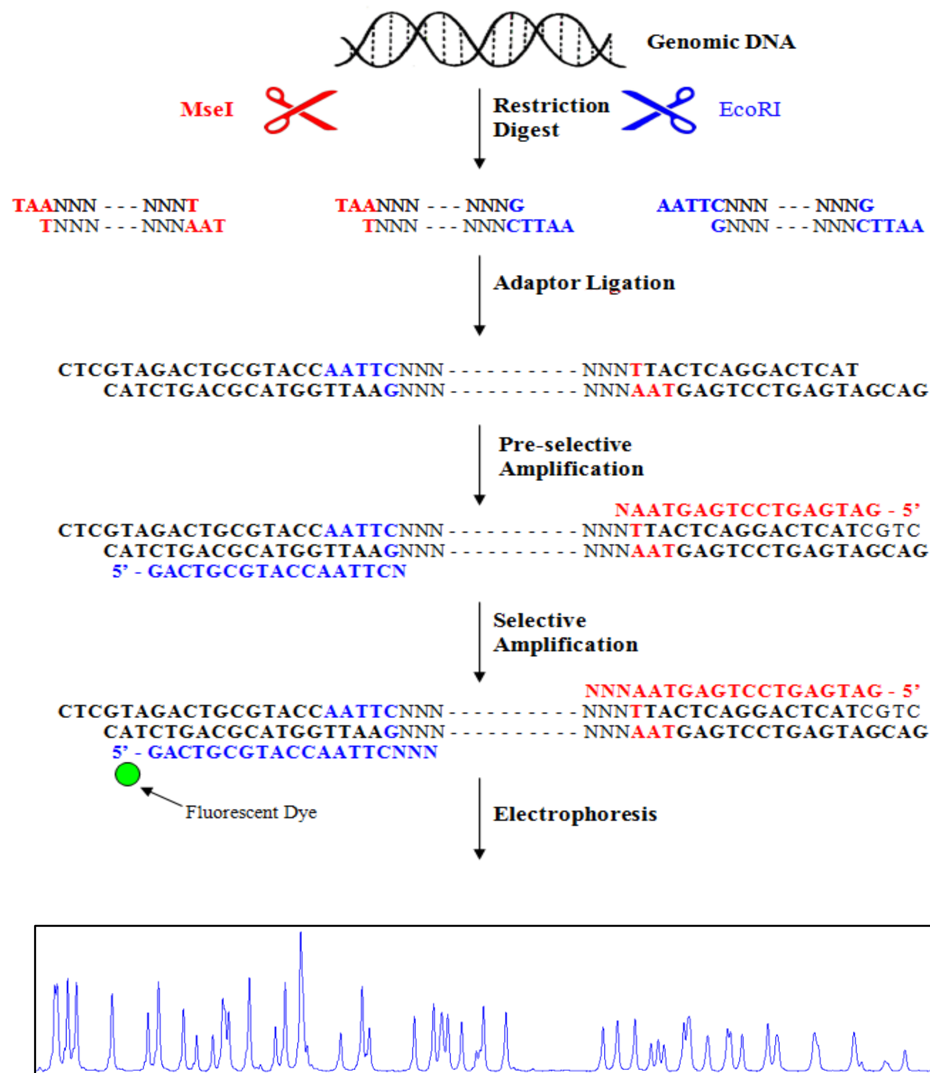


Fig. 2.1 Overview of the AFLP technique. Whole genomic DNA is first digested using two restriction endonucleases (typically EcoRI, in blue and MseI, in red) which cut the DNA at specific recognition sequences (coloured bases). Following digestion, double stranded adapters (black), are ligated to the complementary 'sticky ends' (unpaired nucleotides on the terminal ends of the adapters) of the restriction fragments which introduce primer annealing sites onto the fragments. Two different kinds of adapters are ligated - one with a complementary end to the EcoRI restriction site and the other complementary to the MseI site. Then using primers complementary to the two adapters, but with the addition of one nucleotide at the 3' end (N), a subset of the restriction fragments is amplified through the polymerase chain reaction (PCR). The PCR products of the pre-selective amplification are then used as the template for a second round of PCR using primers with up to three selective nucleotides (NNN) at the 3' end further reducing the number of amplified fragments to a level suitable for visualisation by electrophoresis. The EcoRI+3N primer in the selective amplification is labelled with a fluorescent dye to enable the visualisation of fragments. Note that only fragments having an EcoR1 binding site at one end and an Mse1 binding site on the other are detected, because fragments with two EcoR1 ends do not amplify well and fragments with two Mse1 ends are not marked with a fluorescent tag. The resulting fragments are then separated on a polyacrylamide gel or using a capillary sequencer generating a unique banding pattern for each individual due to substitutions, insertions or deletions within the DNA. An electropherogram for a Californian specimen of *Sargassum muticum* produced by an ABI capillary sequencer is shown here.

When comparing with the marker system of random amplified polymorphic DNA (RAPDs) markers several studies indicate that fewer primers are usually required to produce an equivalent if not greater number of polymorphic loci (Robinson and Harris, 1999). For example in a study of genetic diversity within species of the *Salix* genus, 20 RAPD primers generated a total of 170 polymorphic bands compared with 645 bands generated by only 4 AFLP primers (Barker et al., 1999). A study of the genetic structure of the non-native infaunal anemone, *Nematostella vectensis*, populations using 6 RAPD primers generated a total of 31 polymorphic bands (Pearson et al., 2002) compared with 154 polymorphic loci using 2 AFLP primer pairs (Darling et al., 2004). However the latter study sampled populations from the suggested native range of *N. vectensis* which may account for the greater levels of genetic diversity recorded.

Furthermore the capacity for producing large numbers of polymorphic loci means AFLPs are particularly useful when a lack of variation is suspected (Meudt and Clarke, 2007). Therefore they have the potential to be a valuable tool in studies of recently introduced species where reduced diversity is often predicted (Dlugosch and Parker, 2008). The high variability afforded by AFLPs has also proven useful in detecting differentiation over relatively small spatial scales and in some cases has provided greater resolution than compared with microsatellites (Garoia et al., 2007). For example, Kusumo and Druehl (2000) used AFLPs in their study of the winged kelp, *Alaria marginata* and demonstrated that the technique could genetically differentiate between both adjacent plants and plants separated by 185km.

The ability of AFLPs to provide resolution at much finer scales in contrast to other techniques such as direct sequencing of gene fragments was important in resolving questions regarding the introduction of the invasive alga *Caulerpa taxifolia* into regions of southern Australia (Murphy and Schaffelke, 2003). ITS sequence data generated from a previous study (Schaffelke et al., 2002) was unable to distinguish individuals from Lake Conjola, New South Wales, with those from a native population and from the invasive Mediterranean strain. However, AFLP genotyping revealed that individuals from Lake Conjola clustered with those from a native population refuting the hypothesis of an introduction from the Mediterranean (Murphy and Schaffelke, 2003).

Another major advantage of AFLPs compared with other fragment techniques such as microsatellites is that no *a priori* sequence information from the target species is required (Vos et al., 1995, Robinson and Harris, 1999). Thus, start-up times for investigations employing AFLPs are relatively rapid, unlike those involving microsatellites, where lengthy periods are often required to create DNA libraries in order to screen for polymorphic loci. Evidence also suggests an advantage of a higher level of reproducibility compared with RAPDs (Mueller and Wolfenbarger, 1999, Nybom, 2004). In tests of the AFLP method across a network of nine different European laboratories, Jones et al. (1997) demonstrated that AFLP banding patterns could be reproduced with only a single incidence of a band difference in one track.

The main disadvantage, however, of AFLPs as with RAPD markers is that they are dominant markers, meaning that homozygous and heterozygous states cannot be distinguished when scoring fingerprints (Mueller and Wolfenbarger, 1999). It is simply the presence or absence of a DNA fragment at a particular locus that can be determined. Hence the per-locus type of genetic information obtained by AFLPs is therefore considerably reduced in comparison with multi-allelic microsatellite loci. It is suggested that when considering sample sizes, 2-10 times more individuals per population would need to be genotyped to achieve comparable results with microsatellite markers (Mba and Tohme, 2005). However, the sheer number of loci generated by AFLPs when compared with microsatellites gives them the level of statistical power required for their effective application (Mariette et al., 2002, Meudt and Clarke, 2007). Therefore a trade-off exists when using AFLP markers compared to microsatellites for example in terms of the per-locus type of genetic information (Bensch & Akesson 2005).

Another issue concerning the analysis and interpretation of AFLP data is that of band-size homoplasy. Co-migrating fragments within AFLP profiles are generally assumed to be homologous, which is an essential pre-requisite for estimating genetic parameters from AFLP data (Bensch and Akesson, 2005, Mendelson and Shaw, 2005). However, presence of same-sized bands may in fact consist of bands from different regions of the organism's genome representing two or more different AFLP loci. Conversely, mutations such as indels at a particular locus may produce different size fragments which may be mistakenly scored as an allele for a different locus rather than two alleles for the same locus (Bensch and Akesson, 2005). Null or absent alleles also

present similar problems for homology as nucleotide substitutions, insertions or deletions in regions flanking the restriction sites can lead to band absence. However, estimations of genetic distance derived from similarity of shared band presence (e.g. Nei and Li, 1979) help to circumvent this problem. In addition studies that have looked at the extent of homoplasy in AFLP fragment data using sequencing, have demonstrated high levels of fragment homology (Mendelson and Shaw, 2005).

The statistical analysis of AFLP data can be principally divided into estimators of genetic diversity and population structure. AFLP profiles can be analysed using either a band-based or an allele frequency-based approach. Essentially band-based approaches compare the pattern of presence and absence of bands between individuals to produce similarity estimates which include the Jaccard coefficient (Jaccard, 1908), the Nei and Li coefficient (Nei and Li, 1979) and the simple matching coefficient (Sokal and Michener, 1958). Of these the latter maximises the information taken from the AFLP profile as it considers all scored loci (Bonin et al., 2007). The allele frequency-based approach involves estimating allele frequencies at each AFLP locus. They include the square-root procedure, Lynch and Milligan procedure (Lynch and Milligan, 1994), the moment-based procedure (Hill and Weir, 2004), the Holsinger procedure (Holsinger et al., 2002) and the Bayesian procedure (Zhivotovsky, 1999). Subsequently these metrics can then be used to estimate heterozygosity either as percentage of polymorphic loci or using Nei's average gene diversity (Nei, 1973) which is equivalent to the average expected population heterozygosity. However, many of the allele frequency based methods are only accessible with information regarding the level of inbreeding and assumption of Hardy-Weinberg equilibrium (HWE) (Bonin et al., 2007). The Bayesian approach to calculating allele frequencies make no assumptions regarding HWE and have been shown to be more useful when dealing with monoecious species that undergo selfing (Zhivotovsky, 1999).

In conclusion because low levels of genetic variation are common within introduced species, the use of a single marker system may by inadequate to provide sufficient resolution and it is becoming increasingly common to utilise a combination of molecular techniques to investigate hypotheses (Murphy and Schaffelke, 2003, Petersen, 2006). Depending on the questions asked, markers that differ in their relative evolutionary rates are normally selected (Ashton, 2006). Despite some clear benefits of

AFLP markers for evolutionary studies of populations, to date they have only been used in a handful of invasive species studies, only two of which have been from invasive macroalgae (see Table 2.1 (Murphy and Schaffelke, 2003, Bjærke, 2004)). Also, reports of a low genomic frequency of nuclear microsatellites from seaweeds provide a further reason for the application of AFLP markers in population studies of macroalgae (Wattier et al., 1997, Wattier and Maggs, 2001).

Table. 2.1. Use of AFLPs in marine macroalgal studies including the research questions proposed. Studies focused on introduced macroalgae are indicated in bold.

Species	Research question	Source
Ahnfelti opsis pusilla	Population genetic structure	(Couceiro et al., 2011)
Alaria marginata	Population structure	(Kusumo and Druehl, 2000)
Caulerpa taxifolia	**Source population identification**	**(Murphy and Schaffelke, 2003)**
Chondrus crispus	Population genetic structure	(Donaldson et al., 2000)
Gracilariopsis lemaneiformis	Genetic diversity	(Pang et al., 2010)
Grateloupia lanceola	Genetic diversity, population structure	(Maneiro et al., 2011)
Heterosiphonia japonica	**Population structure**	**(Bjærke, 2004)**
Hizikia fusiformis	Strain identification	(Shan et al., 2009)
Laminaria japonica	Genetic diversity, genetic mapping	(Liu et al., 2009, Heng et al., 2010)
Laminaria spp.	Phylogeny, genetic mapping	(Erting et al., 2004, Li et al., 2007)
Lithophyllum margaitae	Strain identification	(Schaeffer et al., 2002)
Mastocarpus papillatus	Genetic diversity	(Ludington et al., 2004)
Porphyra spp.	Strain identification	(Sun et al., 2005)
Porphyra yezoensis	Strain identification	(Iitsuka et al., 2002, Niwa et al., 2004)
Postelsia palmaeformis	Population structure	(Kusumo et al., 2004)
Saccharina japonica	Genetic diversity	(Shan et al., 2011)
Undaria pinnatifida	Strain identification	(Shan and Pang, 2009)

2.1.3 DNA extraction from macroalgae

There are now a multitude of different chemical methods and commercial extraction kits that allow rapid isolation of high molecular weight DNA from a whole range of starting material. In contrast, the extraction of high quality DNA from macroalgae has proven problematic (Olsen, 1990, Wattier and Maggs, 2001). One of the

main reasons for the difficulty relates to the high polysaccharide and polyphenolic content of many marine algae which are often co-extracted resulting in highly viscous DNA extracts (Shivji et al., 1992, Alberto et al., 1997, Wattier and Maggs, 2001). These contaminants in the DNA extract can potentially inhibit downstream applications such as restriction digests and PCR (Alberto et al., 1997, Jin et al., 1997). Consequently several DNA extraction protocols have been developed specifically for different marine macroalgae and specific taxonomic groups (Saunders, 1993, Wattier et al., 2000, Phillips et al., 2001, Varela-Alvarez et al., 2006, Hoarau et al., 2007). For example Hong et al. (1997) utilised a Lithium chloride based protocol for extraction of seaweed nucleic acids of PCRable quality. However, despite attempts to produce a generalised DNA isolation protocol applicable to all groupings of macroalgae, some species and genera still present extraction difficulties for use in downstream applications e.g. *Sargassum* spp. (Ho et al., 1995) when using the LiCl methodology. In a study by Jin et al. (1997) testing inhibitory effects of extracts from over 25 different species those belonging to the *Sargassum* genus were the most common species containing PCR inhibitory compounds. As such many of the published DNA extraction protocols developed for macroalgae involve a preliminary step of grinding the tissue in liquid nitrogen (Coyer et al., 1995, Wattier et al., 2000, Phillips et al., 2001, Wang et al., 2005, Varela-Alvarez et al., 2006) in order to release viscous soluble polysaccharides (Varela-Alvarez et al., 2006).

In addition, as certain DNA fingerprinting techniques are more susceptible to variations in template quality (e.g. RAPDs), the development of efficient DNA isolation methods for seaweeds has been a necessary step in the advancement of genetic diversity analysis of marine macroalgae (Wattier and Maggs, 2001). It is for the reasons described above concerning the difficulites of extracting high quality DNA that genetic analysis of seaweeds has lagged behind animal and other plant taxa. Common to many DNA-based studies involving seaweed is the need to include further DNA purification procedural steps once the crude DNA extract is isolated. Typically this has involved the use of caesium chloride (CsCl) gradient ultracentrifugation (Coyer et al., 1997, Wright et al., 2000, Engelen et al., 2001). For example in a study by Mizukami et al. (1998) only *Porphyra yezoensis* DNA extracts that underwent further purification with CsCl were of sufficient quality to generate reproducible banding patterns with a selection of RAPD primers. It appears that inclusion of CsCl ultracentifugation is a vital step

necessary to ensure the effective removal of potential inhibitors from macroalgal template DNA.

Extraction of DNA has been particularly problematic for species of brown seaweed (Phillips et al., 2001, Hoarau et al., 2007, Snirc et al., 2010). Furthermore, among the brown algae the fucalean taxa have been shown to be particularly problematic (Phillips et al., 2001, McDevit and Saunders, 2009). This is due to the large amounts of acidic polysaccharides contained in brown algae, which are more water soluble than the typical polysaccharides of land plants (Wang et al., 2005). Methods to overcome these problems include using organelle harvesting or extracting solely from reproductive tissues (Mayes et al., 1992, Shan and Pang, 2009). However despite the development of more efficient DNA extraction protocols for brown algae many of them still require the application of a final CsCl density gradient ultracentrifugation step before the DNA can be used in subsequent downstream applications. In contrast, several population genetic studies on members of the Rhodophyta including those employing RAPD markers (known to be highly sensitive to variations in DNA template quality and quantity) have been successful without the application of further DNA purification techniques (e.g. Alberto et al. (1999)). For example, Alberto and Leitao (1997) utilised a modified DNA extraction protocol originally developed for fungi, to isolate DNA from *Gelidium sesquipedale*. The technique required no final purification steps and the resulting DNA was suitable for population based studies with RAPD markers.

The presence of epibionts on macroalgae is also a major factor that needs to be controlled for when conducting extractions as DNA from any undetected epibiont is likely to co-extracted with the algal genomic DNA. Schofield et al. (1991) has suggested that epiphyte contamination in algal DNA extracts usually constitutes less than 5% of the total extract. However, contamination by epibiont DNA can lead to problems in downstream applications especially when using universal primers in PCR reactions. Therefore the initial phases of cleaning surface tissue of algal material for DNA analysis is a necessary step in the extraction process and a number of cleaning protocols have subsequently been developed.

Although no commercial extraction kits have been developed specifically for macroalgae, several kits for plants are available (e.g. DNeasy Plant Mini Kit, QIAGEN,

Nucleospin Plant II, MACHEREY-NAGEL, E.Z.N.A Plant DNA Kit, OMEGA Biotek) some of which have been successfully applied in macroalgal genetic studies ((Engel et al., 2003, Voisin et al., 2005, Miller et al., 2007, Tatarenkov et al., 2007, Zhao et al., 2007, Lee et al., 2011, Yow et al., 2011). Interestingly, a recent extraction protocol developed for use on brown algal species originated from modifications to a commercial extraction kit (Snirc et al., 2010). Despite several benefits of commercial kits such as a reduced processing time and the absence of hazardous chemicals, the higher costs makes their use prohibitive for larger scale population based studies (Wattier and Maggs, 2001, Hoarau et al., 2007, McDevit and Saunders, 2009). Of the chemical based methodologies used, the CTAB technique (Doyle and Doyle, 1987) has been one the most popular method for the extraction of DNA from macroalgal material (Coyer et al., 1995, Phillips et al., 2001, Varela-Alvarez et al., 2006, Hoarau et al., 2007). Most studies have modified certain aspects of the protocol; however the fundamental processes of the method remain consistent.

2.1.4 Current genetic status of *S. muticum*

To date information on the genetics of *S. muticum* is limited to a handful of DNA sequences isolated from predominantly phylogeny based macroalgal studies (Stiger et al., 2003, Phillips et al., 2005, Lee et al., 2011) and DNA extraction development protocols (Phillips et al., 2001, McDevit and Saunders, 2009). The first assessment of genetic variability at the population level within *S. muticum* based on RAPD and ISSR marker data revealed significantly higher genetic variability among populations than within populations (Zhao et al., 2008). With a monoecious life history and dispersal capacity of germlings limited to within a few metres of the parental plant (Deysher and Norton, 1982), these patterns of genetic diversity reflected well, the expectations based on the life history strategy of *S. muticum* (Loveless and Hamrick, 1984, Zhao et al., 2008). The most recent genetic study on *S. muticum* included material from both the native and introduced ranges of the algae (Cheang et al., 2010c). Using a combination of three molecular markers spanning all three genomes (nuclear, plastid and mitochondrial) a clear lack of genetic variability was revealed in this alga. Only a fragment from a mitochondrial spacer region (*Trn*W_I) displayed any variation with only two distinct haplotypes revealed, of which Hap A was common to all introduced populations and those from the central and western Japan (Cheang et al., 2010c). The

lack of variation displayed in the different markers used in their study lead to the author's suggestion that additional data, incorporating more rapidly evolving markers would be required to further resolve the relationships between native and invasive populations of *S. muticum*.

The aim of this study was to utilise a combination of DNA sequencing markers from the plastid and mitochondrial genomes together with AFLPs to assess the level of genetic diversity within and between populations of *S. muticum* from both the introduced and native ranges of the alga. These markers will be used to detect any patterns of genetic variation and structure with a view to identifying the likely source population(s) of *S. muticum* to its introduced range. More specifically, with regards to the occurrence of the alga within Wales, these data will be used to discover any patterns of genetic structure among the Welsh populations which could indicate the likely vectors of introduction and/or pathways of spread.

Despite the advantages and disadvantages of both AFLPs and microsatellites (as previously discussed in section 2.1.2.1) the reduced developmental time (and therefore monetary cost) for AFLPs in comparison to microsatellites (especially in studies of non-model organisms, such as *S. muticum* for which there is little genetic data available) was one of the main reasons behind the choice of this marker for this investigation. Furthermore, given previous records of low genetic variability within introduced populations of *S. muticum* (Cheang et al. 2010c) AFLPs were selected based on the fact that they assess variability across the whole genome and it was therefore hypothesised this would increase the probability of detecting variability between and potentially within populations.

2.2 MATERIALS AND METHODS

2.2.1 Sample collection and preservation

Samples of *S. muticum* were collected from 30 locations spanning the global distribution of the alga (Table 2.2); this included 7 populations from the alga's native range in Japan and 23 non-native populations. Within Wales 13 populations were sampled, covering both the extent of the alga's distribution and diversity of colonised habitats. All locations within Wales and England were surveyed during spring tides and only samples were collected from the intertidal zone. Two sampling strategies were employed when collecting plant specimens depending on the spatial distribution and extent of plant stands at the selected location. For example, at some sites plants were only found within intertidal pools, in which case 2-3 plants were sampled from each pool at the location. In areas where the plants occurred in a large patch, typically within a shallow lagoon, approximately 20-30 plants were randomly sampled. *Sargassum* germlings are known to have short dispersal distances (Norton, 1992, Kendrick and Walker, 1995). According to Deysher and Norton (1982) recruitment of *S. muticum* germlings is low even within 2 metres of fertile parent plants and virtually negligible at a distance of 5 metres. The authors also demonstrated an abrupt decrease in plant recruitment density beyond 3 metres of the parental plants (see Fig. 2 in Deysher and Norton, 1982). Therefore samples were collected at a minimum spacing distance of 5 metres to avoid sampling individuals from the same parental plant. Seaweed collections involved taking a small fragment (approximately 5 cm length) from the apex of a growing lateral, blotting dry and placing in an individually labelled ziploc bag. GPS data was used to provide a detailed record of the location of populations and harvested individuals within sites. All samples were kept on ice upon return to the laboratory where the fragments were examined under a stereo-microscope and cleaned of visible debris and epiphytes before being snap frozen in liquid nitrogen and stored at -80°C until DNA extraction. Details of the sampling sites and the total number of individuals collected are shown in Table 2.2.

Table. 2.2 Location of sampled populations, number of individuals collected (N), sampling date and collector. e = samples in 70% ethanol, d = silica dried samples, all the rest collected as fresh tissue. Collectors: P.H = Paul Hallas, T.H = Takeaki Hanyuda, P.B = Paul Brazier, L.C = Liz Cook, J.V = Joaquin Vierna, B.H = Birgit Hussel, A.S = Allan Schoenherr, H.A = Hannah Appleyard.

Location	OS Grid Ref/ Coordinates	N	Sampling Date	Collector	Location code
Native Range					
Oiso, Awaji City, Hyogo Pref.		10^{d}	14/03/09	T.H	OI
Yura, Sumoto City, Hyogo Pref.		5^{d}	12/03/09	T.H	YU
Tsui, Minamiawaji City, Hyogo Pref.		8^{d}	27/03/09	T.H	TS
Tomari Port, Marugame City, Kagawa Pref.		5^{d}	28/03/09	T.H	TO
Ushimado, Setouchi City, Okayama Pref.		10^{d}	12/04/09	T.H	US
Mukaijima, Kurashiki City, Hiroshima Pref.		10^{d}	12/04/09	T.H	MU
Katsuma, Fukuoka City, Fukuoka Pref.		10^{d}	25/04/09	T.H	KA
Introduced Range					
Pwlldu Bay, Gower, UK	SS 574868	23	17/03/07	P.H	PWL
Newgale Sands, Pembrokeshire, UK	SM 855197	37	16/04/07	P.H	NEW
Broad Haven, Pembrokeshire, UK	SM 859142	46	16/04/07	P.H	BRD
Freshwater East, S. Pembrokeshire, UK	SS 018974	5	17/04/07	P.H	FRE
West Angle Bay, Pembrokeshire, UK	SM 850032	31	17/04/07	P.H	WST
Dale, Pembrokeshire, UK	SM 812061	25	18/04/07	P.H	DAL
Tenby, Pembrokeshire, UK	SM 125984	8	19/04/07	P.H	TEN
Cei Bach, Cardigan Bay, UK	SN 406601	6	17/05/07	P.B	CEI
Morfa Bychan, Cardigan Bay, UK	SN 566777	20	22/02/08	P.H	MOR
Rhosneigr, West Anglesey, UK	SH 315725	30	10/03/08	P.H	RHO
Porth Dinllaen, North Lleyn Peninsula, UK	SH 277412	8	04/08/07	P.H	DIN
Trearddur Bay, West Anglesey, UK	SH 252790	20	10/03/08	P.H	TRE
Inland Sea, Anglesey, UK	SH 279787	4	02/08/07	P.H	INL
Tal y Foel, Menai Strait, Anglesey, UK	SH 474645	40	03/08/07	P.H	MEN
Sandbanks, Poole, Dorset, UK	SZ 031871	30	08/04/07	P.H	POL
Ilfracombe, N. Devon, UK	SS 515480	52	07/05/07	P.H	ILF
Croyde Bay, N. Devon, UK	SS 432395	17	07/05/07	P.H	CRO
Bembridge, Isle of Wight, UK	SZ 659879	44	11/06/07	P.H	BEM
Wembury, Devon, UK	SX514483	44	06/07/07	P.H	WEM
Clyde Marina, Ardrossan, UK	NS 226422	3^{e}	05/09/07	L.C	CLY
Ortigueria Estuary, La Coruna, Spain	43°44'N 7°45'W	30^{d}	04/03/07	J.V	SPA
The Sylt, Germany	54°49'N 8°21'E	4	26/03/07	B.H	GER
Laguna Beach, California, USA	3°32'N 117°47W	1	24/10/2007	A.C	CAL
Venice, Italy		1	23/05/09	H.A	VEN

The reason for the low sample number (N = 1) for two of the locations (CAL and VEN) was attributable to the opportunistic nature of the sampling procedure and limited resources of the collectors during the time of sampling. Despite these low sample numbers for these two locations it was not considered a significant problem in the context of the study as the results from other similar investigations have not been significantly affected in the wake of small sample numbers (e.g. Donaldson et al., 2000, Uwai et al. 2006, Lee et al., 2012)

2.2.2 DNA extraction trials from *S. muticum*

Given the technical difficulties of extracting high quality DNA from brown macroalgae, as previously mentioned, several extraction techniques were trialled during the study. Following the selection of the best protocol a range of markers (from the mitochondrial, nuclear and plastid genomes) were targeted for amplification and sequencing to examine the extent of their variability.

The first steps in the investigation involved trialling a selection of the different extraction methods available from the published literature and to critically evaluate each method in terms of quality and quantity of DNA. Two standard cetyl trimethyl ammonium bromide (CTAB) based extraction protocols were trialled, one of which had successfully been applied with *S. muticum* (Varela-Alvarez et al., 2006) and the other one successfully applied in a study of the kelp, *Undaria pinnatifida* employing RAPD markers (Wang et al., 2006). In addition, four commercial kits (DNeasy Plant Mini Kit (Qiagen), and NucleoSpin Plant II Kit (Macherey & Nagel), EZNA Plant DNA Miniprep Kit (Omega Bio-Tek) and the Sigma GenElute Plant DNA (Sigma Aldrich) were tested following the manufacturers protocol. Samples of isolated DNA from all methods were run on 0.8% agarose gels stained with ethidium bromide to check DNA quality. Concentration of the DNA was measured using a Nanodrop ND-1000 Spectrophotometer (Nanodrop Technologies). A further measure of DNA purity was made from the A_{260}/A_{280} ratio of which a value of ~1.8 is generally accepted as “pure” for DNA. Results from the DNA extraction trials are presented below.

In terms of both the quality and quantity of DNA isolated, the CTAB based protocol of Wang et al. (2006) provided the best results (see Fig. 2.2). Furthermore,

Nanodrop analysis of the CTAB extracted DNA extracted via this method produced absorbance profiles characteristic of high purity nucleic acids compared to the other methods (Fig. 2.3).

Following a cost benefit analysis of the resulting DNA extraction protocols it was decided to proceed using the CTAB methodology adopted by Wang et al. (2006) with some modifications for processing of the sample set which is described below. The average concentration of the DNA recovered using this CTAB based protocol was ~75 ng/µl, with typical A_{260}/A_{280} ratio purity readings between 1.77 and 1.92.

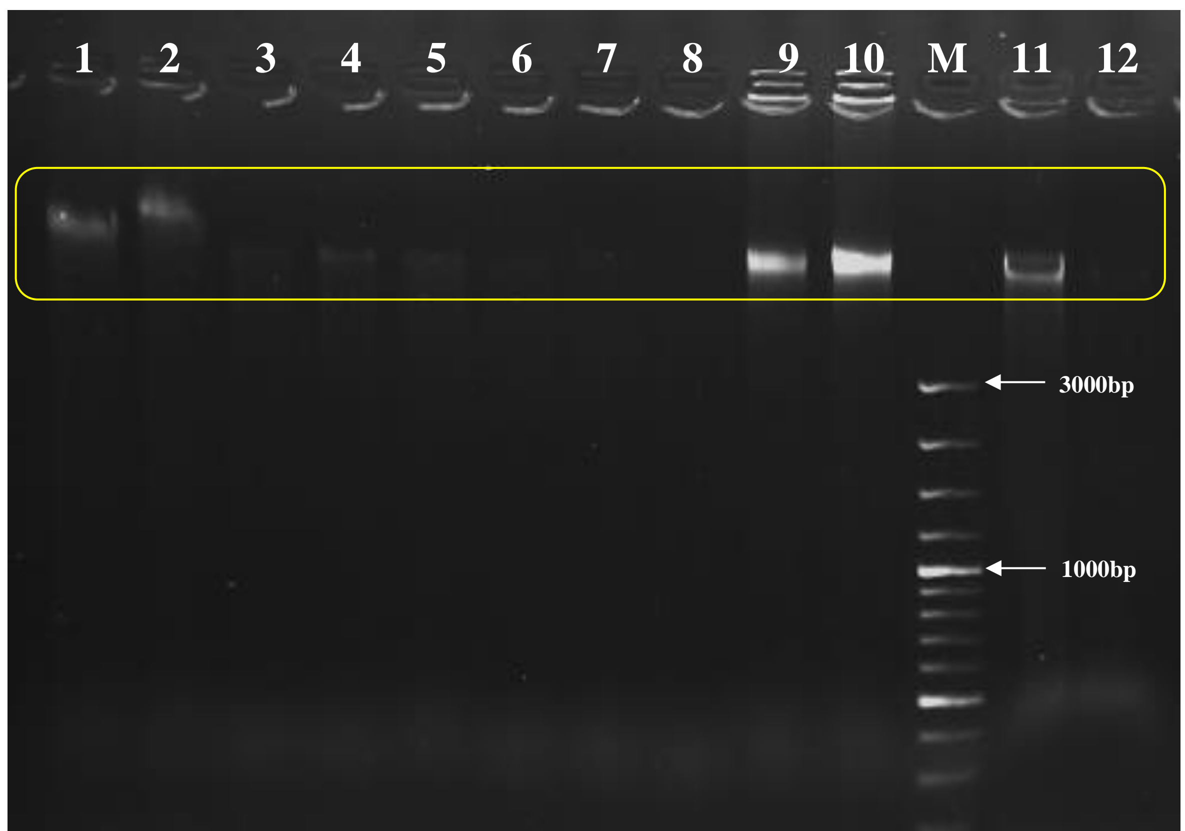


Fig. 2.2 Gel photo of DNA extracts isolated using 4 different commercial extraction kits plus a modified CTAB based protocol. Lanes 1-2, NucleoSpin Plant II Kit; Lanes 3-4, Sigma GenElute Plant DNA Miniprep Kit; Lanes 5-6, EZNA Plant DNA Miniprep Kit; Lanes 7-8, Qiagen DNeasy Plant Mini Kit; Lanes 9-10, CTAB protocol (Wang et al., 2006) and Lanes 11-12, CTAB protocol (Varela-Alvarez et al., 2006). M = GeneRuler 100bp Plus DNA Ladder (Fermentas). Arrows indicate size (base pairs) of two selected fragments from DNA ladder. Yellow box indicates region of the gel containing bands of genomic DNA. All methods were trialled using two replicates of the same *S. muticum* sample (BRD P1) using ~50 mg fresh tissue. All kit extractions were performed according to the manufacturer's instructions.

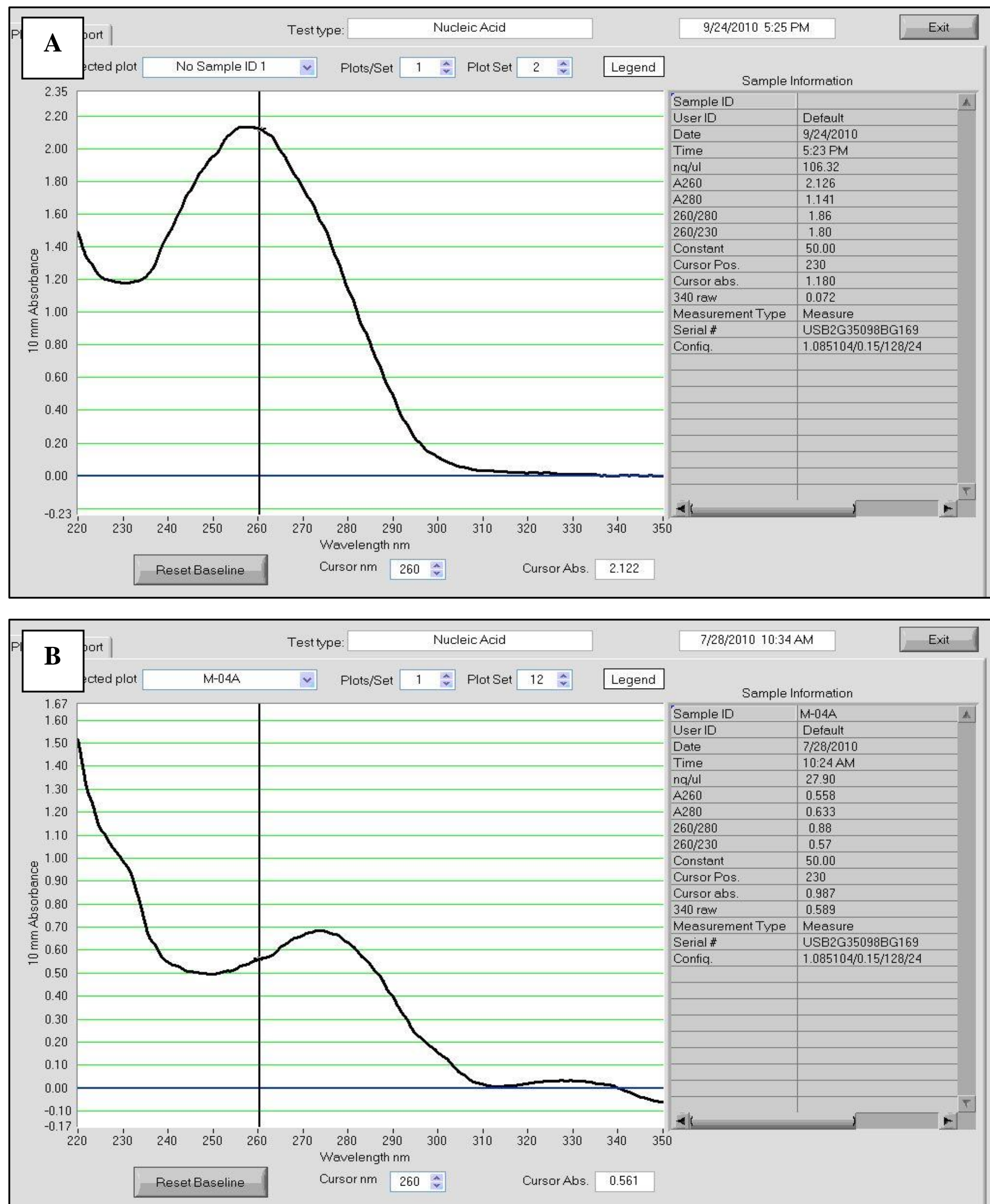


Fig. 2.3 Examples of Nanodrop ND-1000 DNA profile output. **(A)** Characteristic profile of high quality DNA extract (OD 260/280 = 1.86) isolated using CTAB, applicable for use in further downstream reactions. **(B)** Profile characterising poor quality DNA (OD 260/280 = 0.88) isolated using Sigma GenElute Kit discarded from use in further reactions.

2.2.3 CTAB DNA extraction protocol

From the fragments of growing laterals sampled, around 10-15 leaf-like blades were taken from each frozen sample (see Fig. 2.4) and using a dissecting microscope (at 20X magnification) cleaned of any visible epiphytes using several sterile H_2O washes.

Fig. 2.4 Photograph showing the typical leaf-like blades (outlined) that were harvested from the frozen algal samples for the purposes of DNA extraction. Photo adapted from algaebase.org.

After blotting dry on sterile filter paper, approximately 100 mg of cleaned algal tissue was placed into a 2.0 ml microcentrifuge tube and using a micropestle (Fisher Scientific) ground to a fine powder in the presence of liquid nitrogen. Seven hundred microliters of preheated (65°C) cetyl trimethyl ammonium bromide (CTAB) lysis buffer (3% CTAB (w/v), 1.4 M NaCl, 20 mM ethylenediaminetetraacetic acid (EDTA), 100 mM Tris-HCL, pH 8.0, 1% polyvinyl pyrrolidone (w/v), 1% β-mercaptoethanol (v/v)), plus 10 mg mL^{-1} RNase A was then added to the ground algal tissue, vortexed to mix and then placed in a 65°C heat block for 1 hour with gentle inversion every 10 minutes. Following incubation, one third of the volume of 5M potassium acetate (pH 8.0) was added to the mixture and kept on ice for 15 minutes. The samples were then centrifuged at 10,000 rpm for 10 minutes and the upper phase was then transferred to a clean 1.5 ml microcentrifuge tube. One volume of chloroform-isoamyl alcohol (24:1) was added, and the phases were mixed by gentle inversion for 5 minutes and then separated by centrifugation at 14,000 rpm for 10 minutes. The aqueous phase was transferred to a

new 1.5 ml microcentrifuge tube and the chloroform step was repeated once more. DNA from the resulting aqueous phase was then precipitated with one volume of cold isopropanol at -20°C for 1 hour, and collected by centrifugation at 14,000 rpm for 20 minutes. Finally, the DNA pellet was washed twice with 70% ethanol, air dried and dissolved in 50 µl of 1 X TE buffer (10 mM Tris-HCL, pH 8.0, 1 mM EDTA). DNA concentration and purity estimates were quantified using a Nanodrop 1000 Spectrophotometer (Nanodrop Technologies). The purity of the extracted DNA was assessed by calculating the A_{260}/A_{280} ratio to determine protein impurities (Sambrook et al., 1998). DNA yield was calculated from the A_{260} for the clean DNA samples (i.e. those with an A_{260}/A_{280} ratio between 1.8 and 2.0). Integrity of DNA extracts were also visually assessed by electrophoresis by running 5 µl of extract on a 0.8 % (w/v) agarose gel, stained with ethidium bromide, in a 1X TBE buffer system (Sambrook et al., 1998). Gels were viewed using a UV Transilluminator (BioDoc-It® Imaging System, UVP). Successful genomic DNA extracts were stored at -20°C until required. Prior to use in downstream applications DNA was purified further using the GENECLEAN II Kit (BIO 101 Inc., La Jolla, CA, USA), following the manufacturer's instructions and modification for high molecular weight DNA (Yoshida et al., 2000). Between 20 and 30 µl of original extract depending on concentration was aliquotted for purification. For all samples the DNA-glass milk pellet was eluted in 15 µl of 0.1X TE and 3 µl of purified extract run on a 0.8 % (w/v) agarose gel as a check of clean-up success. Fig. 2.5 shows an agarose gel photo of typical DNA extracts recovered using the CTAB protocol.

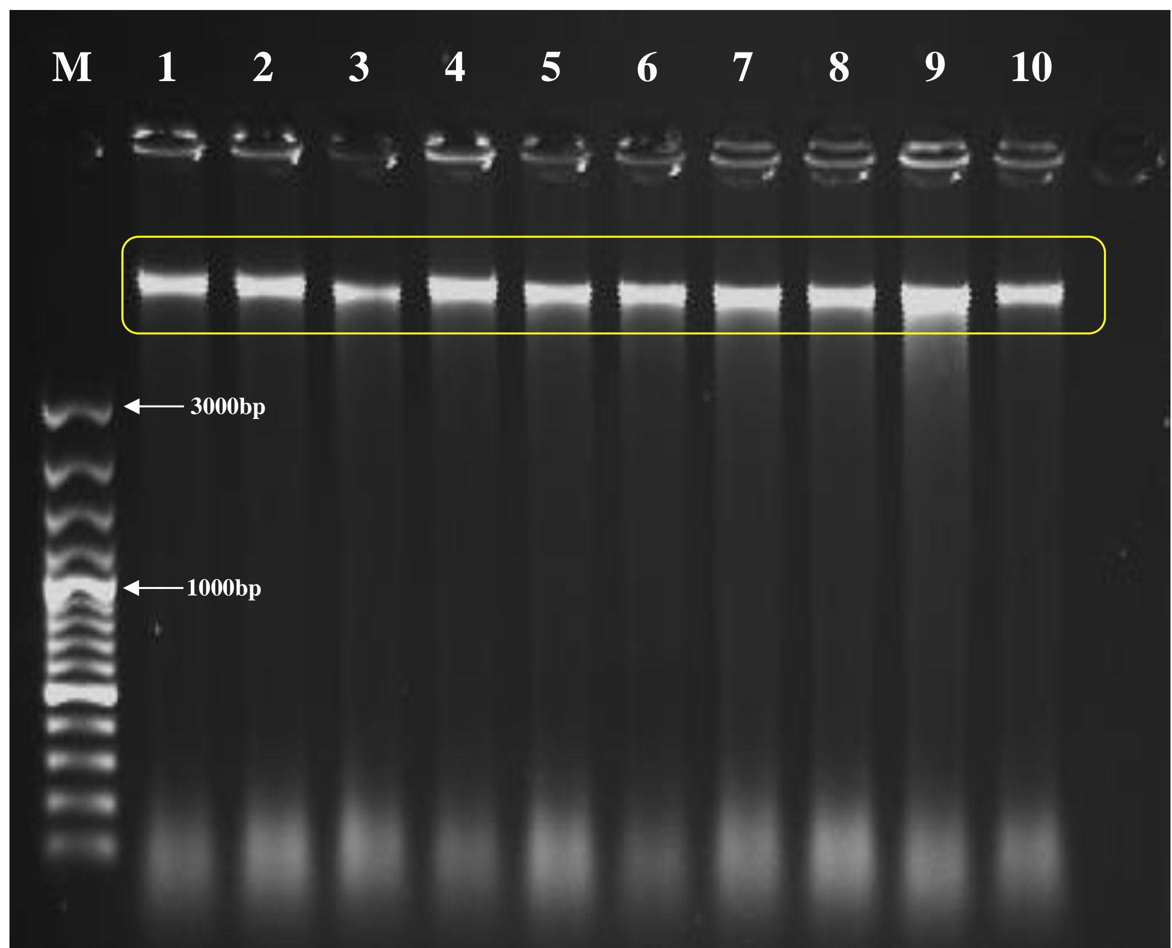


Fig. 2.5 Gel photo showing DNA extracted using the modified CTAB technique of Wang et al. (2006). Lanes 1-10 represent DNA samples from the population of Katsuma, Japan. M = GeneRuler 100bp Plus DNA Ladder (Fermentas). Arrows indicate size (base pairs) of two selected fragments from DNA ladder. Yellow box indicates region of the gel containing bands of genomic DNA.

2.2.4 PCR amplifications of gene fragments

The use of single locus markers in the initial investigations served two purposes; i) as a check to see whether extracted DNA was suitable for polymerase chain amplification (PCR), and ii) to make preliminary assessments of the level of genetic variation within a subset of samples using a relatively rapid methodology. For each marker a series of PCR optimisations was undertaken, initially using the conditions described in the study from which the primer sets were taken (e.g. see Fig. 2.6 for gradient PCR of *cox3* gene fragment). All polymerase chain amplification reactions were performed using a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems). Negative controls (i.e. ddH_2O in replace of template DNA) were included

with at least 1 per 10 reactions. All primers used were synthesised by Sigma Genosys (Cambridge, UK).

2.2.4.1 Mitochondrial *Trn*W_I spacer region

A ~250 base pair DNA fragment corresponding to the intergenic spacer region between the *Trn*W and *Trn*I mtDNA genes was amplified via PCR. Each PCR reaction mixture consisted of 1 µl of purified template DNA, 1X PCR buffer (Promega), 2 mM $MgCl_2$ (Promega), 0.25 mM of deoxynucleotide triphosphates (dNTPs, ABgene), 0.4 µM of each of the primers and 0.5 U *Taq* DNA polymerase (Promega) adjusted to a final volume of 25 µl with ddH_2O. Primers (*Trn*W- F, 5'-GGGGTTCAAATCCCTCTCTT-3', and *Trn*W-R, 5'-CCTACATTGTTAGCTTCATGAGAA-3') were as described by Voisin et al. (2005). Thermal cycling conditions were as follows: initial denaturation at 95°C for 2 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 52°C for 30 seconds and extension at 72°C for 60 seconds, followed by a final extension at 72°C for 5 minutes.

2.2.4.2 Mitochondrial *cox3* gene region

A ~600 base pair fragment of the cytochrome *c* oxidase subunit 3 gene was amplified via PCR. Each PCR reaction mixture contained 1 µl of purified template DNA, 1X PCR buffer (Promega), 1.5 mM $MgCl_2$ (Promega), 0.2 mM dNTPs (ABgene), 0.2 µM of each of the primers and 0.5 U *Taq* DNA polymerase (Promega) adjusted to a final volume of 25 µl with ddH_2O. Primers (CAF4A, 5'-ATGTTTACTTGGTGRAGRGA-3' and CAR4A, 5'-CCCCACCARTAWATNG TNAG-3') were taken from Kogame et al. (2005). Thermal cycling conditions were as follows: initial denaturation at 94°C for 2 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 45°C for 30 seconds and extension at 72°C for 45 seconds, followed by a final extension at 72°C for 5 minutes.

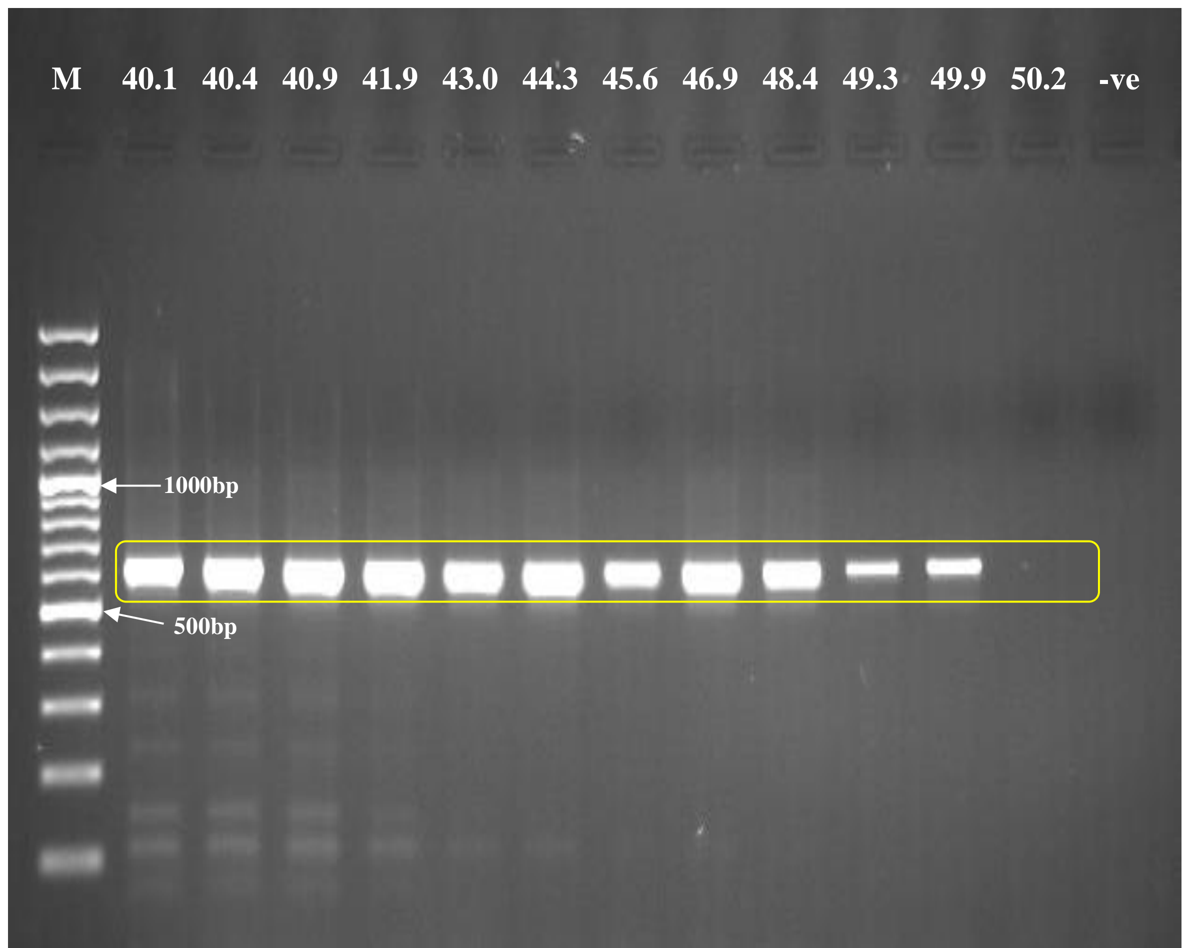


Fig. 2.6 Gradient PCR optimisation for *cox3* gene fragment with the range in annealing temperatures (°C) displayed. , -ve = negative control; M = GeneRuler 100bp Plus DNA Ladder (Fermentas). Arrows indicate size (base pairs) of two selected fragments from DNA ladder. Yellow box indicates region of the amplified PCR products.

2.2.4.3 Rubisco gene region

Due to the difficulties in amplifying this region several primer pairs were utilised to obtain unambiguous sequence reads of the *rbc*L gene fragment. Primers used were *rbc*LS-F, 5'-GATTTTTTTGAGGAAGG-3' and *rbc*LS-R, 5'-CCCCATAGTTC CCAAT-3' (1500 base pair PCR product) (Phillips et al., 2001) and *rbc*LS-F 5'-GAT TTTTTTGAGGAAGG-3' (Phillips et al., 2001) and Rub 2R 5'-GTTTGGATTGCTG TATATCC-3' (500 base pair PCR product) (this study).

For the 1500 base pair fragment thermal cycling profiles was as follows: initial denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds and extension at 72°C for 60 seconds, followed by a final extension at 72°C for 5 minutes.

PCR reaction conditions for the 500 base pair fragment of *rbc*L were as follows: an initial denaturation at 94°C for 2 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 54°C for 30 seconds and extension at 72°C for 45 seconds, followed by a final extension at 72°C for 5 minutes.

For both fragment sizes PCR reactions contained 1 µl of purified template DNA, 1X PCR buffer (Promega), 2.0 mM $MgCl_2$ (Promega), 0.25 mM dNTPs (ABgene), 0.25 µM of each of the primers and 1 U *Taq* DNA polymerase (Promega) adjusted to a final volume of 25 µl with ddH_2O.

2.2.5 Electrophoresis of PCR products

PCR products were run on 1.5% agarose gels stained with ethidium bromide in a 1X TBE buffer system. Before loading on to the gel, 5 µl of PCR was mixed with 2 µl of 6X loading buffer (Fermentas). After loading the samples onto the gel plus a DNA ladder (GeneRuler 100bp plus DNA Ladder, Fermentas) the unit was left to run at 100 V for 45 minutes. PCR products were then visualised using a UV Transilluminator (BioDoc-It® Imaging System, UVP). PCR products were checked for correct length, purity and approximate yield. A clean bright band was taken as a positive result (e.g. see Fig. 2.2). In cases of multiple banding or smears, repeat amplifications of these samples was undertaken with modifications to the PCR reaction conditions (e.g. changing the annealing temperature or the number of PCR cycles) in order to obtain a

single clear band. For example, due to the weak amplification of several samples from the native populations with the *Trn*W_I spacer fragment, the number of PCR cycles was increased to 38 cycles in order to generate an adequate quantity of PCR product suitable for sequencing. Furthermore, in five samples again from native populations the annealing temperature of the *cox3* PCR amplification was increased to 49°C to eliminate the occurrence of secondary banding at the usual temperature (45°C). Gel photos of the different PCR fragments are shown in Fig. 2.7.

2.2.6 PCR purification and sequencing

Unincorporated dNTPs and primers remaining in the PCR product were subsequently removed using an enzymatic purification procedure (Werle et al., 1994). 1 µl of an Exo-SAP-IT mix (2.5 U Exonuclease 1, (VWR) and 0.5 U of Shrimp Alkaline Phosphatase, (Promega)) was added per 10 µl of PCR product. The mixture was incubated in a PCR thermal cycler at 37°C for 60 minutes followed by 15 minutes at 80°C to deactivate the enzymes. Purified PCR products were subjected to commercial sequencing (Macrogen Inc., Seoul, South Korea) in which BigDyeTM terminator cycling conditions were employed through automated sequencer ABI3730XL (Applied Biosystems, Foster City, CA, USA). Samples were sequenced in both directions using the same primers used in the initial PCR amplifications. In a preliminary trial to assess levels of variability within each marker only a random subset of samples from each population were amplified and sequenced for the different regions before proceeding to sequence more individuals.

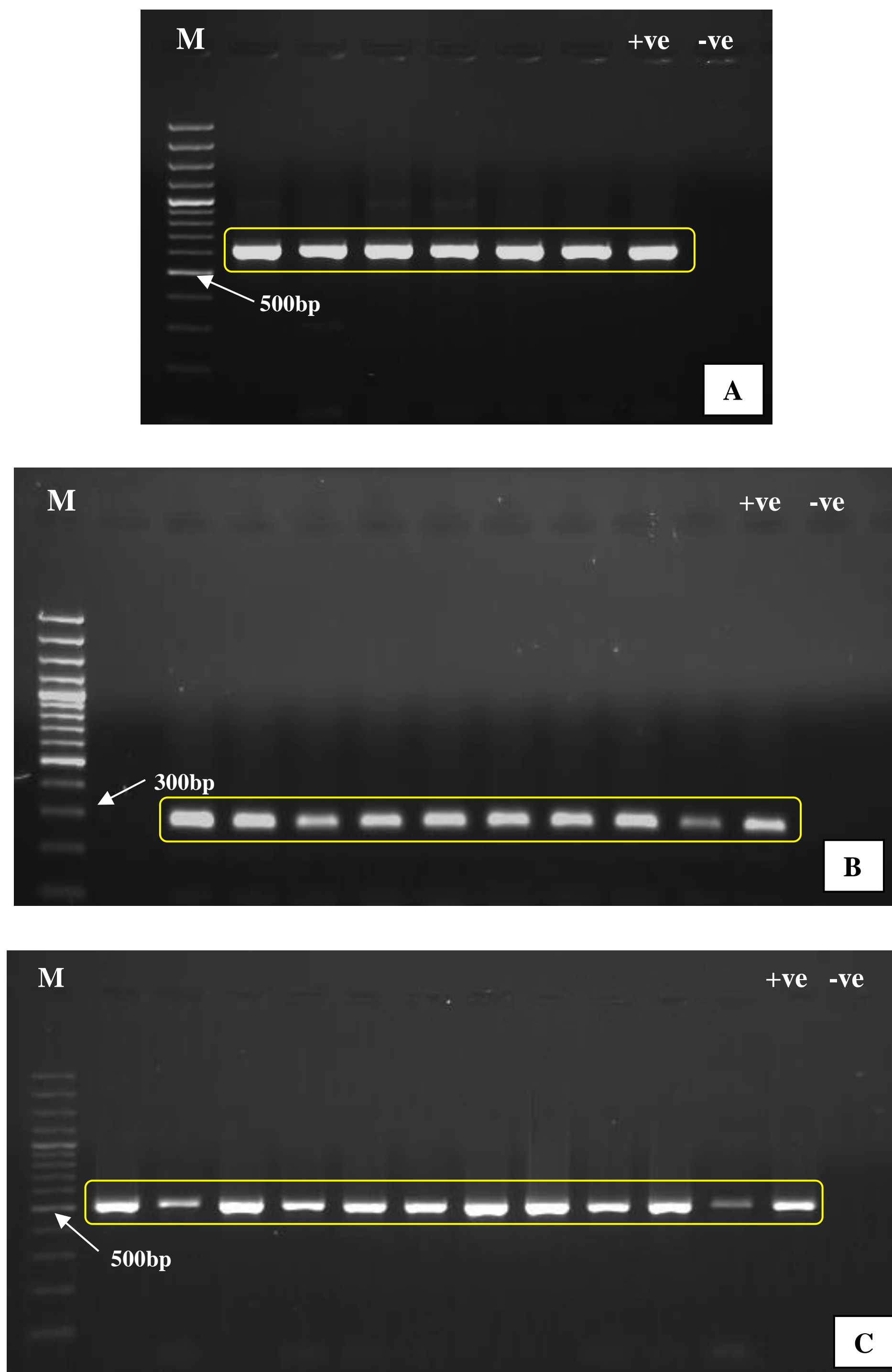


Fig. 2.7 Gel photos of PCR amplification products of **(A)** the *cox3* gene fragment, **(B)** the *Trn*W_I mtDNA intergenic spacer region and **(C)** 500bp fragment of rubisco gene amplified from samples of *S. muticum* collected from the Japanese population of Katsuma. -ve = negative control; M = GeneRuler 100bp Plus DNA Ladder (Fermentas). Arrows indicate size (base pairs) of selected fragments from DNA ladder. Yellow box indicates region of the amplified PCR products.

2.2.7 Sequence alignment and editing

Raw sequence data were first verified as *Sargassum* spp. DNA using the GenBank™ BLASTn search (Altschul et al., 1990). Both forward and reverse sequences were assembled, checked and edited using the BIOEDIT software package (Hall, 1999) and alignments performed using the CLUSTALW programme (Thompson et al., 1994) within BIOEDIT. Additional sequences from GenBank for all four markers were included in the alignments to check for further variation.

2.2.8 Data analysis

Examination of the sequence data revealed that variation was present only in the two mitochondrial gene fragments (*Trn*W and *cox3*). Therefore all subsequent statistical analyses were only conducted using these two data sets. Phylogenetic relationships among populations were assessed via the construction of neighbour-joining (NJ), maximum likelihood (ML), and maximum parsimony (MP) trees using the MEGA version 4.0 software package (Tamura et al., 2007). In addition, the tree of Bayesian inference (BI) was generated using the program Mr Bayes v3.1.2 (Ronquist and Huelsenbeck, 2003). In order to estimate the appropriate substitution model to be used in four tree building methods, Akaike information criterion implemented in Modeltest version 3.7 (Posada and Crandall, 1998) was applied. Akaike information criterion (AIC, Akaike, 1974) is a common method of choosing the most appropriate model for the observed data (Swofford et al., 1996). AIC quantifies the relative 'Goodness-of-fit' of a previously defined model, given a sample of data. The preferred model is that with the lowest AIC. This will be the simplest model (with the fewest parameters) that most closely explains the data. For both mitochondrial markers, Modeltest determined the Hasegawa, Kishino and Yano model to be the optimal substitution model. For the tree building methods of NJ, ML and MP, the significance of the branching was assessed by performing 1000 bootstrapping replicates (Felsenstein, 1985). For BI, analysis was initiated with a random starting tree and run for at least 5,000,000 generations with each data set, until the divergence between two runs became small and stationary. Trees were sampled every 100th generation. With the burn-in value set to retain the last third-fourth sampled trees, a posteriori possibilities were calculated from the sampled trees to illustrate the statistical confidence for the BI tree. Further confidence in the base of the phylogram can be gained by assigning the most distantly related sequence as an

outgroup (Watrous and Wheeler, 1981). *Sargassum thunbergii*, and *S. hemiphyllum*, were designated as outgroups for the *TrnW* data set, and *S. horneri* for the *cox3* data set, since they were phylogenetically closely related to *S muticum* (Stiger et al., 2003, Phillips et al., 2005). The number of haplotypes was recorded for each sampling locality. Relationships among mtDNA sequences were estimated via a haplotype network using the statistical parsimony method (Templeton et al., 1992) in TCS version 1.21 (Clement et al., 2000).

2.2.9 AFLP marker development

For the AFLP marker development trials a random selection of 10 individuals from different populations were used from which two independent DNA extracts were isolated on different days. The reason for this was to check for variations in the AFLP banding patterns due to minor differences in the extraction process. As quality of the DNA has often been cited as the most significant factor for the reproducibility of the AFLP technique (Reineke et al., 1998, Donaldson et al., 2000, Bensch and Akesson, 2005, Heng et al., 2010), only samples with an A_{260}/A_{280} ratio between 1.8 and 2.0 and producing a clear high molecular weight band on agarose gels (see Fig. 2.5) were processed using the AFLP methodology.

2.2.9.1 Restriction/ligation

The AFLP reactions were performed as described by Vos et al. (1995) with minor modifications and using nonradioactive fluorescently labelled primers for the selective amplification step. All enzymes and restriction buffers were obtained from New England Biolabs, unless otherwise stated and oligonucleotide primers were from Sigma Genosys. Total genomic DNA (100-200ng) was digested using 5U each of EcoRI-HF and MseI in a common buffer system (NEB Buffer #4) supplemented with 100 µg ml^{-1} of BSA, at a total volume of 40 µl. Restriction digests were incubated at 37°C for 3 hours and then to 70°C for 15 minutes to inactivate the enzymes. Following incubation, digestion success was checked by running 5 µl of the restriction digest on 1% TBE agarose gel against undigested DNA template (see Fig. 2.8A and B). Before ligation, double stranded adapter pairs were constructed from the complementary single-stranded oligonucleotides (see Table 2.3 and Appendix A). An adapter ligation solution containing 200 CEU (cohesive end units) T4 DNA Ligase, 1X Ligase Buffer

containing 1 mM ATP, 5 pmol EcoRI adapter, 50 pmol MseI adapter and ddH_2O to the volume of 10 µl was added to the restriction digest mixture. The ligation mixture was incubated overnight at 37°C in the PCR thermocycler.

2.2.9.2 Pre-selective PCR amplifications

Following ligation each sample was diluted 1 in 10 in ddH_2O and 5 µl was used in a 20 µl pre-selective amplification reaction containing 1X PCR buffer (Promega), 1.5 mM $MgCl_2$ (Promega), 200 µM dNTPs, 0.2 µM EcoRI + A primer (Table 2.3), 0.2 µM MseI + C primer (Table 2.3) and 0.5 U Taq DNA polymerase (Promega). The amplification profile was: 2 minutes at 72°C, followed by 30 cycles of 30 seconds at 94°C, 30 seconds at 56°C and 60 seconds at 72°C, with a final extension of 2 minutes at 72°C. The reason for a 72°C temperature step at the start of the PCR amplification relates to the fact that the T4 DNA ligase only ligates one of the strands of the adapter to the fragment. The other is held on by base-pair binding to the other adapter strand. By running the first step of the pre-selective PCR reaction at a 72°C hold which allows the Taq polymerase to ligate the other strand to the DNA fragment (AFLP protocol, 2000). To check amplification success and quality, 5 µl of the pre-amplification PCR product was run on a 1.5% TBE agarose gel. Presence of a smear within the range of 100 – 1000 bp was taken as confirmation of a successful AFLP reaction (see Fig. 2.8C). Positive pre-selective PCRs were then diluted ten-fold in ddH_2O before being used in the selective amplification reactions.

2.2.9.3 Selective PCR Amplifications

In the selective PCR reaction 2.5 µl of the diluted pre-amplification product were used in a total reaction volume of 10 µl containing 1X PCR buffer (Promega), 2.0 mM $MgCl_2$ (Promega), 200 µM dNTPs, 1.0 U Taq DNA polymerase (Promega) and a single primer pair combination of 0.2 µM EcoRI + AG and 0.2 µM MseI + CAA (Table 2.3). The EcoRI primer was labelled on the 5' end with the 6-FAM fluorochrome (Applied Biosystems). The thermal cycling profile for the selective amplification involved a touchdown PCR. Touchdown PCR is a form of the standard polymerase chain reaction designed to reduce non-specific amplification by optimising annealing temperatures. In a touchdown PCR the early stages of the reaction uses a high annealing temperature which is decreased by 1°C for every subsequent cycle to a ‘touchdown’

annealing temperature which is then used for further cycles (Don et al., 1991). The touchdown PCR profile used in the selective amplification was as follows: an initial denaturation at 94°C for 2 minutes followed by 13 cycles of 94°C for 30 seconds, 65°C for 30 seconds, decreasing by 1°C after each cycle down to 56°C, and 72°C for 60 seconds, followed by 23 cycles of 30 seconds at 94°C, 30 seconds at 56°C and 60 seconds at 72°C with a final extension of 10 minutes at 72°C. A 1% TBE agarose gel used to visually check the success of each selective amplification reaction with 3.0 µl of the PCR sample loaded on to the gel (see Fig. 2.8D). Amplified PCR products were stored at 4°C.

Table. 2.3 Adapter and primer sequences used in the AFLP reactions.* Indicates primer is labelled with FAM (blue dye). Bases in bold indicate additional selective nucleotides.

	Adapter Sequence
EcoRI-F	5'- CTC GTA GAC TGC GTA CC -3'
EcoRI-R	5'- AAT TGG TAC GCA GTC TAC -3'
MseI-F	5'- GAC GAT GAG TCC TGA G -3'
MseI-R	5'- TAC TCA GGA CTC AT -3'

	Pre-selective Primer Sequence
EcoRI + A	5'- GAC TGC GTA CCA ATT C**A** -3'
MseI + C	5'- GAT GAG TCC TGA GTA A**C** -3'

	Selective Primer Sequence
*EcoRI + AG	5'- GAC TGC GTA CCA ATT C**AG** -3'
MseI + CAA	5'- GAT GAG TCC TGA GTA A**CAA** -3'

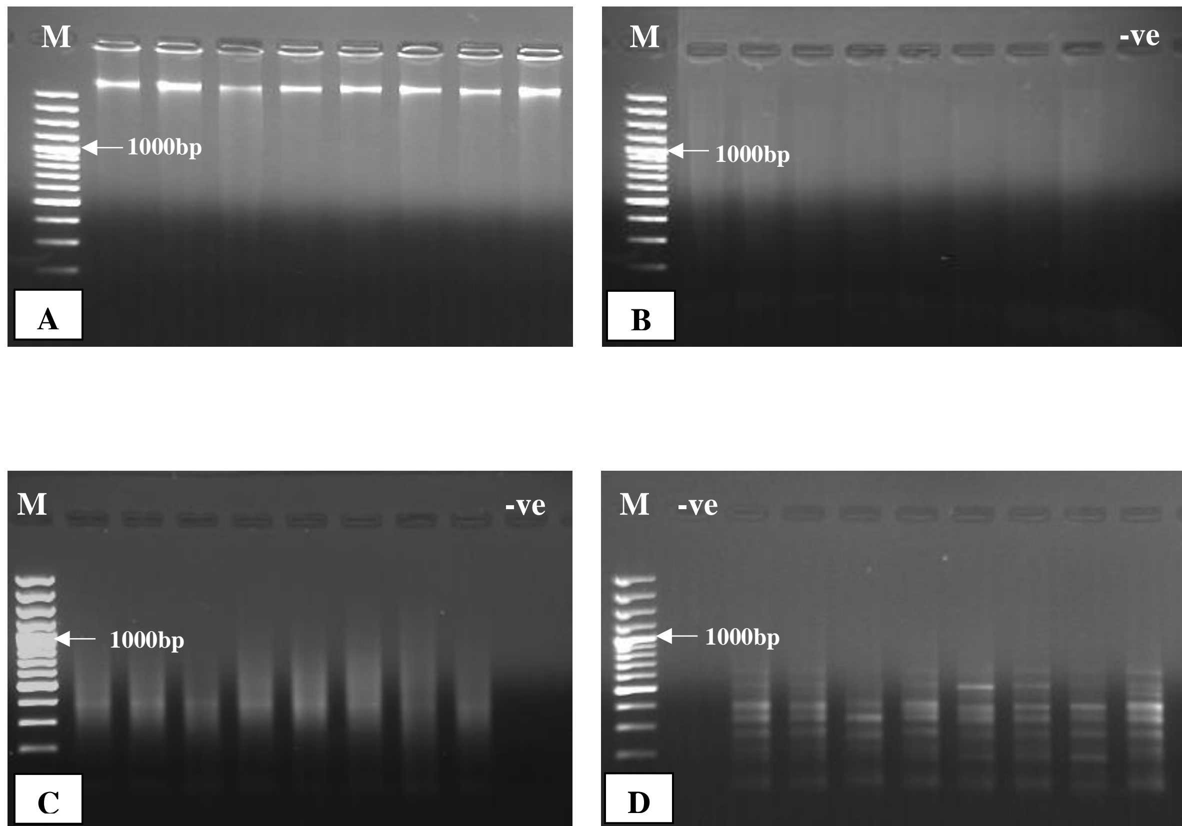


Fig. 2.8 Gel images representing various stages in the AFLP process. All gel images were generated from undiluted DNA solutions run on a 1.5% agarose gel. (**A**) 8 samples of *Sargassum muticum* genomic DNA. (**B**) Restriction digested DNA of the same eight samples. (**C**) Preselective PCR amplification products (using primer pair EcoRI + A and MseI + C) in which only a subset of restriction fragments are amplified. (**D**) Products of the selective PCR amplification reactions (using primer pair EcoRI + AG and MseI + CAA). –ve = negative control reactions (ddH$_2$O in place of genomic DNA). M = GeneRuler 100bp plus DNA ladder (Fermentas). Arrow indicates size (base pairs) of selected fragment from DNA ladder.

2.2.9.4 AFLP Fragment Sizing and Evaluation

One microliter of each selective amplification reaction was mixed with 0.5 µl of Genescan-500 ROX size standard (Applied Biosystems) and 8.5 µl of Hi-Di deionised formamide (Applied Biosystems). The ROX 500 size standard is composed of a set of labelled DNA fragments of known sizes and is always run with each sample allowing for the calibration of the AFLP signals. The mixture was then denatured by heating to 95°C for 5 minutes and then placed immediately on ice. Fragments were separated on a 16 capillary ABI PRISM 3130*xl* genetic analyser (Applied Biosystems) with 100 relative fluorescent units (RFU) units set as a minimum height threshold for peak detection. Raw data were imported into the software program GeneMarker v. 1.75 (SoftGenetics, LLC.) for analyses. The program was also used to create a binary matrix for the estimation of genetic differences between samples using presence/absence of fragments. A consensus banding pattern was evaluated by scoring fragments present in at least two of the three replicates above a threshold of 50 RFU from each of the two samples per population used in the reproducibility trials. Generally a defined threshold value is assigned when scoring AFLP fragments in order to accurately discriminate DNA fragments from the oscillations of background noise. However this procedure when using automated scoring can often lead to erroneous peak detections especially when the overall signal strength is low and there is high fragment sharing between samples. Differences in banding patterns between samples were only counted if they were reproducible i.e. if the differing fragment was present in all three replications (2 repeats from first extract plus repeat using new second extract). Non-reproducible fragments likely represent artefacts rather than actual polymorphisms. Samples showing the same multilocus AFLP phenotype were considered to be the same genotype.

However due to issue surrounding the reproducibility of the technique, a full complementary AFLP data set could not be generated within the time frame of the project. Therefore the potential reasons for these failures are discussed together with suggestions for the future optimisation of the protocol.

2.3 RESULTS

2.3.1 DNA Sequencing

A total of 240 base pairs of the mtDNA *Trn*W_I intergenic spacer region were recovered after alignment and editing. A total of three haplotypes were revealed by the sequence analysis of the mitochondrial *Trn*W_I intergenic spacer fragment (Table 2.4 and Figs. 2.9 and 2.10). Haplotypes were distinguished by two base pair substitutions (1 transition, A to G and 1 transversion, A to T). All trees generated via the various phylogenetic methods exhibited identical topologies (Fig. 2.11). Sequences of the two out-group species differed from those of *S. muticum* by 17 and 13 base pairs for *Sargassum thunbergii* (FJ712743) and *S. hemiphyllum* (FJ712725) respectively. In total 21 variable sites were identified when out-group sequences were incorporated into the analysis.

Table. 2.4 Variable nucleotide positions and base pair changes present in mtDNA *Trn*W_I spacer haplotypes of *S. muticum* revealed by this study.

	Sequence position	
Haplotype	173	204
*Trn*W_I Hap A	A	A
*Trn*W_I Hap B	T	G
*Trn*W_I Hap C	T	A

The same haplotypes as obtained from the recent study by Cheang et al. (2010c) were confirmed by this investigation with Hap A dominant, and found in samples of *S. muticum* from all introduced areas plus several from the native range (see Figs. 2.12-2.14). The other main Haplotype, Hap B, contained all samples from the native population of Yura, Hoyogo Prefecture (Fig. 2.12). However, in contrast to the analysis by Cheang et al. (2010c) where all populations sampled were fixed for one haplotype, one Japanese population in this study (Katsuma, Fukuoka Prefecture) revealed two distinct haplotypes defined by a single base pair substitution (see Table 2.4 and Fig.

2.12). One of these haplotypes (Hap A, typical of central and western Japanese populations, and all introduced populations sampled to date) represented 60% of the individuals screened from Katsuma. An additional haplotype (Hap C) as yet unrecorded in previous studies (Cheang et al., 2010c) was also revealed, representing 40% of individuals screened from the Katsuma population. Preliminary inferences drawn from these results could be that the population of Katsuma is situated within a region subject to mixing of currents that bring in propagules or drift material from two genetically differentiated source populations to leading to the occurrence of population composed of mixed haplotypes. However, in order to confirm this more sampling from regions along the northern coast of Japan and from the coast of Korea would be necessary to test this hypothesis.

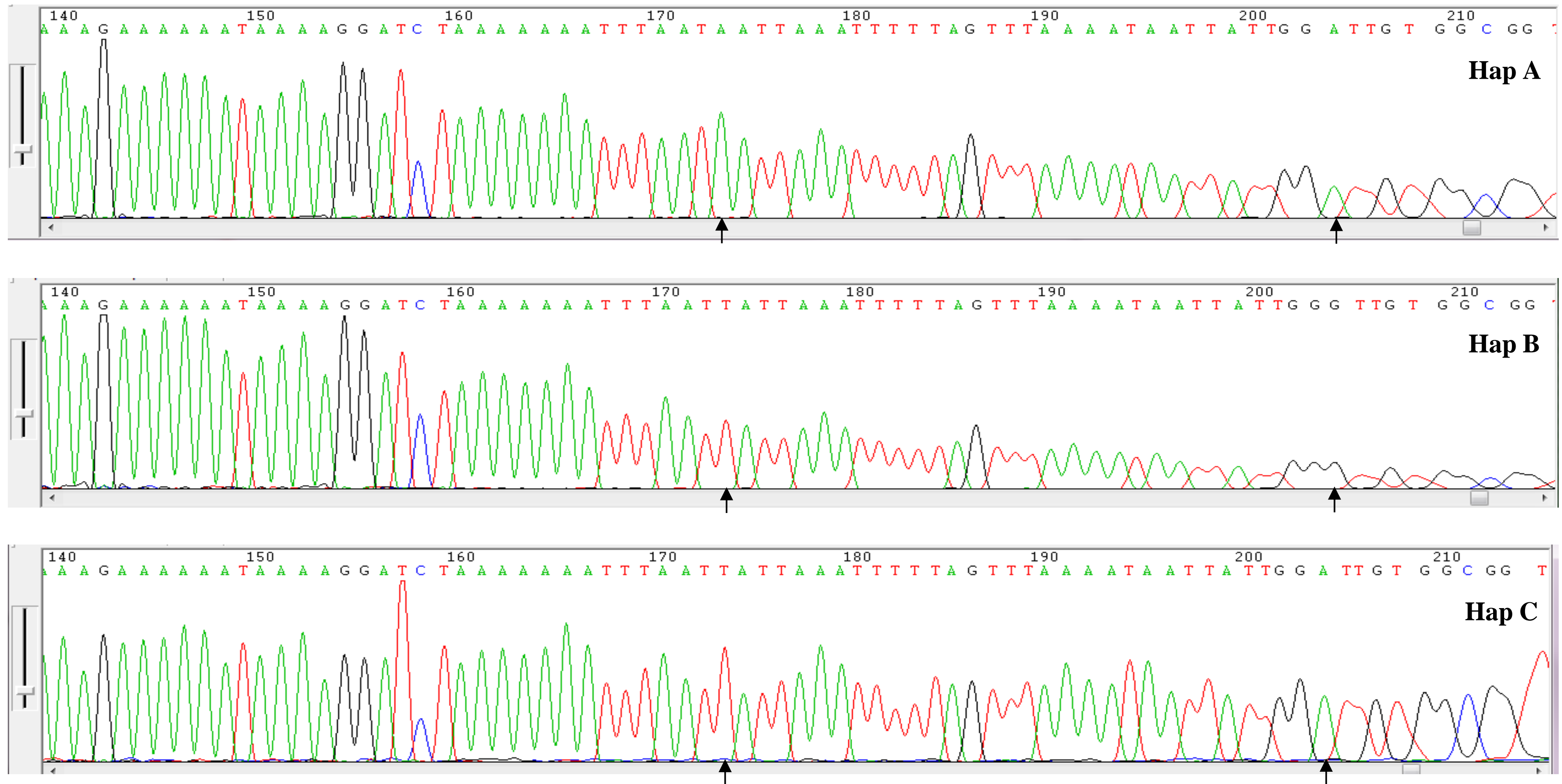


Fig. 2.9 Example chromatograms representing the three *S. muticum* *Trn*W_I spacer haplotypes revealed in the study. Region displayed in chromatograms contains the variable sites (labelled with an arrow) between haplotypes.

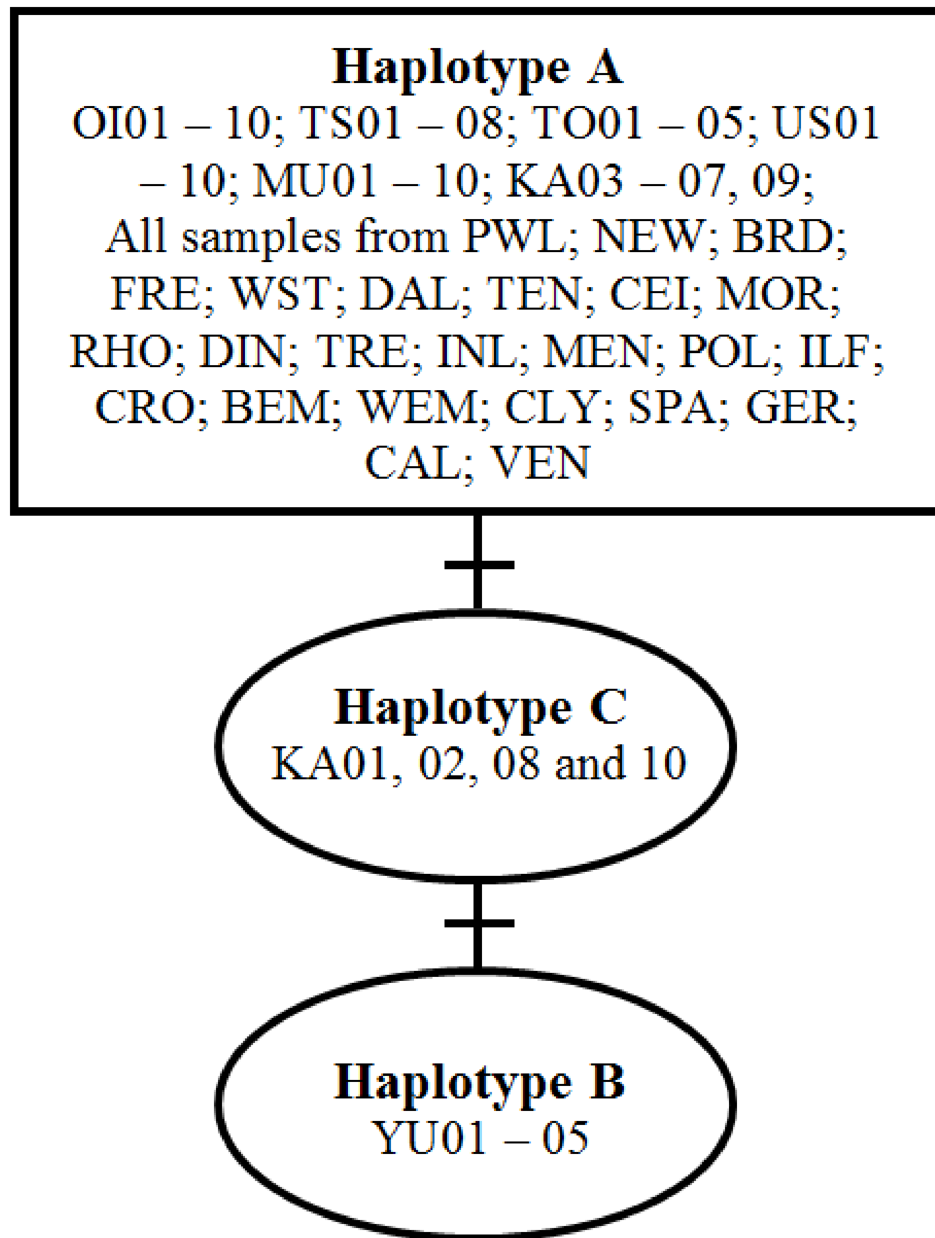


Fig. 2.10 Haplotype network based on the mitochondrial *Trn*W_I intergenic spacer region. Sampling localities of the haplotype are indicated within the haplotype node. Connecting lines show mutational pathways among haplotypes with short cross lines representing single base pair substitutions. See Table 2.1 for the population location codes.

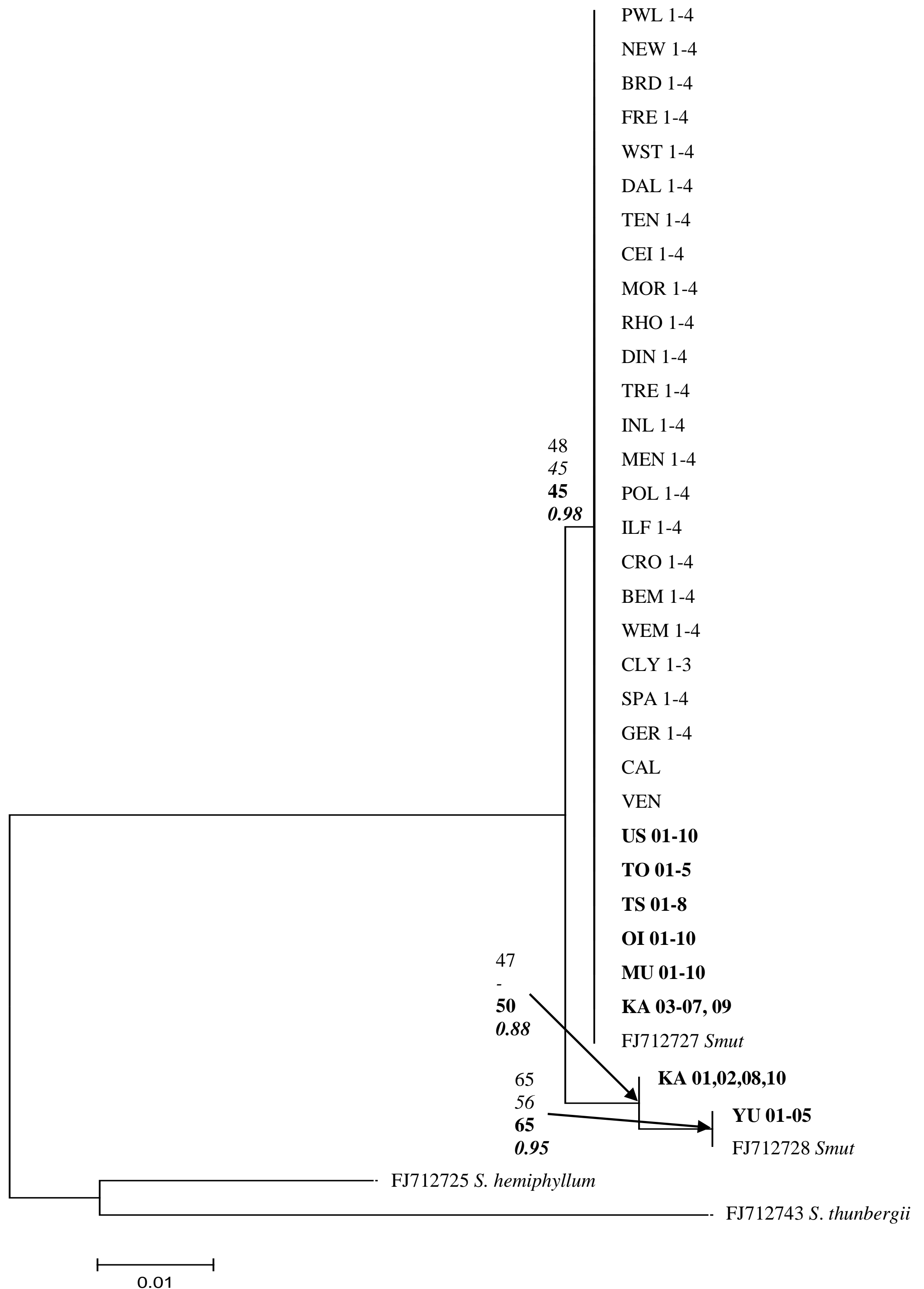


Fig. 2.11 Genetic relationships between populations of *S. muticum* depicted by maximum likelihood tree based on the mitochondrial *Trn*W_I intergenic spacer data set. All four methods tested neighbour-joining (NJ), maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI), generated trees with identical topologies. Bootstrap values (1000 replicates for NJ, ML and MP) for NJ (regular), MP (italic), and ML (bold) methods and the posteriori probabilities BI (bold and italic) are indicated at the nodes. Additional *S. muticum* sequences from GenBank are included in the tree. Populations from the native range are indicated in bold type.

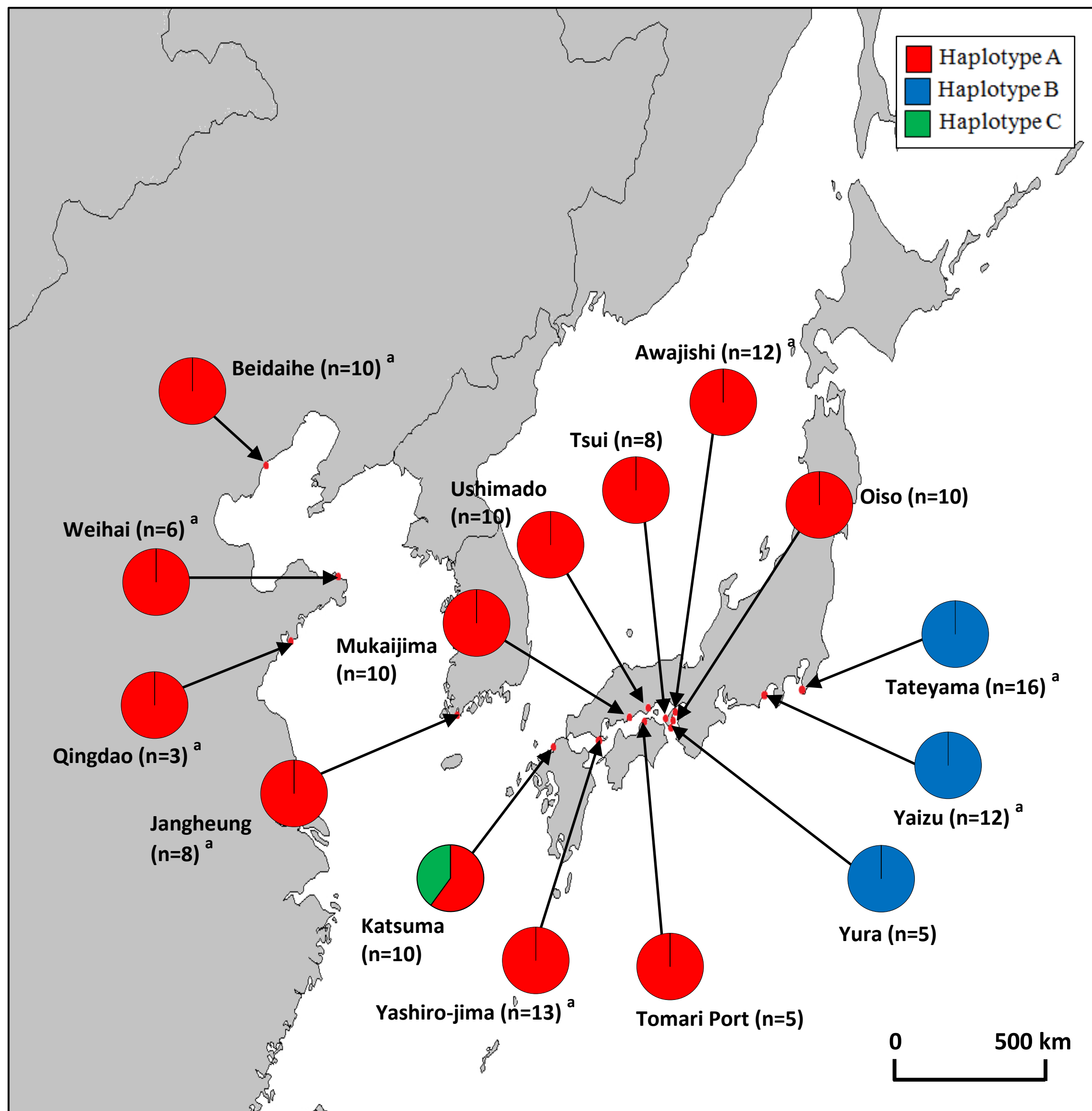


Fig. 2.12 Haplotype distribution map for the mtDNA *Trn*W_I spacer fragment of *S. muticum* populations sampled from the native range in the northwest Pacific. Colour motif pie charts display relative frequencies of each haplotype in each population and number of individual's sequenced shown in brackets. [a] Haplotype frequency data taken from Cheang et al. (2010c).

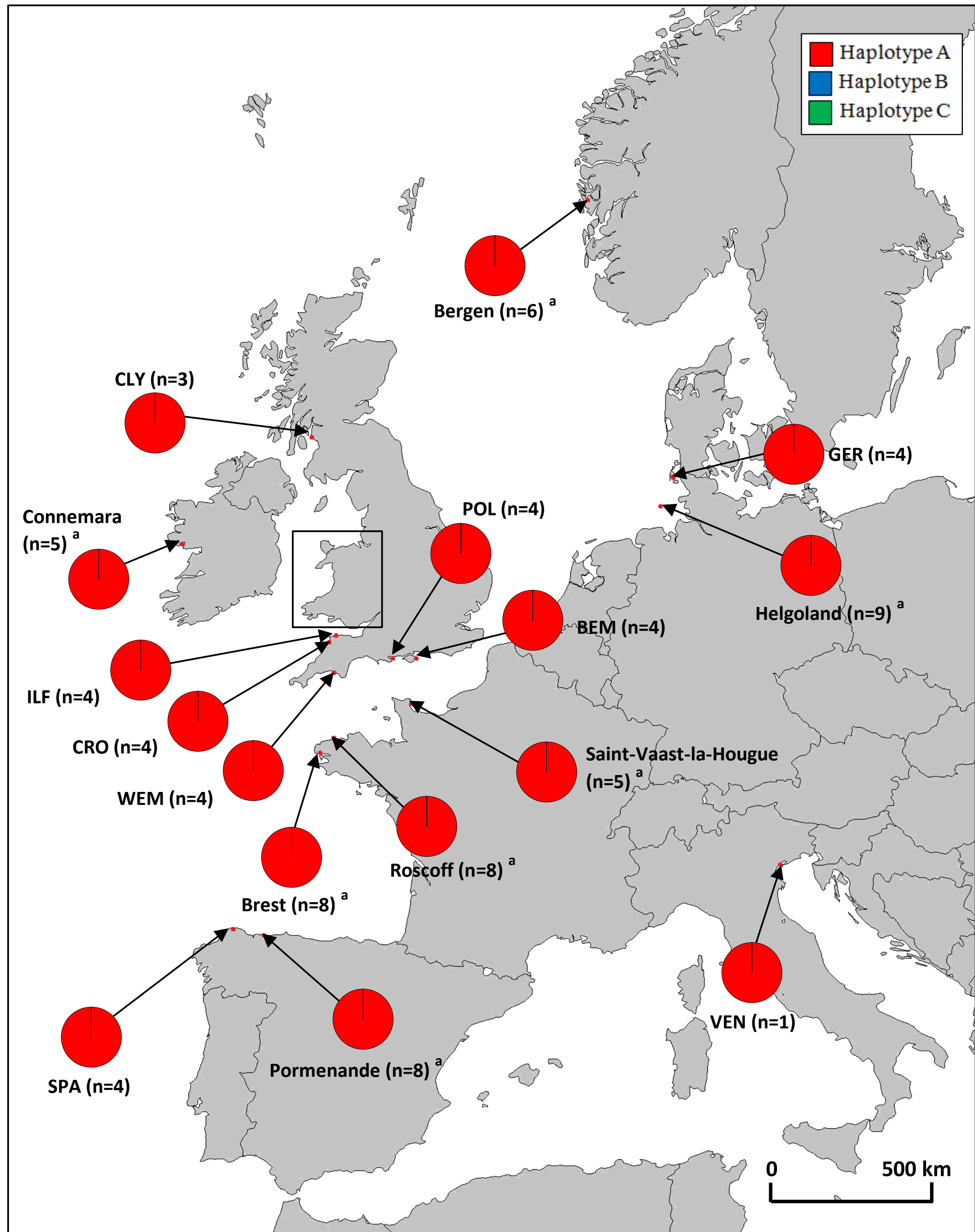


Fig. 2.13 Haplotype distribution map for the mtDNA *Trn*W_I spacer fragment of *S. muticum* populations sampled from various European introduced populations. Colour motif pie charts display relative frequencies of each haplotype in each population and number of individual's sequenced shown in brackets. Note all populations displayed the dominant haplotype (Hap A) only. [a] Haplotype frequency data taken from Cheang et al. (2010c). See Table 2.1 for the population location codes. For distribution of haplotypes within Wales (boxed inset) see Fig. 2.14.

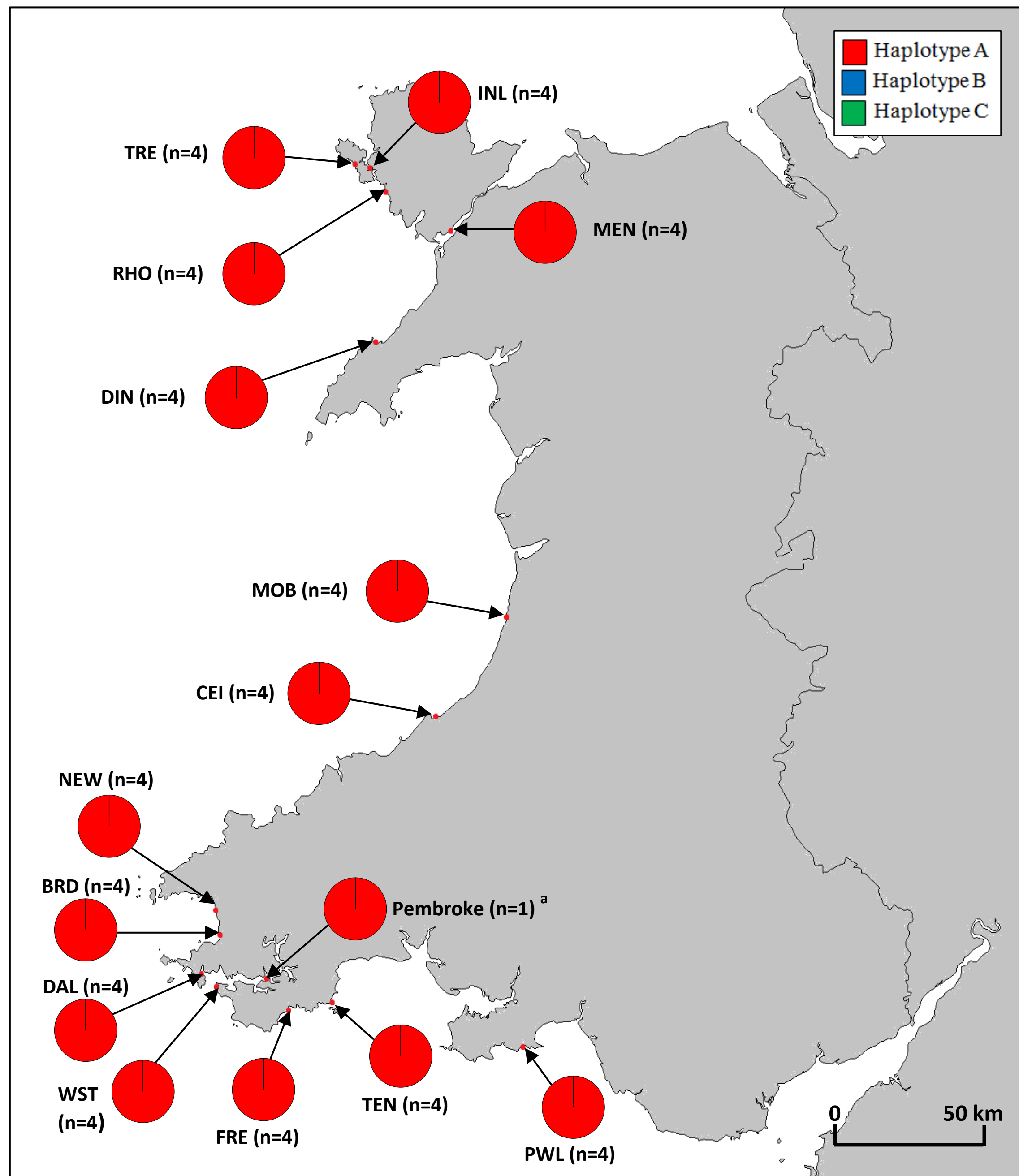


Fig. 2.14 Haplotype distribution map for the mtDNA *Trn*W_I spacer fragment of *S. muticum* populations sampled from various introduced populations within Wales. Colour motif pie charts display relative frequencies of each haplotype in each population and number of individual's sequenced shown in brackets. Note all populations displayed the dominant haplotype (Hap A) only. [a] Haplotype frequency data taken from Cheang et al. (2010c). See Table 2.1 for the population location codes.

A total of 580 base pairs of the nucleotide sequence of the gene encoding subunit 3 of cytochrome *c* oxidase in *Sargassum muticum* were recovered after alignment and editing. Variation was revealed in the *cox3* gene fragment with a total of 2 haplotypes recovered from the investigation plus an additional haplotype when combined with GenBank sequence data. The two *cox3* haplotypes recovered from the main study differed by 3 base pair substitutions (2 transitions, C to T and 1 transversion, T to G) at nucleotide sites 142, 379 and 445 on the original sequence alignment (Table 2.5 and Fig. 2.15). The base pair changes were confined to individuals from the Yura population only. When two additional *cox3* fragment sequences from the GenBank database (EU681463 and AB430582) were included in the alignment 4 variable nucleotide site positions were detected (Fig. 2.16). The out-group sequence, *S. horneri* (AB430550) differed by 39 base pairs from those of *S. muticum*. Similar to the *Trn*W data set all trees generated via the various phylogenetic methods exhibited identical topologies (Fig. 2.17). *Cox3* haplotype A corresponds to the haplotype common to all introduced populations and those from central and western Japan. *Cox3* haplotype B was only found in specimens taken from the Yura population of Japan. *Cox3* haplotype C is based upon inclusion of GenBank sequence AB430582 derived from a Japanese specimen from Kan-non-zaki, Yokosuka, Kanagawa prefecture. Haplotype C displayed the three same base substitutions as found in the Yura isolates plus an additional base pair change when placed in the alignment (a transversion T to G) at nucleotide site 475, from original alignment (Table 2.5 and Fig. 2.15). Therefore analysis of the *cox3* gene marker has revealed the presence of three haplotypes (Figs. 2.16 and 2.17). However caution must be taken when stating these results as the additional *cox3* haplotype was only revealed from a single nucleotide sequence not derived via sampling in the main study. When translated into the corresponding amino acid sequence no stop codons were present which is consistent with protein coding genes such as *cox3* and COI. However, the translation of the sequence corresponding to haplotype C and alignment of the resulting amino acid sequence with those from the other two haplotypes revealed a change in amino acid from cysteine to tryptophan. Still, no stop codons were detected. Changes in amino acids within the *cox3* gene sequence has been demonstrated in *Sargassum horneri* (Uwai et al., 2009). Overall in terms of the mitochondrial DNA fragments no ambiguous base compositions were encountered

suggesting that heteroplasmy is largely absent in *Sargassum muticum* (Coyer et al., 2004).

Table. 2.5 Variable nucleotide positions and base pair changes present in mtDNA *cox3* haplotypes of *S. muticum* revealed by this study including an additional *cox3* sequence (AB430582) taken from the GenBank database.

	Sequence position			
Haplotype	142	379	445	475
cox3 Hap A	C	C	T	T
cox3 Hap B	T	T	G	T
cox3 Hap C	T	T	G	G

A preliminary alignment of the five *rbc*L sequences for *S. muticum* available on GenBank revealed a number of single nucleotide substitutions between individuals from different geographical locations. When the sequence data from this study was included in the alignment, all sequences were found to be identical to a published sequence from the Netherlands (Accession No. AJ287854). Furthermore, when the sample collected from Laguna Beach, California was aligned against a GenBank sequence from California (Accession No. AF244331), we identified 5 nucleotide substitutions relative to the published sequence. This is of particular interest as it may suggest that potentially two or more introductions to the California region may have occurred, although much more comprehensive sampling from this area would be required before making a conclusive statement. Overall the preliminary sequence data for the two mitochondrial DNA fragments and the large subunit of the plastid ribulose-1, 5-bisphosphate carboxylase (*rbc*L) gene have so far revealed no variability at the spatial scale of both Wales and the UK.

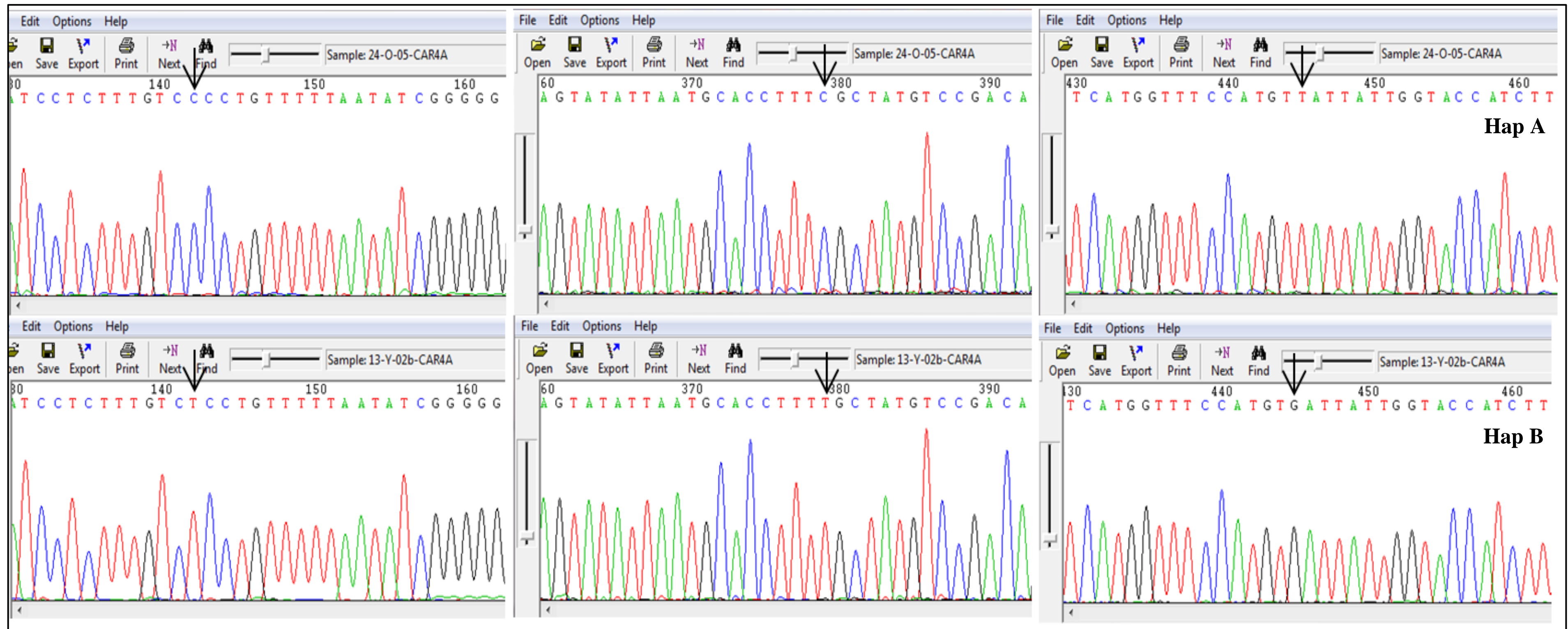


Fig. 2.15 Example chromatograms representing the two main *cox3* haplotypes (Hap A and Hap B) revealed in this study. Regions displayed in chromatograms contain the variable site positions (labelled with an arrow) between haplotypes.

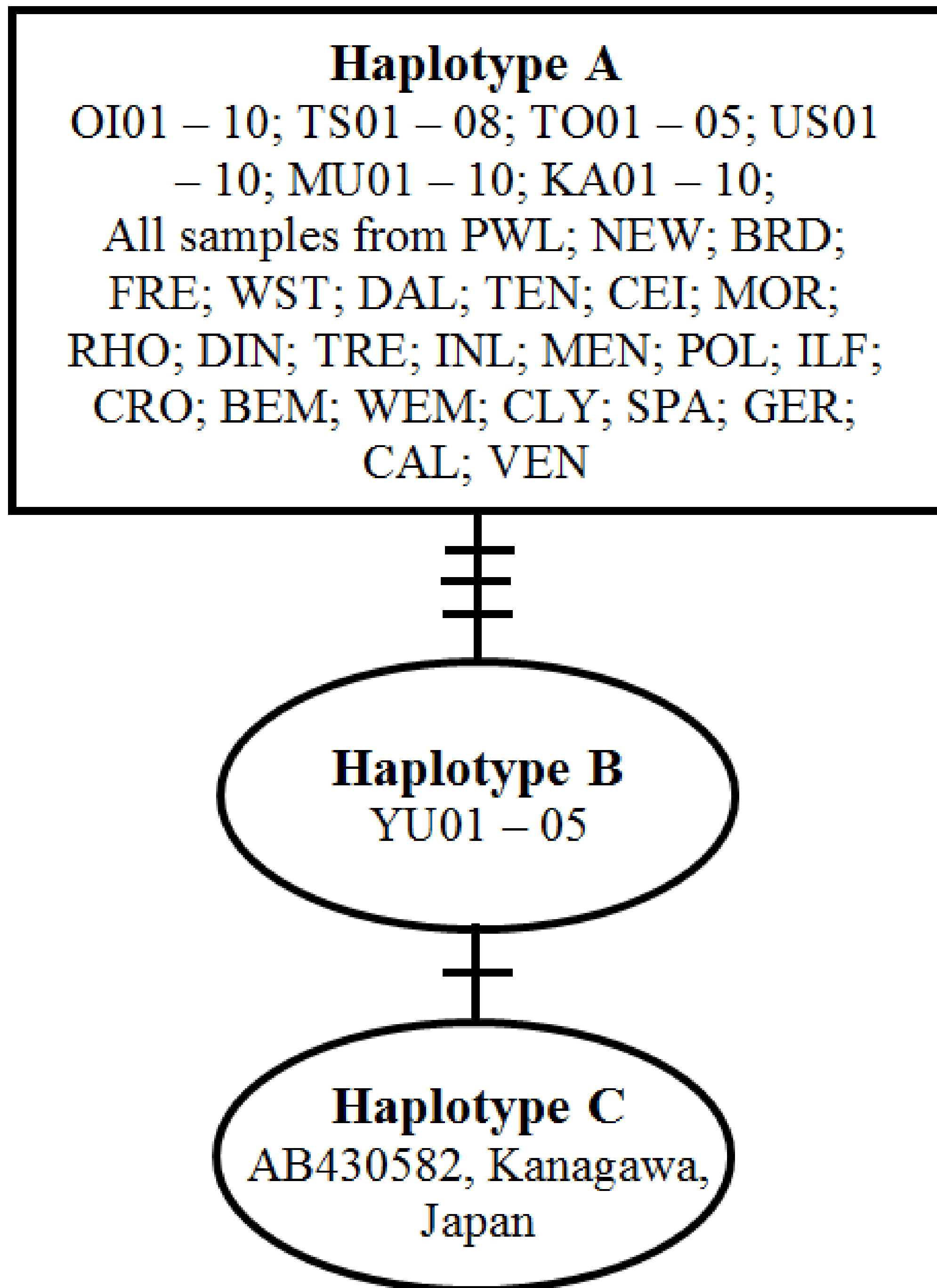


Fig. 2.16 Haplotype network based on the mitochondrial *cox3* gene fragment. Sampling localities of the haplotype are indicated within the haplotype node. Connecting lines show mutational pathways among haplotypes with short cross lines representing single base pair substitutions. See Table 2.1 for the population location codes.

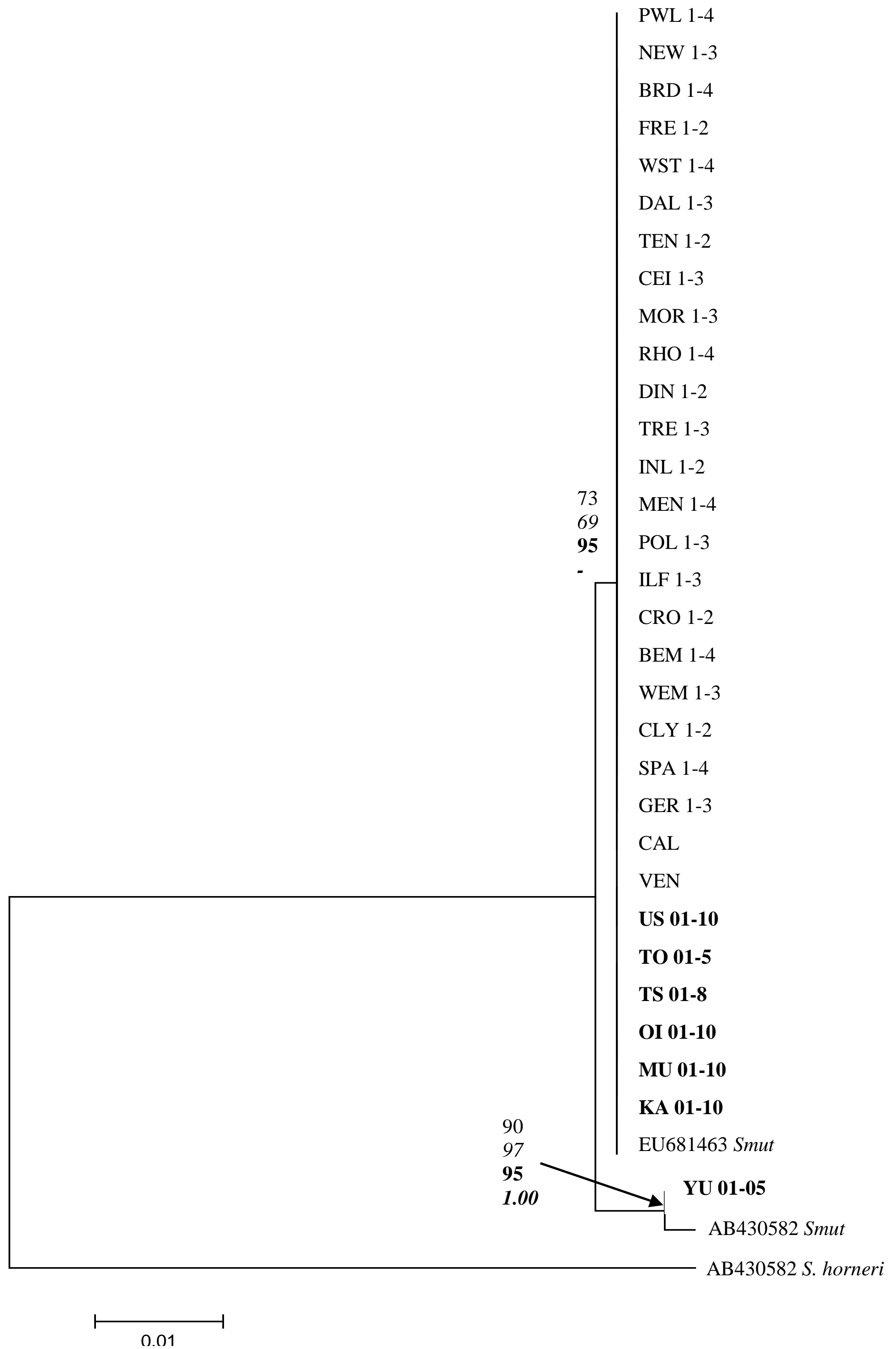


Fig. 2.17 Genetic relationships between populations of *S. muticum* depicted by maximum likelihood tree based on the mitochondrial *cox3* data set. All four methods tested neighbour-joining (NJ), maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI), generated trees with identical topologies. Bootstrap values (1000 replicates for NJ, ML and MP) for NJ (regular), MP (italic), and ML (bold) methods and the posteriori probabilities BI (bold and italic) are indicated at the nodes. Additional *S. muticum* sequences from GenBank are included in the tree. Populations from the native range are indicated in bold type.

2.3.2 AFLP development results

Given the lack of variability detected within the sequenced molecular markers, the AFLP technique was selected to try to obtain a more enhanced resolution of any existing genetic variability within *Sargassum muticum*. However, during this course of the AFLP development the reproducibility of the technique became a significant problem. In numerous samples, peaks were not shared between individuals and duplicate runs did not produce the same AFLP profile (see Fig. B.1A, Appendix B). In terms of the AFLP reproducibility, there were several recurrent problems. Firstly, the intensity of the amplified fragments was not often reproduced within an individual sample. On occasions, a strong band observed in one run was weak, falling below the RFU threshold cut-off value, and barely discernible from background noise in the second run. Variations in individual band signal intensity was also noted across samples adding to further issues surrounding the reproducibility of the technique. Secondly, some individual samples were not reproducible across the whole technique, with failed runs in addition to weak amplifications where no fragments greater than 300 bp were observed (see Fig. B.1B, Appendix B). Reasons as to the lack of reproducibility and optimisation of the technique are considered in the discussion (section 2.4.2). However, from the 60 samples tested, encompassing all sampled populations, reproducible AFLP profiles using a single primer pair (E + AG/M + CAA) were successfully generated for 21 of the *S. muticum* samples from the study. An example of a sample showing good reproducibility is displayed in Fig. B.2 (Appendix B). Moreover, only samples from introduced populations were among the 21 samples that displayed successfully reproducible AFLP profiles.

Overall, the samples from which successful AFLP profiles were generated displayed a significant lack of variability, comparable to the trends observed in the DNA fragment sequencing study. However, following a comparative analysis of six samples collected from the population at Sandbanks (Poole) one variable and reproducible AFLP fragment was consistently detected in three of the samples (Fig. 2.18).

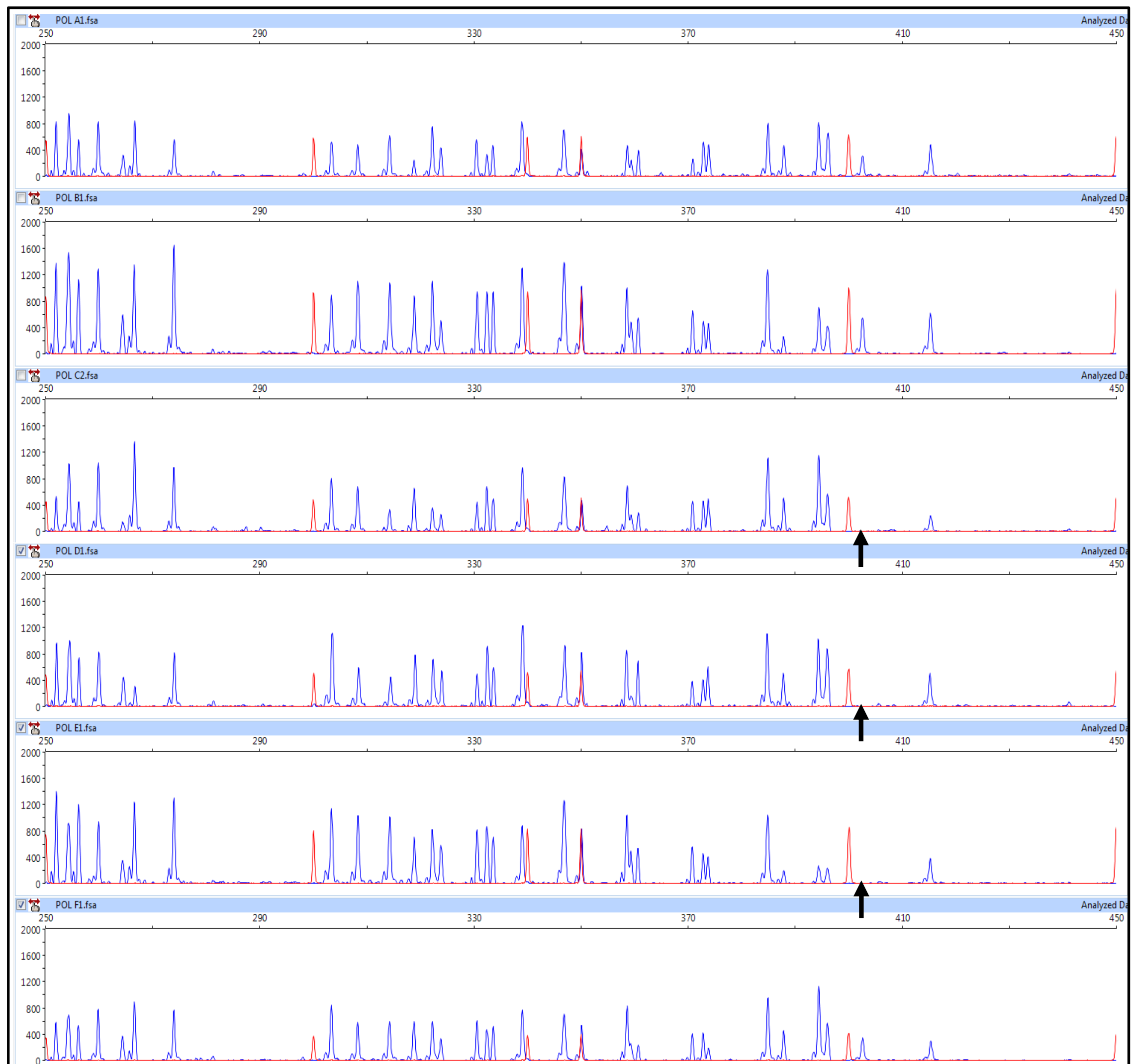


Fig. 2.18 Electropherograms produced from samples of *S. muticum* collected from Sandbanks, Poole. Each sample was collected from a separate groyne starting at the chain ferry. Consistency of the absence of a 402 bp fragment was noted from 3 samples (see arrows) within the POL samples analysed by AFLP using the selective primer pair *Eco*RI + AG and *Mse*I + CAA. Horizontal scaling indicates fragment sizing (base pairs); vertical scaling indicates fluorescence signal intensity of amplified fragments (rfu). Blue peaks represent sample fragments; red peaks indicate internal size standard (ROX-500) added to the samples.

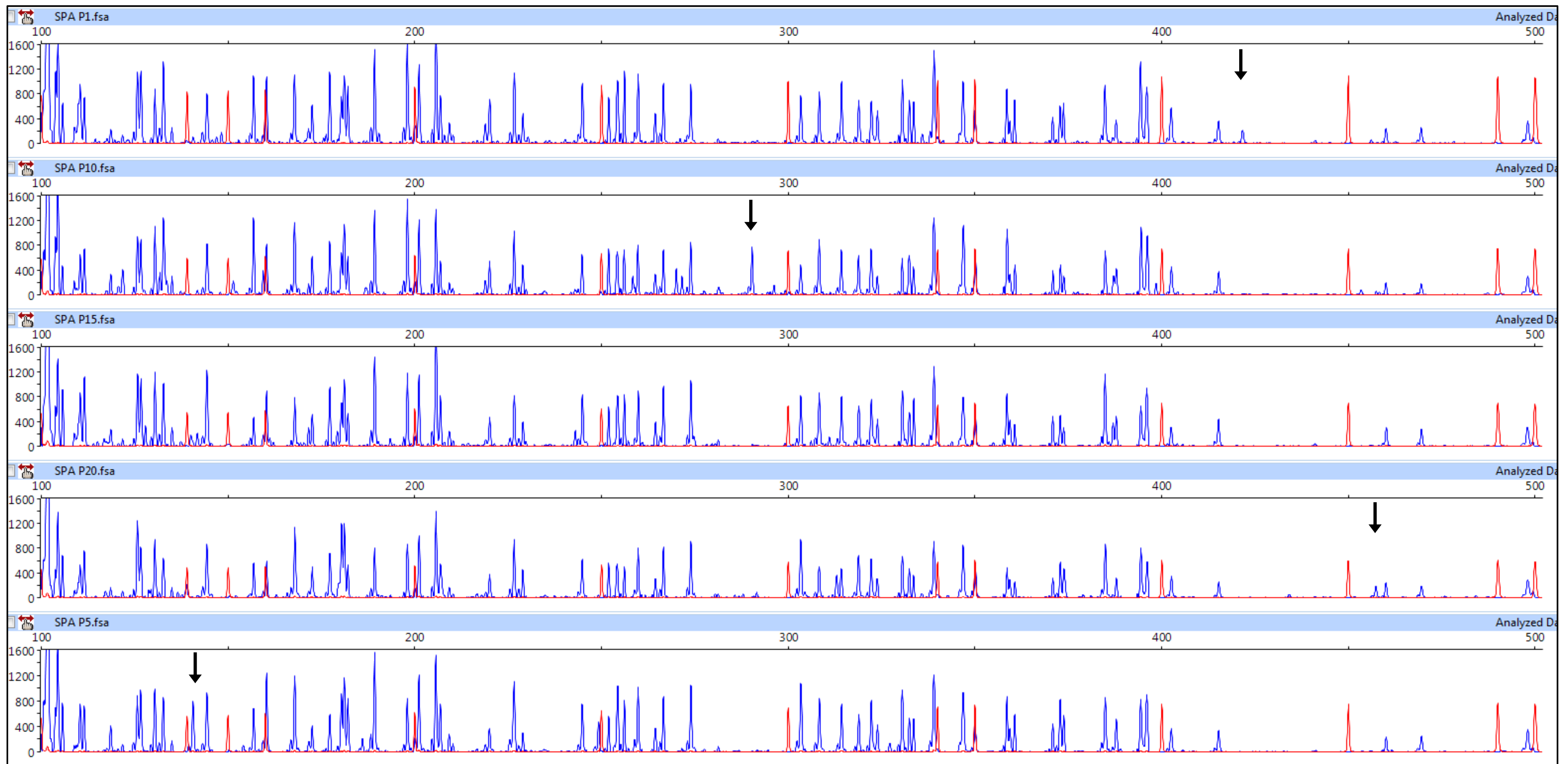


Fig. 2.19 Electropherograms produced from samples of *S. muticum* collected from Ortigueria Estuary, Spain that gave a discernible AFLP fragment profile using the selective primer pair *Eco*RI + AG and *Mse*I + CAA. Arrows indicate intra-population polymorphism and all occurrences of polymorphisms were reproducible within samples. Horizontal scaling indicates fragment sizing (base pairs); vertical scaling indicates fluorescence signal intensity of amplified fragments (rfu). Blue peaks represent sample fragments; red peaks indicate internal size standard (ROX-500) added to the samples.

Further within population variability assessments also revealed polymorphic fragments within the samples collected from the Ortigueria Estuary, Spain (Fig. 2.19). Again these variable fragments were reproducible following repeat AFLP reactions with newly extracted sample DNA. Other than these instances of a single polymorphic fragment present within a sample, variability on the whole was very low. When the profiles from successful AFLP reactions produced by additional samples from various introduced populations were compared a genetically homogenous population was suggested (see Fig. B.3, Appendix B).

Due to reproducibility problems, no discernible AFLP profiles from any native population samples could be generated within the time frame of the investigation. Therefore the genetic diversity with the native range of Japan, as characterised by AFLP marker remains unresolved. Furthermore, the identification as to the potential source region(s) for introduced populations cannot yet be concluded from this study's data set.

2.4 DISCUSSION

The results from this study demonstrated a clear lack of genetic variability in *S. muticum* within its introduced range which was also shown in the results of Cheang et al. (2010c).

2.4.1 Genetic diversity within the sequencing markers

2.4.1.1 Mitochondrial *Trn*W_I region

In concordance with the results of Cheang et al. (2010c) sequence data confirmed all samples from both introduced and native populations in this study as *S. muticum*. Sequencing results from the *Trn*W_I mitochondrial intergenic spacer region confirm those previously reported by Cheang et al. (2010c). However an additional haplotype (Hap C) was revealed from the results of the current study. This haplotype connects the two main haplotypes and occupying the missing intermediate originally presented in the haplotype network of Cheang et al. (2010c; see Fig. 3C). Furthermore this haplotype, only detected in the Japanese population of Katsuma, co-occurred with the main *Trn*W_I haplotype (Hap A, see Fig. 2.12). This occurrence of intrapopulation variability within the mitochondrial *Trn*W_I fragment represents its first recorded

occurrence in *S. muticum*. A recent phylogeographic study of the brown algae, *Ishige okamurae* in the northwest Pacific region detected two *cox3* haplotypes present on the Fukuoka Prefecture of Japan, and in similar proportions to the two *Trn*W_I haplotypes identified from the Katsuma population in this study (Lee et al., 2012). The presence of converging water currents off the Fukuoka Prefecture coast may be a possible explanation for the existence of two *Trn*W_I haplotypes within the Katsuma population sampled in this study (see Fig. 2.20). The Tsushima current, a branch of the Kuroshiwo current flows northwards alongside the west coast of Japan. Also within the waters between Korea and Japan flows the East Korean Warm current. The convergence of these two currents may therefore increase localised mixing within the region and may be a suggested reason for the occurrence of intra-population variability within Katsuma.

The Japanese population of Yura from the native range displayed the same *Trn*W_I haplotype (Hap B, Fig. 2.12) as that revealed from populations located further north on the east coast of Japan noted in the study by Cheang et al. (2010c). Therefore the genetic divide between the east coast and central Japanese populations of *S. muticum* is likely to occur somewhere near the entrance to Seto Inland Sea. An examination of the currents in this region may provide an indication of mechanisms contributing population genetic boundaries. Only with further sampling and genotyping of *S. muticum* populations from along the stretch of coast at the entrance to the Seto Inland Sea will the boundary and potential overlap of these mitochondrial haplotypes likely be resolved.

According to Yoshida (1983) two morphological variants of *S. muticum* exist within the alga's distribution in Japan. The results from the study by Cheang et al (2010c) suggested the possibility that the two main *Trn*W_I lineages identified may in fact correspond to the two morphological variants. One variant with larger basal leaves was suggested to correlate with the *Trn*W_I haplotype A, with the other variant possessing smaller basal leaves and correlating with the *Trn*W_I haplotype B (Cheang et al., 2010c). However, due to the absence of significant morphological variation between the samples collected in their study further morphometric analyses were recommended on specimens from both mtDNA lineages. The identification of an additional *Trn*W_I haplotype (Hap C) from this investigation could also suggest the existence of an additional morphological variant. Therefore any future morphometric

analyses of *S. muticum* should include samples from the Fukuoka prefecture where the *Trn*W_I Hap C was detected.

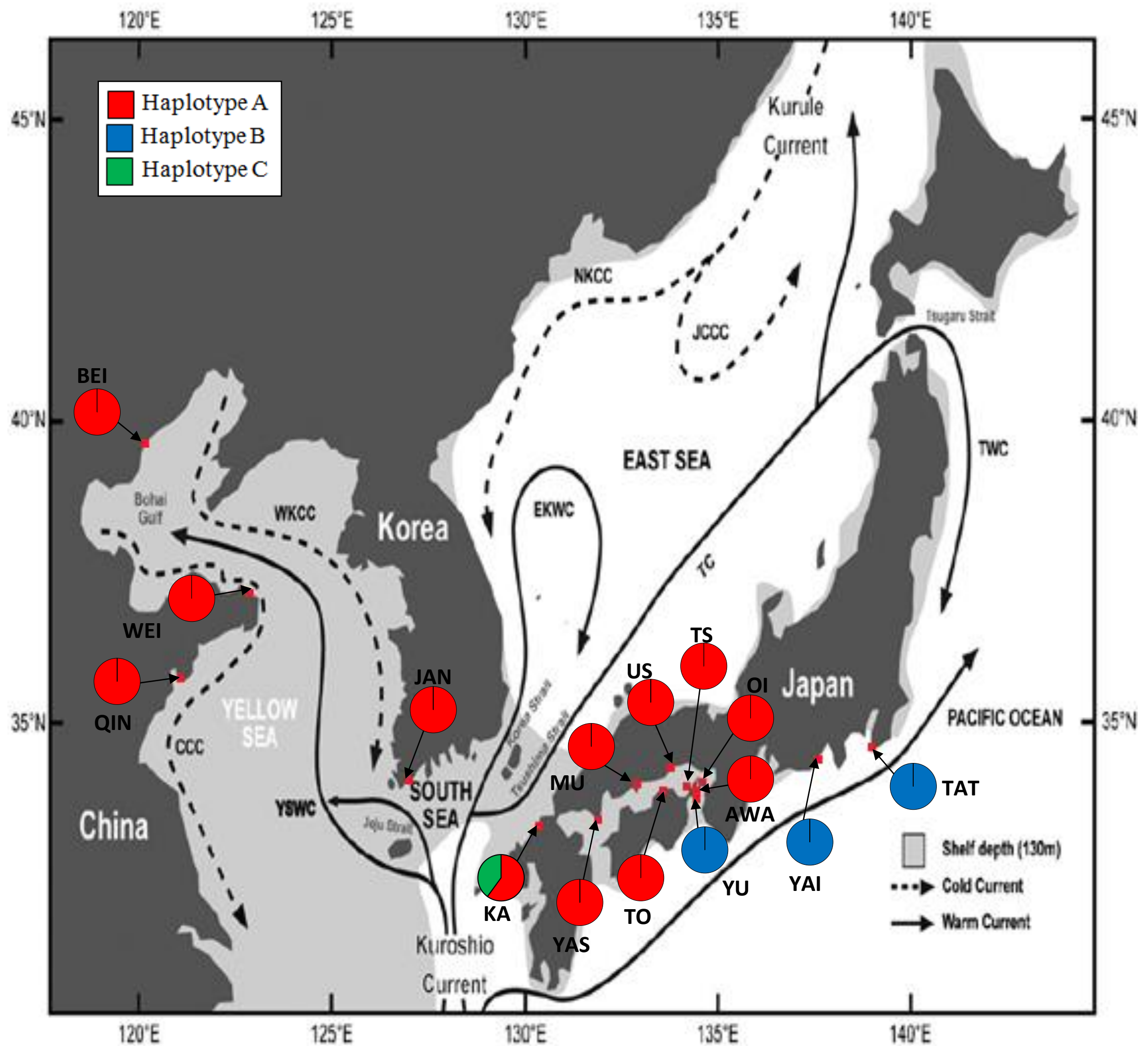


Fig. 2.20 Map showing the main water currents within the northwest Pacific together with the *Trn*W_I frequency data from this study and that from Cheang et al. (2010c) overlaid on the map. Colour motif pie charts display relative frequencies of each haplotype in each population. For data from Cheang et al. (2010c) TAT = Tateyama, YAI = Yaizu, AWA = Awajishi, YAS = Yashiro-jima, JAN = Jangheung, QIN = Qingdao, WEI = Weihai and BEI = Beidaihe. See Table 2.1 for the population location codes from this study. Dotted arrow lines indicate cold currents, and solid arrow lines show warm currents. CCC, China Costal Current; EKWC, East Korea Warm Current; JCCC, Japan Central Cold Current; NKCC, North Korea Cold Current; TC, Tsushima Current; TWC, Tsugaru Warm Current; WKCC, West Korea Costal Current; YSWC, Yellow Sea Warm Current. (Figure adapted from Kim et al., 2012).

Comparisons between the levels of genetic variability in *S. muticum* to the kelp, *Undaria pinnatifida* (the only other introduced macroalgae which has been genotyped using the *Trn*W_I mitochondrial spacer region) revealed a considerable lack of diversity in *S. muticum* compared to *U. pinnatifida* (Voisin et al., 2005). In contrast, *Sargassum thunbergii*, also native to the northwest Pacific displays similar levels of genetic diversity to *S. muticum* with the occurrence of two *Trn*W_I haplotypes (FJ712742 and FJ712743; GenBank database). However, as no information exists within the GenBank database as to the general location of the samples from which these two haplotypes were derived (Cheang et al., 2010a), no correlations between the patterns of distribution of the *Trn*W_I haplotypes in both *S. muticum* and *S. thunbergii* can be currently inferred.

Sargassum hemiphyllum has also been investigated using the *Trn*W_I marker and shown to possess two distinct haplotypes (Cheang et al., 2010b). In contrast to results from this study, the only population where both haplotypes were detected was located near Kyoto on the Wakayama Prefecture. In terms of water currents within this region the main current is the Kuroshio Current that flows NE along the coast of Japan. This main current may be interacting with localised coastal currents contributing to the mixing of genotypes within the region. Given the poor dispersal capacity of *Sargassum* zygotes (Kendrick and Walker, 1995), drift plant material is most likely the main mechanism by which mixing of genetically differentiated individuals occurs. Similar to the findings of this study, the *Trn*W_I marker was shown to be the most variable marker in *Sargassum hemiphyllum* (Cheang et al., 2010b).

2.4.1.2 Mitochondrial *cox3* region

Similar to the findings from the *Trn*W_I data set, two main *cox3* haplotypes were revealed by this study (Figs. 2.16 and 2.17). The dominant *cox3* haplotype (Hap A) corresponded to all samples from the introduced range plus almost all those from native populations sampled with the exception of Yura whose samples displayed the other main haplotype (Hap B). Also comparable with the *Trn*W_I data, a third haplotype was detected following the inclusion of a *cox3* sequence taken from the GenBank database (AB430582). According to the information provided on the GenBank database, this sequence was obtained from a sample collected from Kan-non-zaki, Yokosuka located on the Kanagawa prefecture. The prefecture of Kanagawa on the east coast of Japan is positioned between the prefectures of Chiba and Shizuoka

where populations were sampled in the study by Cheang et al. (2010c). Therefore it is probable that additional *cox3* genetic analyses of the populations from Chiba and Shizuoka used in the study by Cheang et al. (2010c) may confirm the existence of this third *S. muticum cox3* haplotype.

The occurrence of only a single *cox3* haplotype within each of the native *S. muticum* populations contrasts with studies of other *Sargassum* species from the same region. For example a recent study by Uwai et al. (2009) examining the phylogeography of the *Sargassum horneri/filicinum* complex reported four *cox3* haplotypes from a *S. horneri* population located at Yura, corresponding to the same location as sampled in this study. In contrast, the *cox3* subclades 2-1 and 2-5/2-6 detected in the *Sargassum horneri/filicinum* complex showed similar distribution patterns with the two *S. muticum cox3* haplotypes detected in this study.

With regards to the spatial scale of the Welsh populations no variability was detected both within and between any of the populations using the sequencing markers. Again these findings confirm those of the recent study by Cheang et al. (2010c) who also showed that introduced populations of *S. muticum* on a worldwide scale are genetically homogenous with respect to markers examined from both the nuclear and extranuclear genomes.

2.4.2 AFLP development in *S. muticum*

Despite the incomplete optimisation of the AFLP marker, results from a limited number of successful AFLP reactions obtained with various samples of *Sargassum muticum*, also suggest that introduced populations are largely genetically homogenous. In fact, a small degree of variability was detected within a number of samples from two introduced populations (Poole and Spain) included in this study. Only one polymorphic fragment (402 base pairs) was detected within three individuals sampled from Sandbanks, Poole (see Fig. 2.18). Furthermore, the fact that the absence of this 402 base pair fragment was detected in 3 different samples from Sandbanks provides support against occurrence due to any technical artefact of the AFLP reaction. In the Spanish samples a total of four polymorphic fragments were identified but each one only occurred in a single individual (Fig. 2.19). The reliability of these polymorphisms was again supported by their reproducibility within the individuals tested and the overall

average level of fragment intensity within samples. This previously undetected genetic variability within two introduced populations may therefore provide the first indication that multiple introductions have played a role in the invasion history of *S. muticum*. However, given the use of single selective primer pair and the limited number of samples involved, this result is highly speculative and overall the preliminary results of the successful AFLP reactions suggest that introduced populations of *S. muticum* are genetically homogenous. This genetic homogeneity exhibited by various introduced population samples could be a sign of a founder effect, as has been seen in populations of other introduced species including species of marine macroalgae (Jousson et al., 1998, Amsellem et al., 2000, McIvor et al., 2001, Colautti et al., 2005, Hamner et al., 2007). However, given the limited sample numbers, lack of comparative native sample data and the use of a single selective primer pair any inferences made regarding the AFLP results must be treated with caution.

The apparent lack of any detectable variation is surprising given the fact that AFLP markers are one of the most variable classes of genetic markers known, and typically show wide variation at the intraspecific level (Mba and Tohme, 2005, Meudt and Clarke, 2007). Although according to a recent report (MARINEXUS, 2010) the application of 12 newly developed *S. muticum* microsatellite markers failed to reveal any polymorphism in two introduced populations from France and one from England, with all individuals sharing the same multi-locus genotype. However, the same 12 microsatellite loci did show variability when a set of samples from the native range were genotyped. So it could be that the lack of AFLP marker variability is not atypical for this alga. Again these recent findings would concur with previous genetic diversity assessments of *S. muticum* that have highlighted genetic homogeneity throughout the introduced range of this seaweed (Cheang et al., 2010c).

A possibility that cross-contamination of samples led to the false impression of genetic uniformity in the successful AFLP reactions, can be ruled out, as reproducible results were generated on different days, using different set of samples. A second possibility exists that AFLP markers are not suited for the genetic study of *S. muticum* given the inherent lack of variation. However, as only a handful of successful AFLP reactions were generated from samples from introduced populations only, the presence and extent of variability within the native range of this alga remains to be tested. Based

on the variability revealed within the sequencing data set, it is likely that successfully generated AFLP data from native samples should demonstrate further genetic polymorphism. Evidence based on preliminary variation detected within microsatellites loci from native populations also suggests the likelihood of this scenario.

Overall, the main issue encountered during the AFLP development in this study was the lack of reproducibility in many of the samples tested. A vital step in the AFLP protocol is the complete restriction digest of the template DNA, as an incomplete digestion may result in banding pattern differences between individuals interpreted as false polymorphisms (Bensch and Akesson, 2005). To ensure complete digestion, template DNA must therefore be of high quality and free from inhibitors (Chen and Ronald, 1999, Bonin et al., 2005, Meudt and Clarke, 2007). DNA concentration is not thought to be a crucial factor in AFLP assays as several protocols have been developed using a wide range of starting DNA template amounts (Vos et al., 1995, Bensch and Akesson, 2005, Trybush et al., 2006). In addition, technical differences are affected by the purity and quality of DNA, which has been found to be the limiting factor in obtaining high quality AFLP fingerprints (McLenachan et al. 2000, Mannschreck et al. 2002, Bonin et al. 2004) and influences the quality of the digest. The observation that non-reproducible fragments were mainly found between patterns of the two different DNA extractions and not within the two patterns of the same DNA extraction in this study provides support for this scenario. Therefore it is likely that the problems encountered during the optimisation of the AFLP markers in *S. muticum* are mostly attributable to the quality of extracted DNA.

As the AFLP technique is highly susceptible to contamination by foreign DNA (Dyer and Leonard, 2000) it is important to reduce this risk at all stages of the method. Therefore several precautions were taken during the DNA extraction, restriction/ligation and PCR procedures, including the use of ‘sterile conditions’ on a clean bench and the use of a PCR workstation (Bigneat Ltd., Hampshire, United Kingdom). Therefore the probability of contamination by foreign DNA, acquired during the various methodological steps remains relatively low. Within this study the chances of contamination by foreign DNA were most likely during the DNA extraction itself. This may provide an explanation as to the poor reproducibility observed in native sample material as small quantities of additional epiphytic material were observed with the

silica dried native tissue samples. Hence when taking subsamples for DNA extraction purposes each portion was visualised under a dissecting microscope in order to minimise any contamination by non *S. muticum* material. However, any minute quantities of foreign DNA within the extracts may have been sufficient to cause complications with the AFLPs without affecting the standard DNA sequencing methods.

The reason for successful AFLP fragment generation in a study by Shan and Pang (2009) may be partially attributable to the use of laboratory culture derived tissue samples of *Undaria pinnatifida* which would have minimised the chances of contamination by foreign organismal material. Although the presence of contaminating DNA has been reported to cause suppression in fragment amplification for RAPDs (Dyer and Leonard 2000), no evidence of a similar such effect has been reported for AFLPs. The fact that no a priori sequence information is required to implement AFLPs also means that the technique is not selective for specific types of DNA (e.g. no species specific primer sets are typically utilised). As such, the presence of any additional DNA from foreign organisms can subsequently lead to the generation of false banding patterns in the AFLP profile.

As quality of DNA is largely reported as the main cause of reproducibility problems in AFLP based investigations, it is likely the quality of the DNA isolates used in this study were responsible for the lack of reproducibility of the technique. The DNA extraction procedure employed in this study followed a modified CTAB protocol taken from Wang et al. (2006) followed by further purification using the GENECLEAN II Kit (BIO 101 Inc., La Jolla, CA, USA). A similar method was adopted in a study by Ludington et al. (2004) who successfully used AFLP markers to examine the genetic structure of *Mastocarpus papillatus*. However, nowhere in their study do they discuss the level of reproducibility, hence any inferences regarding their findings should be accepted with caution.

Based on the literature, only four AFLP based macroalgal genetic studies have been successfully implemented without the need for additional DNA purification procedures (Murphy and Schaffelke, 2003, Shan et al., 2009, Shan and Pang, 2009, Shan et al., 2011). In fact the extraction technique used in the study by Shan et al. (2009) was based on that from Wang et al. (2006) and very similar to the one adopted in

this investigation. Furthermore, the species under investigation in the study by Shan et al. (2009) was a related species of *Sargassum* providing evidence that the AFLP technique is applicable to the study of macroalgae from this genus. Most of the remaining studies have used some form of post extraction DNA purification or a commercial DNA isolation kit (e.g. Kusumo and Druehl, 2000, Iitsuka et al., 2002, Schaeffer et al., 2002, Pang et al., 2010). However, given the low reproducibility success, it is likely that contaminants still remained in the DNA extracts from this study, despite using only those with good purity readings (i.e. those with an OD260/280 ratio between 1.8 and 2.0). Any contaminants present in the extracts, not interfering with the purity ratio measurements may have been sufficient to affect various stages of the AFLP reaction. It is interesting to note that RAPD markers which have often been criticised for reproducibility problems over AFLPs (Jones et al., 1997, Meudt and Clarke, 2007) were successfully applied to the study of genetic structure in *S. muticum*. Although the authors don't mention how the reproducibility of both markers was assessed, an estimation of both within and among population variability was generated. The reason behind the successful application of these markers is likely attributable to the authors use of a commercial DNA extraction kit which in general tends to yield DNA of high purity. Therefore it would be interesting to assess the suitability of the DNA isolated using the same kit in the application of AFLP markers.

Often cited as one of the main advantages of the AFLP technique is its relatively rapid start up times (Bensch and Akesson, 2005, Mba and Tohme, 2005, Meudt and Clarke, 2007). However, results from this investigation appear contrary. A significant length of time was invested in the development of the technique, but with only limited success and requiring further optimisation in the future. Similar experiences by other researchers during the development and application of AFLP markers suggests that the technique is not always a quick and robust alternative to other molecular markers (Donaldson, 1996, Ashton, 2006, Benefer, 2011). Furthermore, the development time may also depend upon the organism under investigation. However, the generation of reproducible profiles with a limited selection of samples from this study highlights the potential that given further development and optimisation, the AFLP technique may be applicable to future studies into the genetics of *S. muticum*. With the advent of new technologies such as whole genome sequencing and their increasing accessibility to

non-model organisms, the ability to assess genetic variation is likely to become much easier, especially in species previously found to be genetically depauperate.

2.4.3 Conclusions

The low level of genetic variability in *S. muticum* appears to agree with several of the alga's life history characteristics (Zhao et al., 2008). Self-fertilizing plant species like *S. muticum*, commonly have very low genetic diversity within populations (Loveless and Hamrick, 1984, Hamrick and Godt, 1996). Given the typically low dispersal distances of *S. muticum* germlings (Deysher and Norton, 1982, Norton, 1992) to within a few metres of the parental plant, genetic differentiation between population stands would be expected to increase with increasing geographical distance. In fact, a study using both RAPD and ISSR markers to examine genetic structure of natural *S. muticum* populations along a province of China showed this to be the case with the detection of significant isolation by distance (Zhao et al., 2008). However, in the alga's introduced range no evidence to date has demonstrated any degree of genetic structuring due to the prevalence of genetic homogeneity. From the collective results a possible explanation could be that a single introduction event was responsible for the alga's initial incursion into European waters and that further spread of the alga has been primarily via natural drift in surface waters. A further possibility is that the invasion history of *S. muticum* may be similar to that of *Caulerpa taxifolia* in the Mediterranean (Jousson et al., 1998), in that a single invasive strain is responsible for all introduced populations and a range of dispersal vectors, both natural and anthropogenic may have contributed to the spread of the alga.

The term "general purpose genotype" was introduced by Baker (1965) to describe species able to cope with a range of different environmental conditions due to the mechanism of phenotypic plasticity. This is certainly a model that fits the worldwide invasions of *Sargassum muticum* (Cheang et al., 2010c). However, it has been suggested that the role of phenotypic plasticity is more significant during the initial stages of invasion when tolerance of novel environmental conditions is necessary for invader survival. The magnitude of the phenotypic plasticity response may then be reduced following an invaders successful establishment, due to increased selection of the most adaptive phenotypes (Palacio-López and Gianoli, 2011). Populations of invasive species that exhibit low levels of variation at neutral genetic loci may still have

the ability to adapt to new environmental pressure due to higher levels of variability in fitness related traits (McKay and Latta, 2002). Furthermore, low genetic diversity is not always a hindrance in the success of invasive species. For example the success of invasive argentine ants has been attributed to the low levels of genetic variability between colonies, reducing intraspecific aggression leading to the subsequent formation of supercolonies (Tsutsui et al., 2000). Introduced populations of the water hyacinth, *Eichhornia crassipes* in Southern China also display a complete lack of genetic variability suggesting that other adaptability related factors are responsible for the species successful expansion (Li et al., 2006).

Given the genetic homogeneity displayed by *S. muticum*, as revealed from the molecular markers employed to date, both the global introduction pathways and routes of localised spread still remain largely unresolved (Cheang et al., 2010c). The development of more variable markers such as microsatellites should therefore be targeted for future genetic analyses of this global invasive alga. Furthermore, additional populations from within the alga's native range should be included in future studies. In particular, given the occurrence of three mitochondrial haplotypes from both the *Trn*W_I and *cox3* regions, detected within Japan, further populations encompassing the alga's entire Japanese distribution should be targeted for further genotyping using these markers. In that way, a more accurate picture of the phylogeographic structure of *S. muticum* within its native range can be determined.

Based on the shared occurrence of the main *Trn*W_I haplotype (Hap A) among all introduced populations and native populations sampled within the area of the Seto Inland Sea, Cheang et al. (2010c) suggested that Hiroshima (the main oyster growing area within this region) was the most plausible source for the introduction of *S. muticum* to the Pacific coast of North America. The extra *Trn*W_I sequence data obtained from this investigation together with the new complementary *cox3* data set would seem to add further support to this introduction scenario.

It is interesting that the Seto Inland Sea has also been suggested as the source for other introduced macroalgae. For example, specimens of *Sargassum horneri* (previously known as *S. filicinum*) introduced to the Californian coastline have been shown to display identical *cox3* gene sequences to samples taken from regions in the Seto Inland Sea (Miller et al., 2007). However, unlike the suggestion that the world-wide

introductions of *S. muticum* have happened via the transfer of oysters, the authors indicated shipping as a likely vector for the introduction of *S. horneri* to the Californian coast due to the proximity of international shipping ports to established populations of the alga. Comparatively low levels of genetic variation relative to *S. muticum* have also been detected in *Codium fragile* ssp. *tomentosoides* from both native Japanese and introduced populations (Provan et al., 2005). Furthermore, the similarity of haplotypes shared between introduced populations in the Mediterranean and those from Japanese populations located within the region of the Seto Inland Sea pointed to this native area as a likely introduction source for the alga. Regions of the Seto Inland Sea may be the suggested sources for several non-native marine species, but the reverse is also true. According to an article by Iwasaki (2006) the Seto Inland Sea has also been on the receiving end of a large number of exotic species introductions associated with the heavy concentration of shipping in the region.

The aims of this study were to test if a combination of various molecular markers could provide information as to the source(s) of introduced populations of *S. muticum* both on a global scale and at the level of established populations within Wales. A further aim was also to see if genetic data could be used to identify the main pathways of the alga's spread and the likely dispersal mechanisms involved. However, given the lack of variability within the markers assessed and the unresolved technical issues with the AFLP methodology tested, no conclusive answers to the main objectives could be derived at this time.

The limited information that the cumulated genetic data on *S. muticum* has so far contributed is however, balanced by the existence of a good historical record of introduction and spread of *S. muticum* on a global scale (Critchley et al., 1983, Harries et al., 2007a). Therefore we already have a reasonable insight into the alga's introduction and dispersal pathways. Where the current gap in our knowledge concerning the worldwide introductions of *S. muticum* is uncovering how many founding individuals have been involved at a specific site of introduction. Further on-going genetic analyses utilising a suite of newly developed microsatellites may provide further answers to this question (MARINEXUS, 2010). The lack of genetic variability within invasive populations of *Sargassum muticum* reinforces the need to consider other

factors in order to explain the invasion success of this seaweed (e.g. Engelen and Santos, 2009, White, 2010).

CHAPTER THREE POPULATION DYNAMICS OF *SARGASSUM MUTICUM* IN WALES

3.1 INTRODUCTION

Biological invasions represent a significant threat to the biodiversity and functioning of coastal marine ecosystems (Carlton, 1989, Grosholz, 2002, Occhipinti-Ambrogi and Savini, 2003). However, despite the increased frequency of nonindigenous marine species introductions, the vast majority of introduced species fail to establish in their new environment (Lodge, 1993, Williamson, 1996). On average, only around 10% of species introductions are successful (Williamson and Fitter, 1996), but those that are, often contribute to significant ecological changes (Vitousek et al., 1997, Grosholz, 2002). Given the potential impacts posed by non-native species, much research has focussed on determining what factors contribute to invasive success (Lodge, 1993, Kolar and Lodge, 2001, Stachowicz et al., 2002). Many established non-native species display *r*-selected life history characteristics including rapid growth, high fecundity and a high tolerance to environmental heterogeneity (Sakai et al., 2001). For example, a rapid growth rate, high fecundity level and early onset of sexual maturity were recognised traits contributing to the invasive success of the Ponto-Caspian gammarid, *Dikerogammarus villosus* (Devin et al., 2004). Several *r*-selected traits have also been identified for the alga *Codium fragile* ssp. *tomentosoides* including a high growth rate and reproductive output and an asexual mode of reproduction (Chapman, 1998). Despite the recognition of similar such characteristics in many other invasive species, not all successful invaders possess such attributes. Furthermore, the traits contributing to an invader's success may shift during the course of the invasion (Engelen and Santos, 2009). In *Sargassum muticum* a recent investigation has shown that the alga's invasive success is more reliant on *K* rather than *r*-selected life history strategies (Engelen and Santos, 2009). However, it is recognised that determinants of invasive success in *S. muticum*, as with numerous other introduced species are often highly context specific (Valentine et al., 2007, Britton-Simmons et al., 2011).

In order to develop an understanding of the invasion process and the potential impact on the invaded ecosystem, it is vital to accumulate information regarding the life

history and population demography of non-native species (Sakai et al., 2001). Without such information, it becomes difficult to generate effective management strategies specifically addressing whether eradication or containment programs are feasible. Investigations into the seasonal dynamics of nonindigenous species may also provide valuable information as to the functional role of the invader within the invaded ecosystem. However, in the early stages of a species introduction it has been suggested that more effort be focused on eradication and control rather than detailed research into the invader's population biology (Simberloff, 2003b). Although, as prevention of introductions is typically regarded as the ultimate goal, a basic knowledge of the population dynamics of non-native species is a prerequisite for predicting the chances of future introductions (Simberloff, 2003a). In the case of *Sargassum muticum*, many established populations in the UK are well beyond the initial stages of their introduction, and attempts to eradicate and/or control the spread of this alga have had limited success (Farnham et al., 1981, Critchley et al., 1986, Davison, 2009). Hence, the focus of the majority of research conducted has been on determining the impact of the alga within its invaded habitat, and the mechanisms contributing to the alga's invasion success (Critchley et al., 1990b, Strong, 2003, Inderjit et al., 2006, Davison, 2009, Engelen and Santos, 2009, White, 2010). Since the impact of habitat forming invasive species such as macroalgae have the potential to cause significant changes to the both the structure and functioning of invaded ecosystems (Crooks, 2002, Schaffelke and Hewitt, 2007), adequate information about an invader's population dynamics and reproductive phenology are of paramount importance for predicting their impacts

The pseudo-perennial life cycle of *Sargassum muticum* provides a mechanism by which the alga can persist during unfavourable conditions over the winter months (Davison, 1999). The seasonal patterns of growth and reproduction therefore take place at the level of the primary laterals that typically develop throughout spring and summer (Fletcher and Fletcher, 1975). Populations of *Sargassum* seaweed species typically show seasonal variations in abundance with close relationships between periods of vegetative and reproductive growth with environment factors (McCourt, 1984). In comparison to tropical regions where the abundance of *Sargassum* species typically peaks during the cooler periods of the year (McCourt, 1984), the abundance of *Sargassum muticum* from temperate regions attains a peak of abundance typically during the warmer months of spring and summer (De Wreede, 1978, Davison, 2009).

The reproductive phenology of *Sargassum muticum* has been investigated from several locations within the alga's introduced range including England (Fletcher and Fletcher, 1975, Critchley, 1981c, Lewey and Farnham, 1981), Canada (De Wreede, 1978, Norton and Deysher, 1989), the USA (Norton, 1977a, Nicholson et al., 1981, Deysher, 1984), Mexico (Aguilar-Rosas and Galindo, 1990), Spain (Arenas et al., 1995, Arenas and Fernandez, 1998), Ireland (Baer and Stengel, 2010) and Northern Ireland (Strong, 2003). Comparative analyses demonstrate that the reproductive period of *S. muticum* varies with geographical location and appears to be heavily dependent on local environmental conditions e.g. water temperature (Deysher, 1984). When populations of *S. muticum* along the Pacific coast of North America were investigated, Norton and Deysher (1989) typically observed an earlier onset and a longer duration of the reproductive period in the more southerly localities. In most introduced regions the alga displays a seasonal pattern of both growth and fertility although exceptions do occur (e.g. see Aguilar-Rosas and Galindo, 1990). Even within a location there may be small scale variations in the onset and duration of the fertile period of *S. muticum* determined by spatial and temporal variability in environmental conditions. For example, DeWreede (1978) noted timing differences in fertility between rock pool plants and those from adjacent intertidal rock, attributed to the elevated water temperature of the rock pool environment.

Considerable differences in plant size have been reported from the various regions where *S. muticum* has been introduced. For example on the pacific coast of North America, plants may reach 7 metres in length (Nicholson et al., 1981). Off the coast of Brittany, France plants up to 10 to 12 metres have been collected (Critchley et al., 1990b), whereas in British waters the average size is between 2 to 4 metres (Jephson and Gray, 1977, Critchley et al., 1990b). Variations in the local and regional environmental conditions are the most likely explanations for these observed size differences in S. muticum. For example, growth rate, and presumably overall size has been shown to increase with temperature over the range 3°C to 30°C (Norton, 1977, Hales and Fletcher, 1989). Variations in salinity may also be influential in determining size as reductions in growth have been demonstrated during exposure to salinities below 25‰ (Hales and Fletcher, 1989, Steen, 2004). Population stand density effects may be another contributing factor affecting size distributions as Arenas et al. (2002) reported that at high densities, *S. muticum* thalli became longer, thinner and less branched, which

was considered a mechanism to reduce overcrowding within the monospecific canopy. Nutrient availability is another important factor regulating growth of macroalgae (Norton et al., 1981) and enhancement of growth in *S. muticum* has been linked to the occurrence of local nutrient enrichment within areas (Sanchez and Fernandez, 2006). The effects of water flow and tidal currents are also likely influential factors in determining overall size of *S. muticum* plants. For example, several reports of larger sized macroalgal specimens have been documented from areas subjected to strong tidal currents attributable to the enhancement of nutrient delivery and gaseous exchange (Norton, 1969, Reynolds, 1971, Norton et al., 1981).

In comparison to UK native marine algae, *S. muticum* typically exhibits considerably higher rates of growth. For example, under favourable conditions Hales and Fletcher (1989) recorded maximum growth rates of 0.2-0.36 mm per day for *S. muticum*, whereas growth rates of native algae *Ascophyllum nodosum*, *Halidrys siliquosa* and *Fucus vesiculosus* were recorded at 0.02 mm, 0.03 mm and 0.035 mm per day respectively. Variability in the rate of growth of *S. muticum* has been observed from different introduced locations and appears closely associated with water temperature (Davison, 2009).

3.1.1 Current status of *S. muticum* in Wales

On the Welsh coastline, attached populations of *S. muticum* have been recorded since 1998 (see Table 1.1 and Fig. 1.2) although recorded drifting since 1983 (Eno et al., 1997, Davison, 2009). Prior to a study by Grant in 2007, no organised investigations of *S. muticum* within Wales had been carried out and knowledge of the alga's distribution was merely based on occasional observations and anecdotal reports from the public. This lack of baseline data regarding the alga's ecology within areas of the Welsh coastline seems surprising given the extent of coastline included in designations such as (Special Areas of Conservation) SACs and Sites of Special Scientific Interest (SSSIs) (plus one of only three statutory Marine Nature Reserves in UK waters). *Sargassum muticum* is now an established member of the algal community in Welsh coastal waters (Grant, 2007). However, despite this very little is known regarding basic aspects of the alga's biology such as seasonality of growth and reproduction within population stands of the alga along the coast of Wales.

The aim of this chapter was to investigate the population demography of *S. muticum* within Wales by looking at seasonal variations in plant size structure, reproductive status and growth rates. A further objective was to compare these factors over the range of different habitats colonised by populations of the alga. The result of the investigation form part of a general assessment into the potential ecological impacts of the alga in Welsh waters by providing baseline information on the seasonal dynamics of this invasive seaweed.

3.2 MATERIALS AND METHODS

3.2.1 Study Sites

Over the course of a single growth and reproductive season during 2010, *S. muticum* plants were monitored from 4 well established populations distributed along the Welsh coastline (Fig. 3.1). Selected field sites were chosen to represent the range of different habitats that the alga has colonised within Wales.

3.2.1.1 Rhosneigr

Rhosneigr is located on the west coast of Anglesey, with a south-westerly facing aspect (SH 314 762, Fig. 3.2). The substrate at this site consists predominantly of pebbles and stones of a range of sizes resting on or partially buried within sandy sediment (Fig. 3.7A). The intertidal area of Rhosneigr consists of a series of rock protected lagoons and numerous rockpools. The site has variable exposure levels, with the outer rocky outcrops exposed to moderate wave action but with the internal lagoonal areas somewhat sheltered from wave exposure. The reefs at Rhosneigr have been classified as a SSSI due to the high diversity of littoral and sublittoral algae occurring within the area, including less common and rare species such as the red alga, *Laurencia obtusa* (Hudson) Lamoroux (CCW, 1992). Despite this conservation status, *S. muticum* which was first recorded in 2001 (see Table 1.1) has now become well established at the site both within many of the intertidal rockpools and within several of the rock protected lagoonal areas. Plants of *S. muticum* monitored during the investigation formed part of an expansive bed located within a semi-enclosed lagoon at the site. The grid reference of centre of the *S. muticum* stand was SH 3137 7272 (Fig. 3.2A).

3.2.1.2 Tal y Foel

Tal y Foel is located in a sheltered area towards the southwest end of the Menai Strait (SH 474 645, Fig. 3.3). The sediment is predominantly of a sandy substrate with pebbles of various sizes and shell fragments sporadically spread and often buried beneath the sand (Fig. 3.7B). Throughout much of the tidal cycle this site typically experiences a strong current flow. *Sargassum muticum* has been present at this site since 2001 (see Table 1.1 and Fig. 1.1B) and it is likely that the population has provided source material for the colonisation of other areas of the Menai Strait. The alga typically forms a continuous belt along the low intertidal fringe with plants attached to pebbles and shells of a variety of sizes. The individuals in this population can be largely regarded as peripatetic individuals as the *Sargassum* belt often shifts throughout the daily tidal cycle. The surface temperature of the Menai Strait generally varies seasonally between 4ºC and 17ºC and the salinity of the Menai Strait is generally between 32 and 34‰. The grid reference of centre of the *Sargassum* belt was SH 4749 6439 (Fig. 3.3).

3.2.1.3 Cei Bach

Cei Bach is located just west of New Quay in a sandy bay of Cardigan Bay (Fig. 3.4). The main stand of *S. muticum* is located in a large tidal pool located north of the breakwater (Fig. 3.4). The site is of marine conservation importance due to the occurrence of *Sabellaria alveolata* reefs (a UK Biodiversity Action Plan species) within the area (Brazier et al., 2007). This species is recognised as one of the most ecological important biogenic reef forming species in the UK with the *Sabellaria* biotope considered to be of national importance by the Joint Nature Conservation Committee (JNCC) (Maddock, 2008). However, the alga has not been reported as growing on the *S. alveolata* reef structures (P Hallas, personal observation). The substrate at this site is similar to that found at Rhosneigr consisting predominantly of pebbles and stones of a range of sizes resting on or partially buried within muddy sediment (Fig. 3.7C).

3.2.1.4 Broad Haven

Broad Haven is located within St Brides bay off the Pembrokeshire coast with a westerly facing aspect (SM 859 142, Fig. 3.1). At the site there is a large gently shelving sandy beach with an expanse of rocky intertidal area dominated by rockpools at the northern end (Fig. 3.5). Plants of *S. muticum* occur solely within the rockpools at

this site and have colonised numerous pools at various tidal heights from high to low shore levels. *S. muticum* has been present within this area since 1999 when it was recorded from a few pools (see Table 1.1) but has since spread and now occupies up to 20 pools (P Hallas, personal observation). For the purpose of this investigation plants from three rockpools at various shore levels were monitored from during 2010 (Fig. 3.5B). Pool 1 was located on the high shore (Grid ref: SM 86014 14168); Pool 2 was located within the mid-intertidal (Grid ref: SM 85998 14194) and Pool 3 was situtated in the low intertidal (Grid ref: SM 85959 14276). Fig. 3.6 shows photographs of the three rockpools monitored during the study. Plants within the rockpools are typically attached directly to the bedrock of the pool, with the base of the holdfast often covered with a small layer of sandy sediment, up to 2 cm depth (Fig. 3.7D).

3.2.2 Size and reproductive phenology

The length, from the base of the holdfast to the tip of the longest primary lateral of each plant was measured with a tape measure to an accuracy of 10 mm. The number of primary laterals arising from the holdfast was noted for each *S. muticum* plant measured. Reproductive phenology was also monitored during the survey periods at each site. Each plant was examined for the presence of receptacles and fertility was estimated as the percentage of plants bearing receptacles. At the open coast sites between 50 – 100 randomly selected individuals were typically measured on each sampling date. Each site was visited monthly during spring tides during 2010. Plants from all locations were accessed from the shore with surveys undertaken during the one hour period before and after low water.

At the locations of Rhosneigr and Cei Bach where a the population of *S. muticum* occurred in large stands, measured plants were randomly selected using a 10-point clock face and random number table to obtain an approximate direction and distance of travel from within the centre of the algal stand. At Tal y Foel, where the population of *S. muticum* occurred in a narrow belt parallel to the shoreline, measured plants were selected by moving along the belt from one end of algal stand using a random number table to determine distance travelled along the belt. Upon reaching the end of the algal stand the same random sampling process was repeated travelling back along the algal stand. Plants from within the three surveyed rockpools at Broad Haven

were haphazardly chosen for measurement on each sampling period, with 30-40 plants per pool measured. The application of these methods meant that different plants were measured on each sampling survey.

At each survey site a measure of the water temperature was also recorded. Temperatures were recorded using a digital thermometer (ThermoWorks) to the nearest $0.1^{o}C$. General observations of the local weather conditions were also noted.

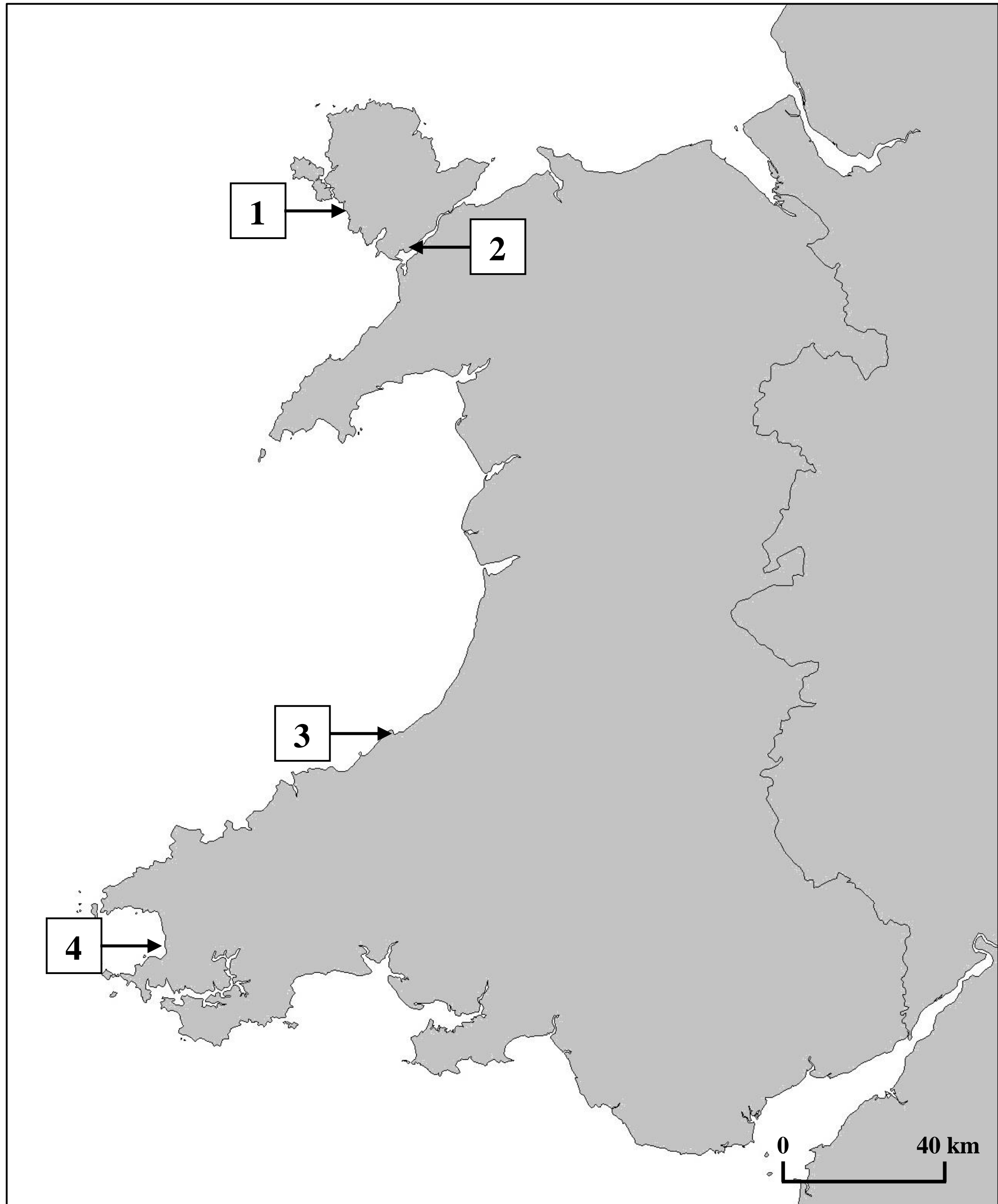


Fig. 3.1 Map of Wales showing the location of the four sites where *S. muticum* was monitored during 2010. 1) Rhosneigr, Anglesey; 2) Tal y Foel, Anglesey; 3) Cei Bach, Cardigan Bay and 4) Broad Haven, Pembrokeshire.

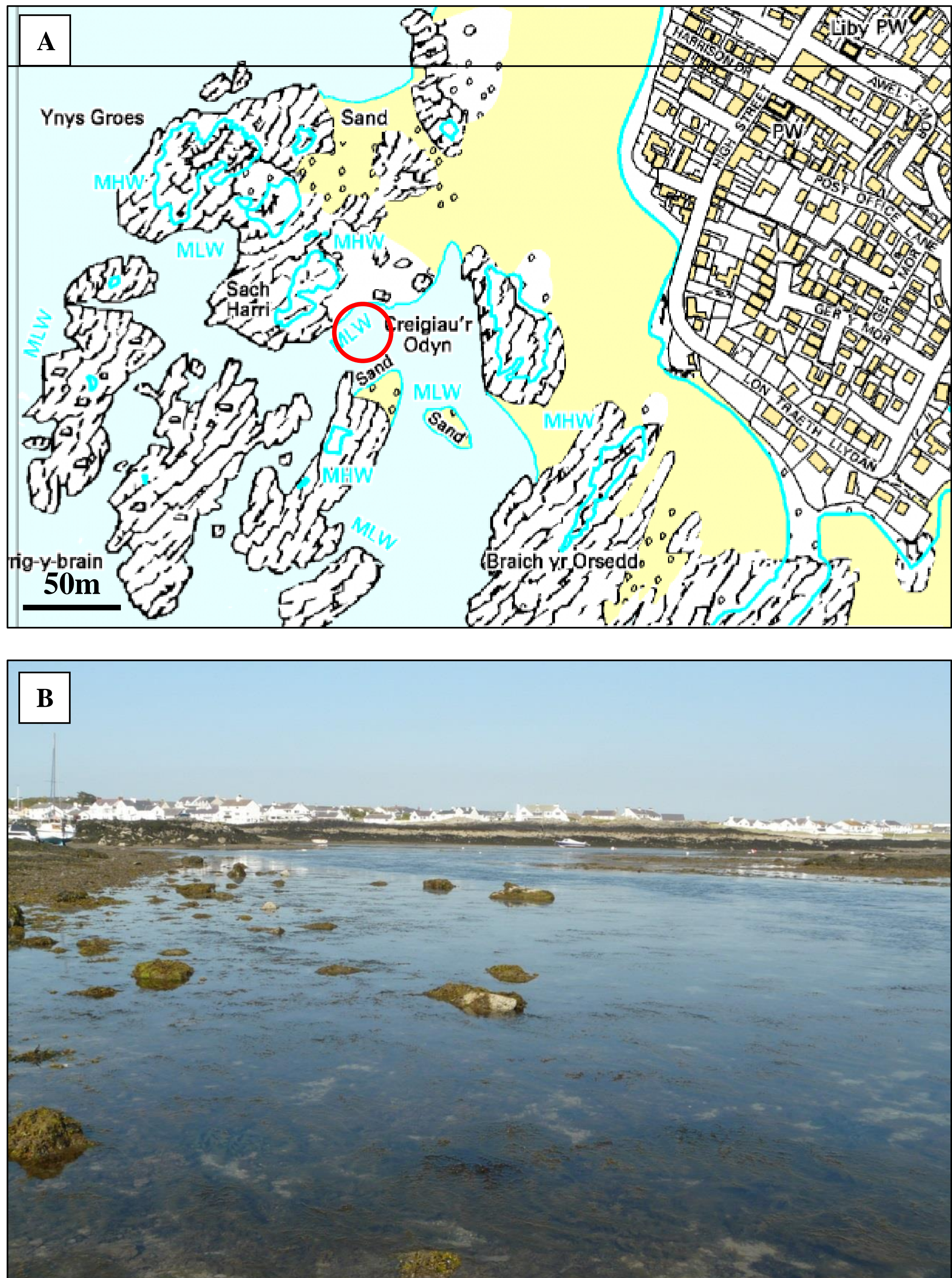


Fig. 3.2 Study site at Rhosneigr, Anglesey. (A) 1:10,000 map showing the area with red dot indicating the location of the survey area (Grid ref: SH 31370 72720) and (B) a photograph of the area looking east within the survey site.

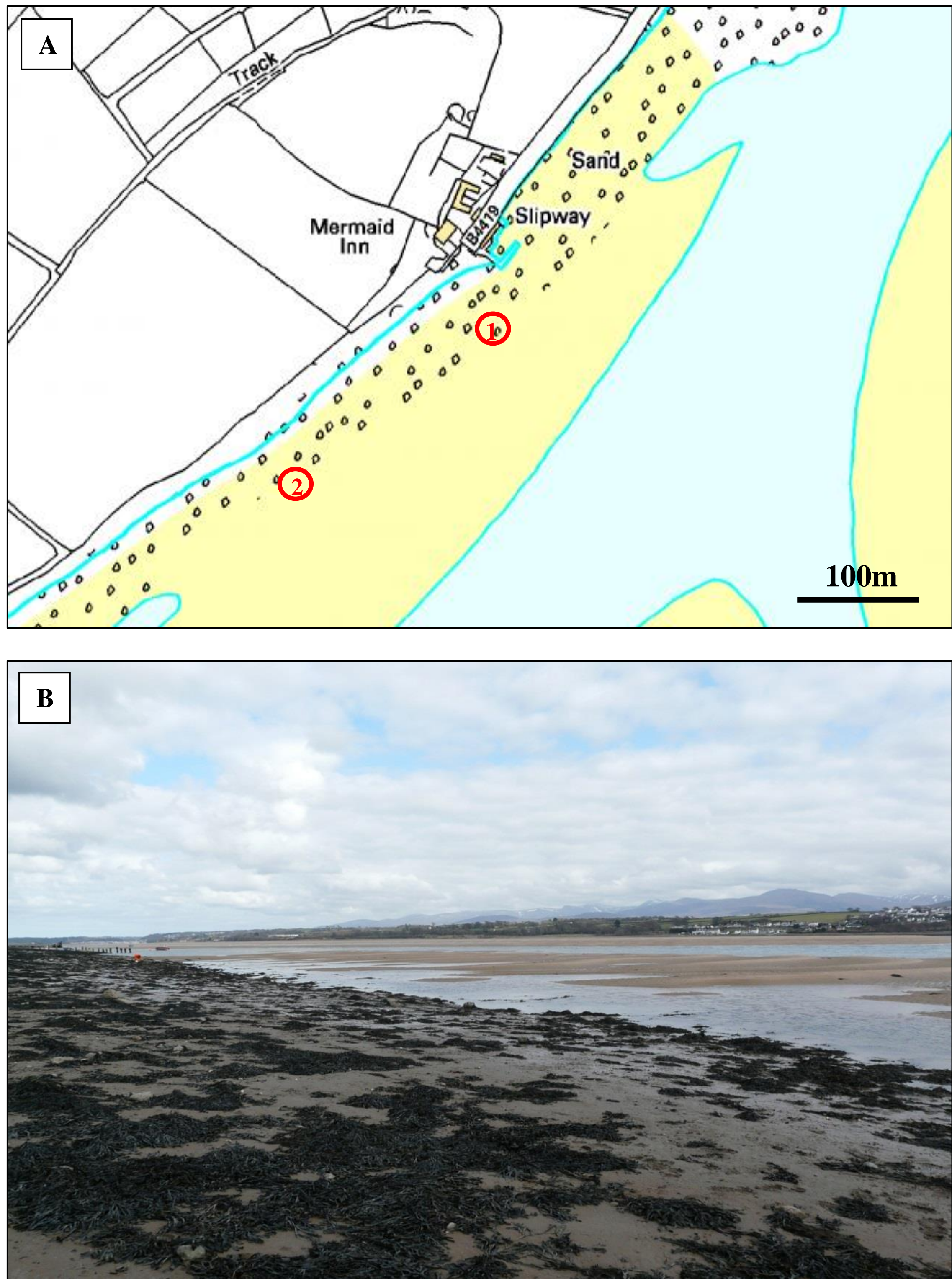


Fig. 3.3 Study site at Tal y Foel, Anglesey. (A) 1:10,000 map showing the area with red dots indicating the location of the two ends of the survey area (Grid refs: 1) SH 47470 64460 and 2) SH 47310 64330) and (B) a photograph of the area looking northeast towards the slipway at the Mermaid Inn.

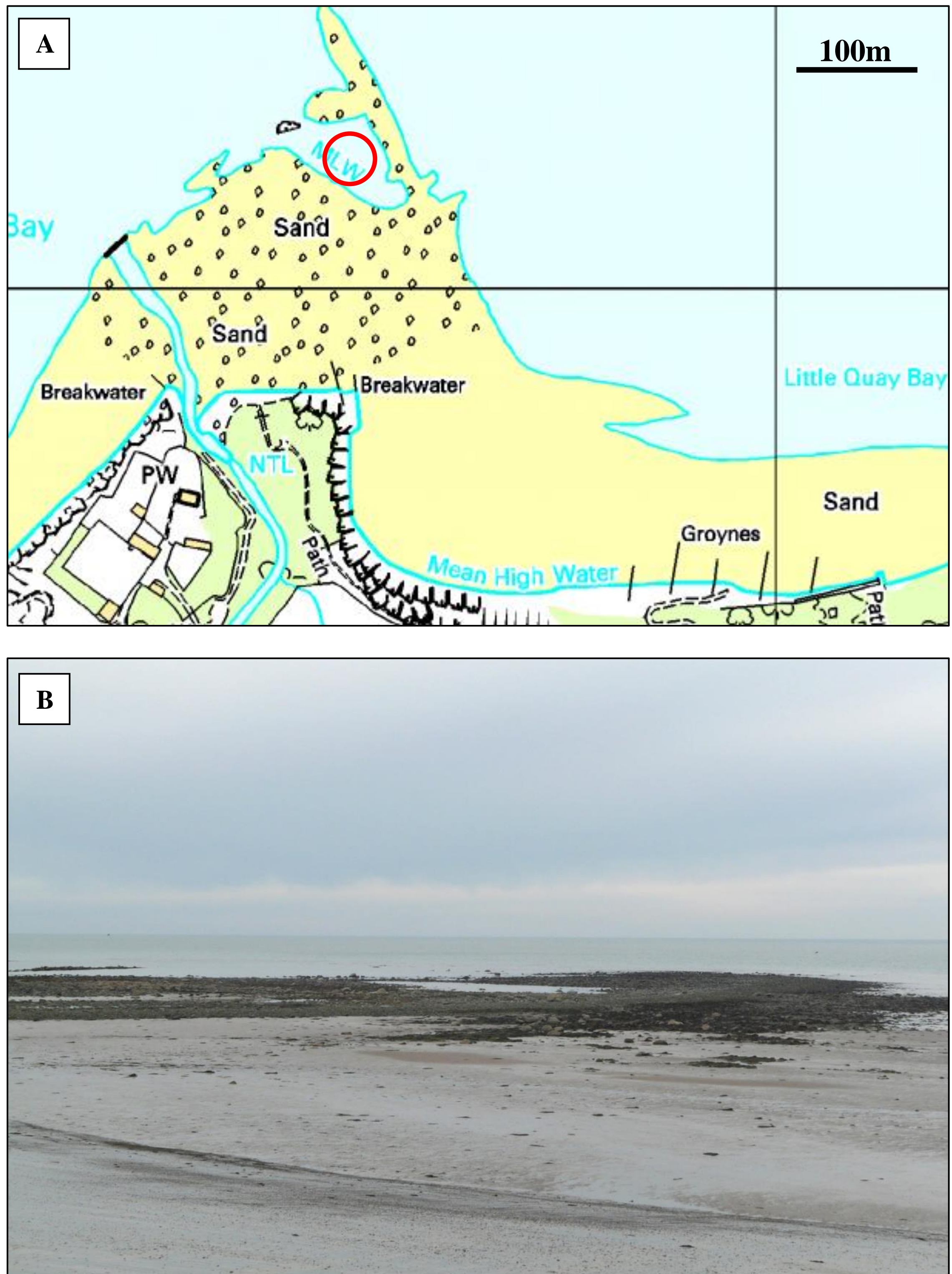


Fig. 3.4 Study site at Cei Bach, Cardigan Bay. (A) 1:10,000 map showing the site with red dot indicating the location of the survey area (Grid ref: SN 40640 60110) and (B) a photograph of the area looking northwest taken from the coastal path near the groynes.

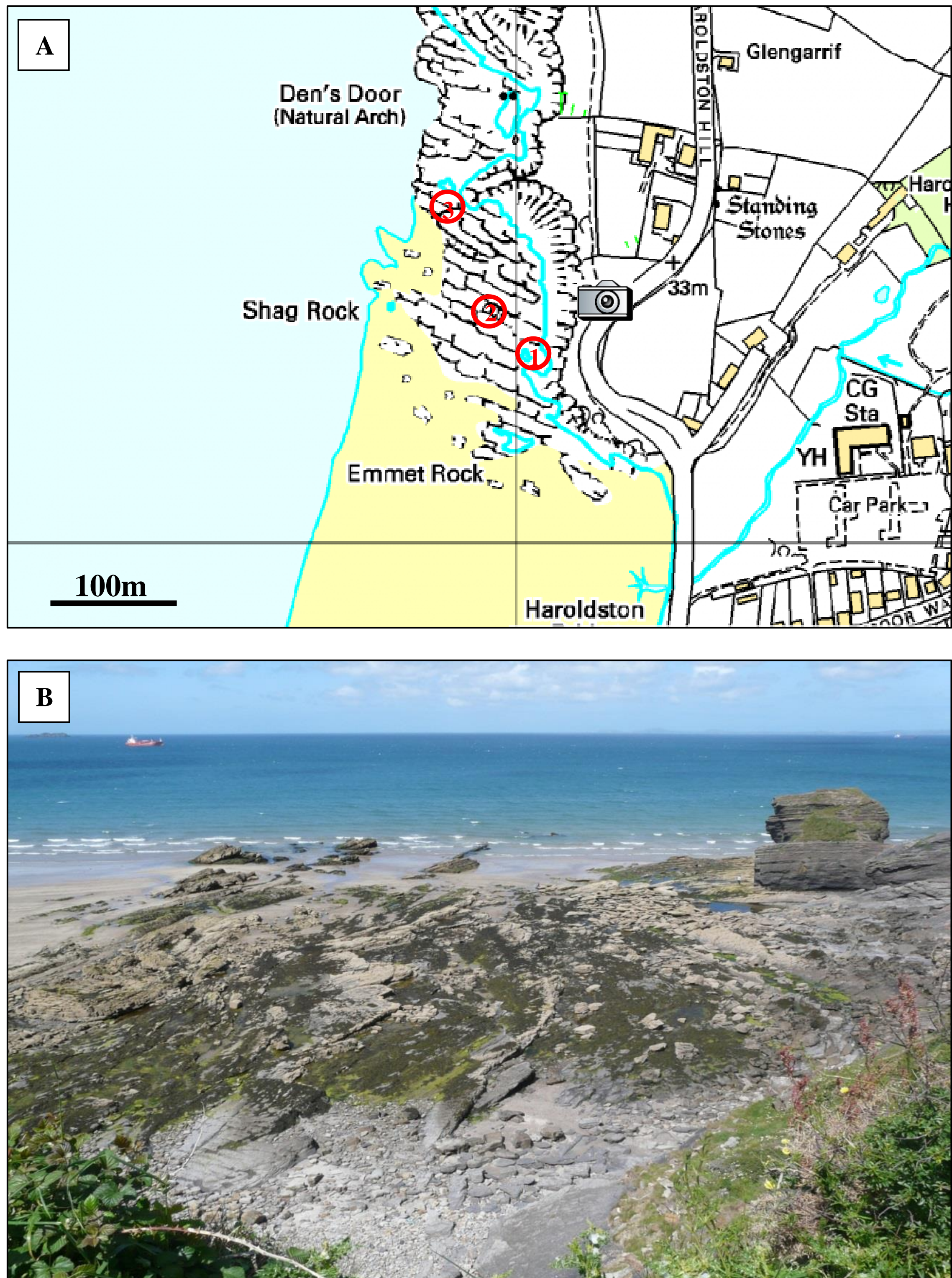
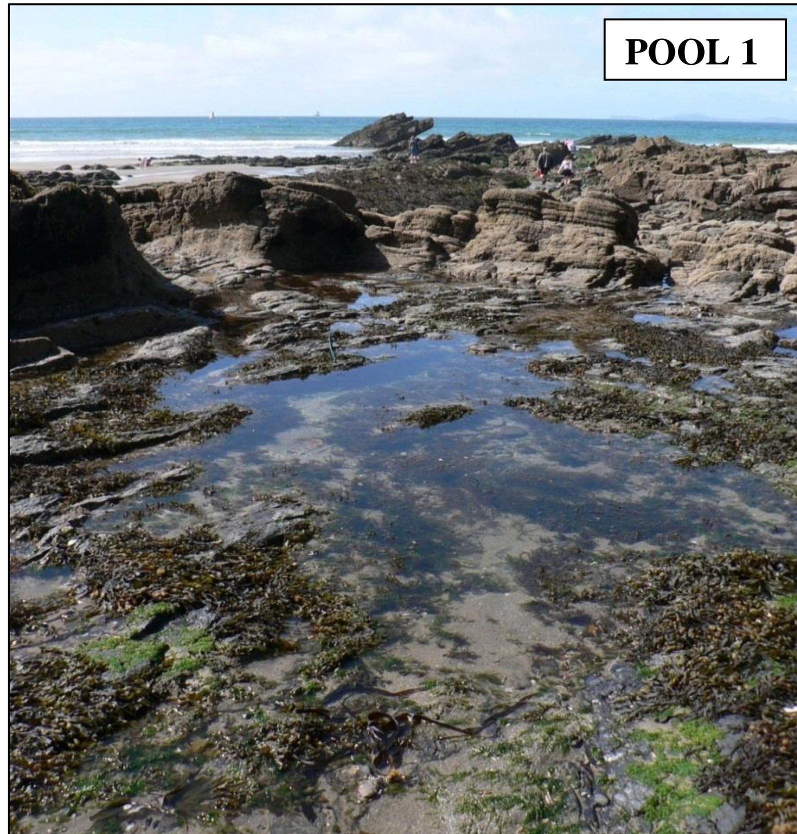


Fig. 3.5 Study site at Broad Haven, Pembrokeshire. (A) 1:10,000 map showing the area with red dots indicating the location of the surveyed rock pools (Grid refs: Pool 1 - SM 86014 14168, Pool 2 - SM 85998 14194 and Pool 3 - SM 85959 14276) plus the position from where subsequent site photo was taken and (B) a photograph of the area looking down from coastal path.



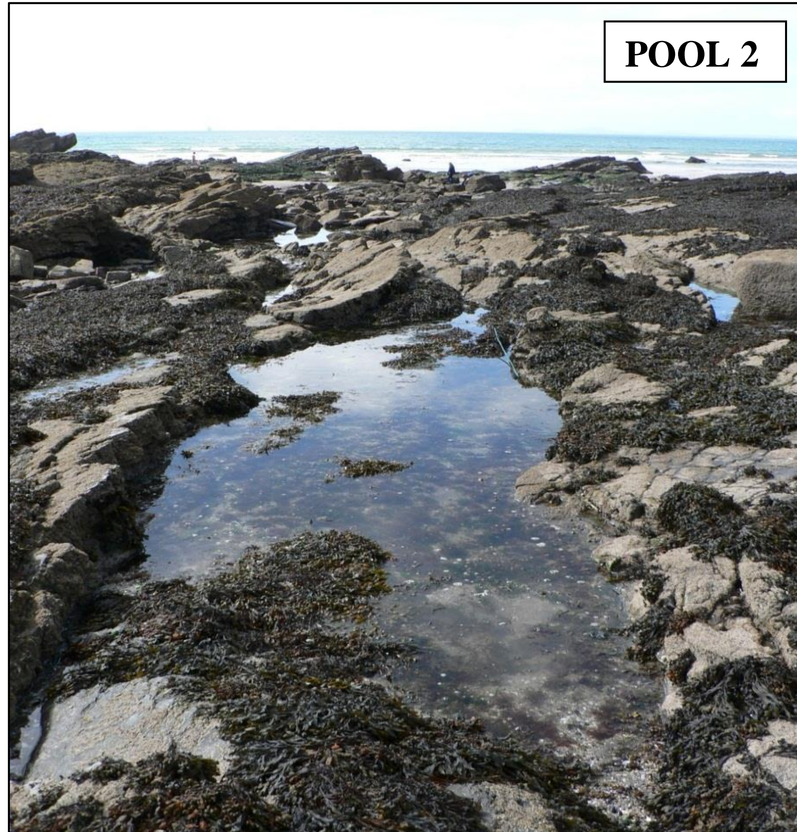


Fig. 3.6 Photos of the three surveyed rockpools at Broad Haven. Pool 1 – high shore looking seaward. Pool 2 – mid shore looking seaward.

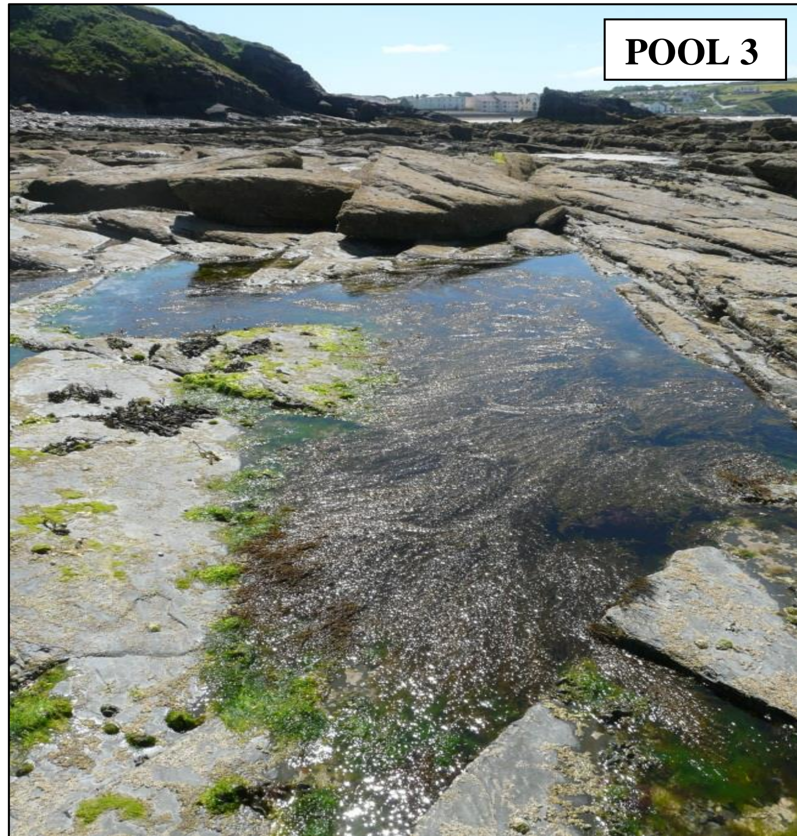


Fig. 3.6 continued Pool 3 – low shore looking south back along shore.

Fig. 3.7 Substratum on which *S. muticum* was found at the four different sites surveyed during the study. (A) loose pebbles and cobbles on sandy substrate at Rhosneigr and (B) sandy substratum at Tal y Foel, to which plants were attached to pebbles typically buried within the sandy substrate at 20-30cm depth (P Hallas, personal observation).

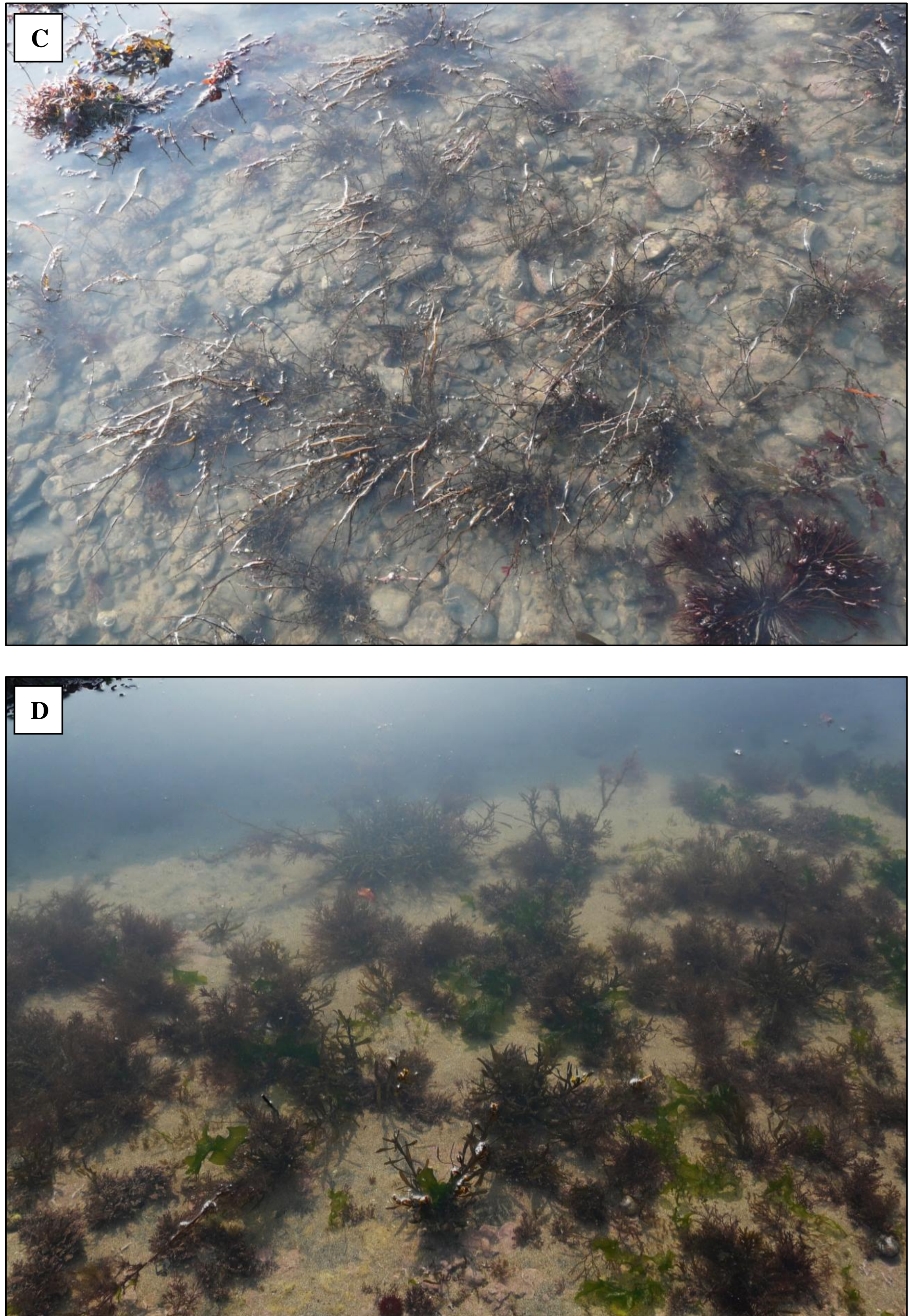


Fig. 3.7 continued Substratum on which *S. muticum* was found at the four different sites surveyed during the study. (C) loose pebbles and cobbles on muddy substrate at Cei Bach and (D) plants attached to bedrock in rockpools at Broad Haven with frequent covering of sandy sediment (2cm depth) overlaying the holdfasts.

3.2.3 Growth studies

In order to measure plant vegetative growth rates over a single season a random selection of *S. muticum* thalli encompassing the size range of plants from each site were tagged. Tags consisted of a standard white plastic plant pot tag (~10 cm) with a hole at one end through which a short cable tie was passed and then fastened around the base of the longest primary lateral, just above the holdfast. Fig. 3.8 shows a photo of how the plants were tagged in the field. Each tag was given a site specific identification number which was recorded along with the length of the primary lateral. The lateral was measured from the base of the holdfast to the tip of the lateral using a standard measuring tape to an accuracy of 10 mm. A note of each plant's geographic coordinates within the site was recorded using a GARMIN etrex handheld GPS device (with an accuracy of 6 metres) to facilitate its location the following month. The relatively large size and conspicuous colour of the tags was intended to ensure ease of relocation. At the start of the experiment 25 individuals from each site were tagged and measured. Any tags that were lost or could not be relocated were replaced at every census survey date in order to maintain suitable replicate numbers. The elongation rate of the alga was calculated as follows:

$$E.R = \frac{L2 - L1}{T2 - T1}$$

Where L1 and L2 are the lengths of the primary lateral at the beginning and end of the period respectively, and T1 and T2 indicate time (in days) at which the lengths were determined.

3.2.4 Statistical Analyses

The monthly collected data for length, laterals and percentage fertility were presented on graphs. Differences in the lengths of the dominant primary lateral and the number of laterals from the four study sites were analysed using a combination of both one and two-way analyses of variance (ANOVA) with site and month as fixed factors. Due to the absence of data from particular months at certain sites only data from the period of February to October were included. The assumptions of normality and homogeneity of variance, typical of parametric tests were tested *a priori* with the

Kolmogorov-Smirnov and the Levene's test respectively. When one or both of these parameters were not met various transformations of the data set were assessed and the normality and variance tests repeated for confirmation. When the ANOVAs indicated significant differences among means, *post hoc* Tukey tests (when variances were homogenous) or Games-Howell tests (when variance were unequal) were carried out in order to determine which means contributed to the effect. In cases where transformation of the data did not produce normally distributed data sets, non-parametric Kruskal-Wallis or Scheirer-Ray-Hare tests followed by Mann-Whitney tests were carried out. Where appropriate a Bonferroni correction for multiple testing was used. Unless otherwise stated significance was set at $P < 0.05$. All statistical analyses were undertaken using a combination of SPSS (SPSS for Windows, Version 18.0) and Microsoft Excel.

Fig. 3.8 Photo showing tagged *S. muticum* plant from rockpool at Broad Haven.

3.3 RESULTS

3.3.1 Seasonal variability in plant length and number of laterals

The seasonal patterns for mean length of thalli were similar among all four survey locations, with a growth period beginning at the start of the year during winter, and continuing throughout the spring months, followed by a decrease into the summer/early autumn months (Fig. 3.9). Site specific differences in growth were also noted with the growth period at Tal y Foel extending for an additional month in comparison to that observed at Rhosneigr (Fig. 3.9A). The largest mean sizes were observed in the month of June from both Rhosneigr and the rockpools at Broad Haven. In contrast, largest mean plant sizes at Tal y Foel and Cei Bach were observed one month later in July (Fig. 3.9A). Timing of the onset of plant senescence was earlier at Rhosneigr than the other two sites located on the open coast. This earlier onset of plant size decrease was also seen in plants from the rockpools at Broad Haven (Fig. 3.9B). The early onset of senescence at Rhosneigr was reflected by the lower number of fertile plants and the earlier occurrence of maximum fertility (Fig. 3.11A). Plant size comparisons between the three open coast sites throughout the year indicated significant differences with regards to both site (Scheirer-Ray-Hare test, df = 2, SS = 110140904, H = 207.67, $P < 0.001$) and survey month (Scheirer-Ray-Hare test, df = 9, SS = 248843345, H = 110.12, $P < 0.001$) plus a significant interaction between the two factors (Scheirer-Ray-Hare test, df = 18, SS = 120326598, H = 31.24, $P < 0.001$). Comparisons of plant lengths (natural log transformed) from open coast sites during July when plant size were at a maximum (except at Rhosneigr) indicated significant differences between sites (One-way ANOVA, $F_{2,247}$ = 24.753, $P < 0.001$) Subsequent *post hoc* Tukey testing indicated the average plant length at Tal y Foel was significantly different ($P < 0.001$) than that recorded from the other two locations. Overall the largest individuals were observed at Tal y Foel with some recorded plants measuring up to 6 metres (Table 3.1).

Table 3.1 Summary statistics of *S. muticum* plant length (cm) recorded from all study sites throughout the study period. Overall total represents length statistics when data from all three rockpools at Broad Haven were combined.

Site	Average	SD	SE	Max	Min	n
Tal y Foel	87.6	75.1	2.56	602	5	860
Rhosneigr	49.5	40.4	1.46	229	3	765
Cei Bach	43.3	32.8	1.24	198	4	697
Broad Haven						
Pool 1	30.8	15.8	0.90	83	4	304
Pool 2	35.2	16.6	0.91	106	3	336
Pool 3	43.7	20.4	1.08	111	4	361
Overall Total	37.0	18.6	0.59	111	3	1001

Within site differences in plant size were also observed between *S. muticum* individuals from the three different rockpools at Broad Haven (Fig. 3.9B). Plants from the low shore pool (Pool 3) were consistently larger in size than those from the rockpools located higher up the shore (Table 3.1). Plant size comparisons (using square-root transformed data) between the three rockpools over the growth season indicated significant differences with respect to both pool ($F_{2,971} = 53.415$, $P < 0.001$) and census month ($F_{9,971} = 68.489$, $P < 0.001$) but no interaction between the two factors ($F_{18,971} = 1.586$, $P = 0.057$). *Post hoc* Tukey analyses showed that all comparisons between all rockpools were significant ($P < 0.001$). Comparisons between rockpools when plant size was at a maximum, during June (Fig. 3.9B) showed significant differences according to results of one-way ANOVA ($F_{2,118} = 9.468$, $P < 0.001$) performed on untransformed data. A subsequent *post hoc* Tukey test showed that the main differences occurred within the low shore pool (Pool 3) whose plant lengths were significantly larger than those from the other two rockpools ($P < 0.001$).

In comparison to plant size changes during the study, trends in the seasonal changes in number of laterals per plant were less well-defined (Fig. 3.10). The open coast sites showed particular contrasting patterns regarding changes in the number of laterals per plant (Fig. 3.10A). Plants at Tal y Foel showed an increase in lateral

development into the summer months; at Rhosneigr following fluctuations during the spring months, a decrease was observed at the start of summer coinciding with the observation of an earlier onset of senescence; in contrast, the number of laterals recorded from plants at Cei Bach showed a more constant level over most of the year, with a final drop from September onwards again due to the onset of plant senescence. A Scheirer-Ray-Hare test showed a significant effect of both site (df = 2, SS = 18867912, $H = 19.24$, $P < 0.001$) and month (df = 9, SS = 55006970, $H = 18.27$, $P < 0.001$) with regards to plant lateral number from the three open coast sites. Moreover, a significant interaction was also noticed between site and month of survey (df = 18, SS = 53199796, $H = 8.89$, $P < 0.001$). Further pairwise Mann-Whitney tests between each site revealed significant differences between all locations ($P < 0.01$). At the Broad Haven rockpools, an overall decrease in the number of laterals per plant was observed during the survey period, with the exception of the mid-shore pool (Pool 2) where a final increase in lateral number was recorded during October (Fig. 3.10B). When a Scheirer-Ray-Hare test was performed on the rockpool data set the results showed no effect of pool location on the number of laterals present on plants (df = 2, SS = 148061, $H = 1.18$, $P = 0.398$). However, significant effects of both survey period (df = 2, SS = 12246882, $H = 20.08$, $P < 0.001$) and an interaction between this factor and pool location were observed (df = 18, SS = 2849281, $H = 2.36$, $P < 0.01$).

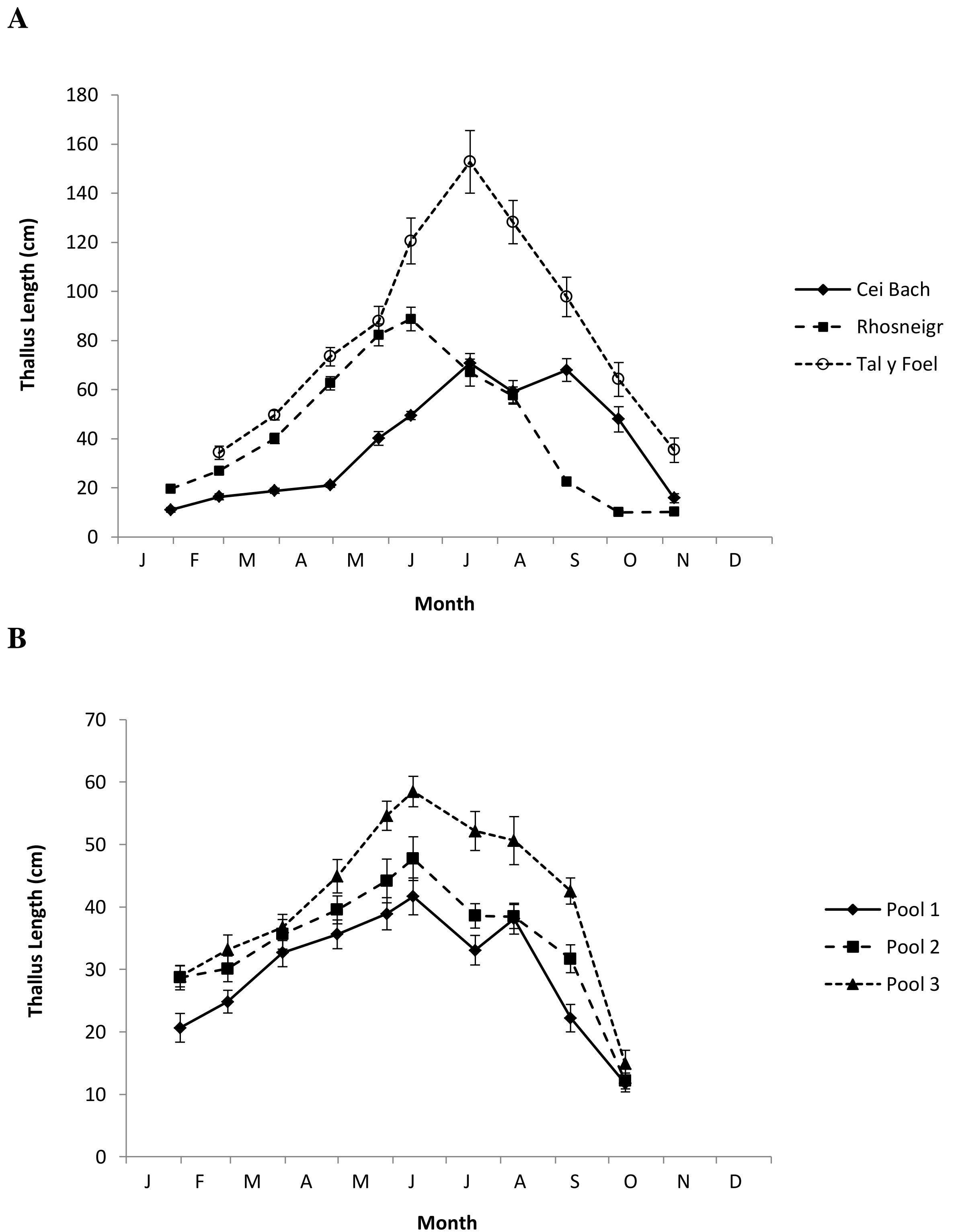
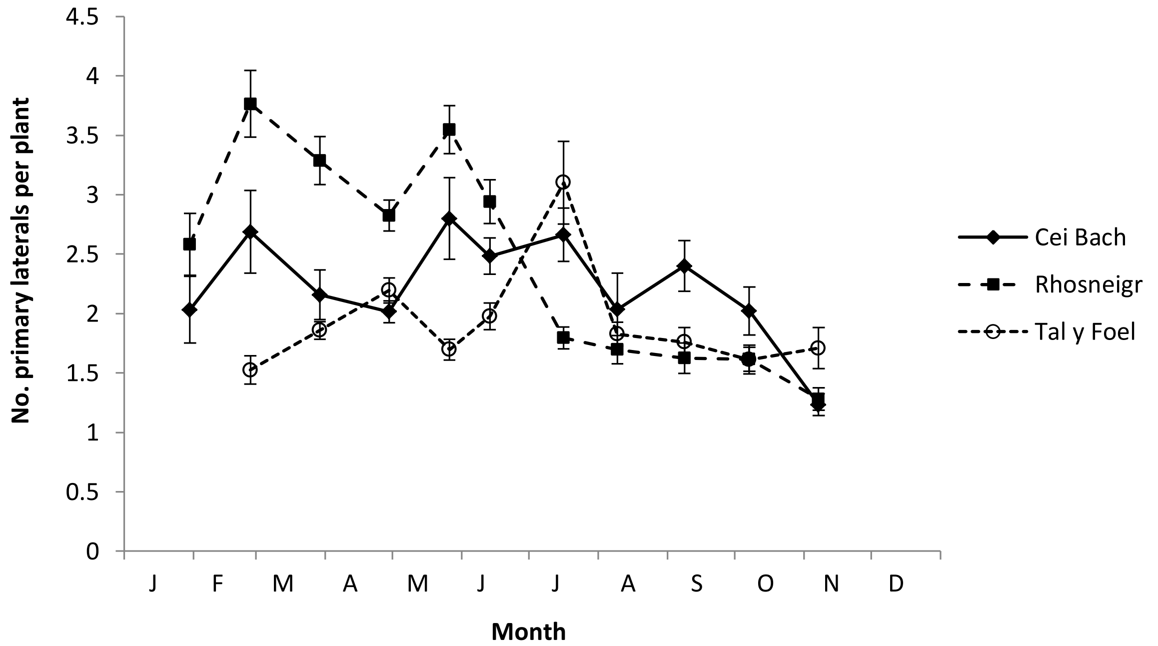


Fig. 3.9 Seasonal development in length of the dominant primary lateral of *S. muticum* plants from (A) open coast sites of Rhosneigr, Tal y Foel and Cei Bach, and (B) rockpools from different tidal levels at Broad Haven. Data presented are means ± S.E.

A



B

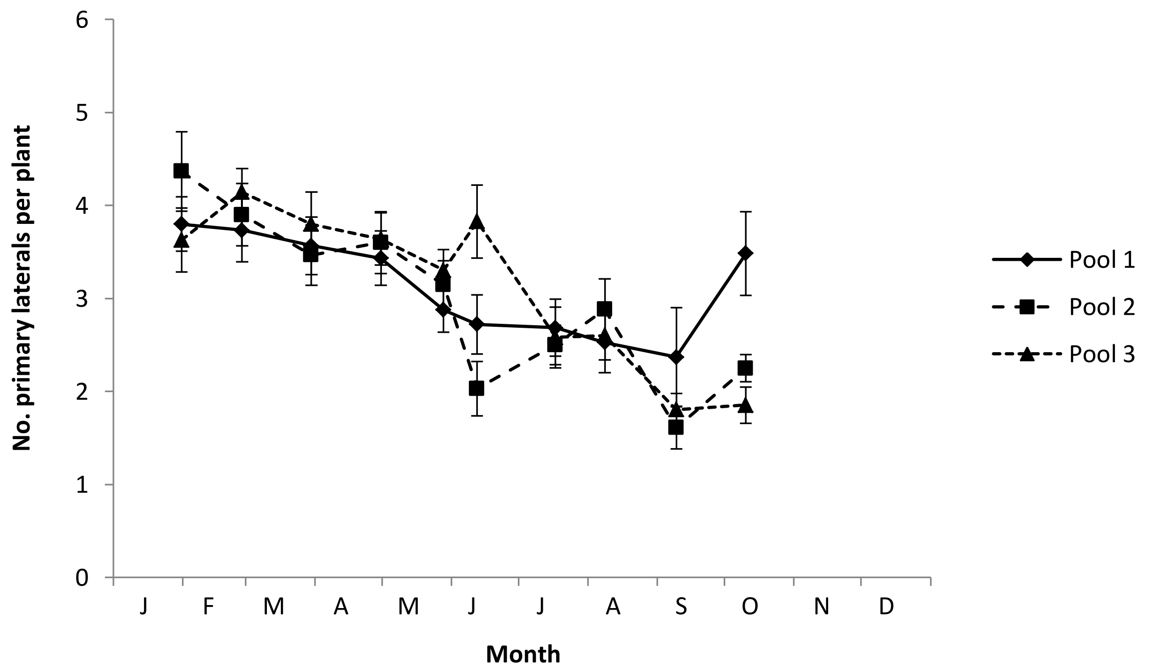


Fig. 3.10 Seasonal changes in the number of primary laterals recorded from *S. muticum* plants from (A) open coast sites of Rhosneigr, Tal y Foel and Cei Bach, and (B) rockpools from different tidal levels at Broad Haven. Data presented are means ± S.E.

3.3.2 Reproductive phenology

Due to the nature of how the reproductive phenology data were collected, no formal statistical analyses could be undertaken, therefore the results are based purely on descriptive comparisons between the sites. With regards to the open coast sites, the two sites located on Anglesey (Rhosneigr and Tal y Foel) began to show signs of fertility from April whereas the reproductive period of plants from Cei Bach in Cardigan Bay initiated two months later in June (Fig. 3.11). Site differences in the timing of maximum fertility were also noted between the two locations on Anglesey, with levels at Rhosneigr reaching a peak in June, compared with maximum fertility at Tal y Foel recorded during July/August (Fig. 3.11A and B). The later onset of the reproductive period in plants from Cei Bach coincided with peak of maximum maturity observed later in the year during September (Fig. 3.11C) in comparison to the other open coast sites.

In all three rockpools at Broad Haven, maximum percentage of fertility was recorded during August (Fig. 3.12) with a similar trend in the seasonal onset of maturity. However, plants from the low shore pool (Pool 3) typically showed a faster rate of reproductive development with higher fertility levels maintained over a longer duration. Furthermore plants from Pool 3 consistently displayed the highest proportion of fertile plants at every survey census period (Fig. 3.12).

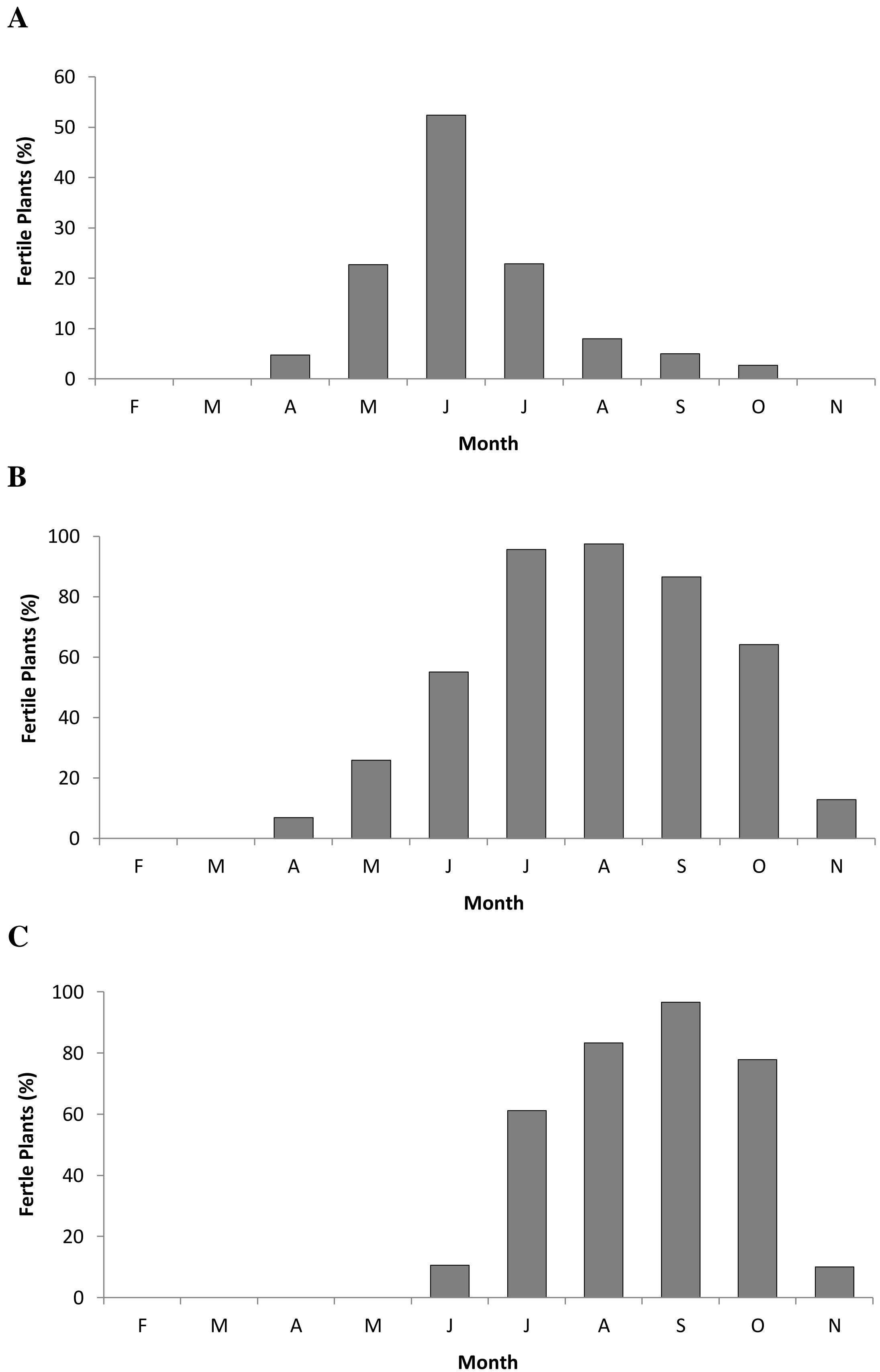


Fig. 3.11 Seasonal variation in presence of receptacles from *S. muticum* plants from the open coast sites at (A) Rhosneigr, (B) Tal y Foel and (C) Cei Bach.

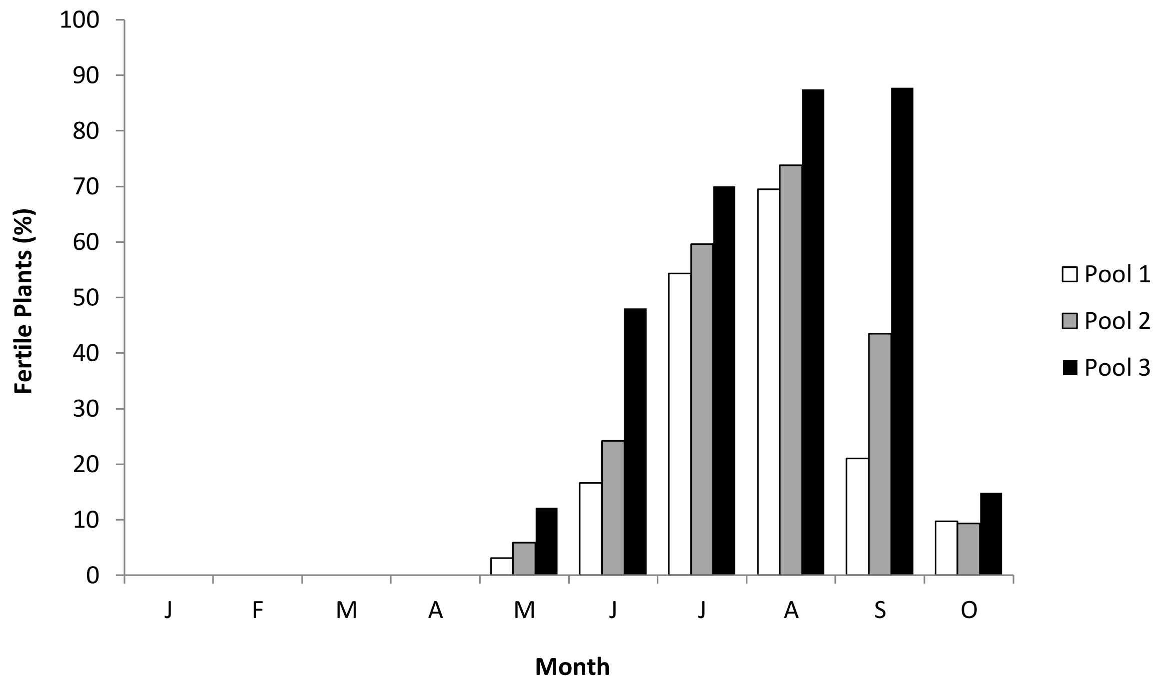


Fig. 3.12 Seasonal variation in presence of receptacles from *S. muticum* plants from the three rockpools at Broad Haven.

3.3.3 Temperature

The seasonal pattern of seawater temperatures from all four habitats surveyed showed similar trends with maximal temperatures recorded during summer months as would be expected from temperate waters (Fig. 3.13). With regards to the open coast sites, summer seawater temperatures were appreciably cooler (up to 5°C) than the other two sites, which may explain the reduced level of fertility seen in the *S. muticum* population at this site (Fig. 3.11).

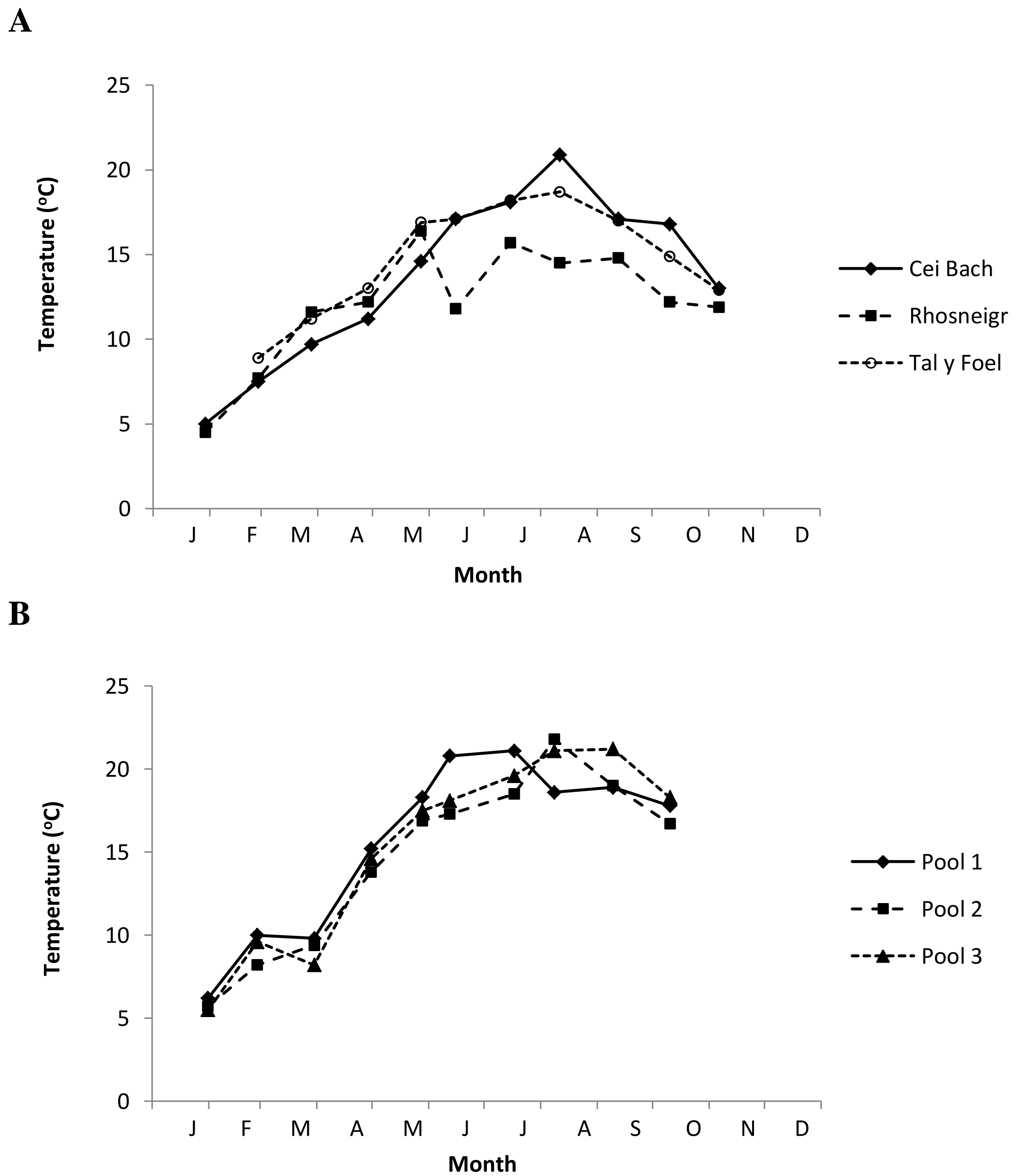


Fig. 3.13 Seasonal changes in seawater temperature (°C) recorded from (A) the three open coast sites (Rhosneigr, Tal y Foel and Cei Bach) and (B) the three rockpools surveyed at Broad Haven.

3.3.4 Growth rates

Due to the nature of the substratum at three of the four selected monitoring sites, a full set of growth rate measurements were only obtained from tagged plants at Broad Haven (Fig. 3.14). At Tal y Foel the shifting nature of the sandbank in which the *S. muticum* holdfasts were typically buried meant that relocation of the tags was largely unsuccessful with the exception of one growth rate period between April and May when nine tagged individuals were retrieved. Tag losses at Rhosneigr were also high, again attributable to the instability of the attachment substrate although estimates were obtained over four growth periods. At Cei Bach where plants were typically attached to pebbles and cobbles of a range of sizes (Fig. 3.7C) no tags could be relocated on each of the replicate surveys despite extensive searches. As a result of the difficulties in ensuring the longevity of the tagged plants an additional estimate of growth rates from the three open coast sites was produced based upon the average length data of the dominant primary lateral recorded each month.

The mean growth rate of plants from the rockpools at Broad Haven varied significantly during the study period (One-way ANOVA, $F_{6,117} = 31.473$, $P < 0.001$, Fig. 3.14). From February to June plants showed a positive increase in length, with the rate of elongation increasing throughout the spring months reaching a peak of 0.5 $cm.day^{-1}$ at the start of summer. From mid-June onwards the mean growth rate became negative, again the rate increasing in magnitude throughout late summer coinciding with the observed decrease in overall mean plant size (Fig. 3.9B). At Rhosneigr the mean growth rate of plants also varied significantly during the period for which tagging was successful (Kruskal-Wallis, $H_3 = 16.468$, $P < 0.01$, Fig. 3.15). Between March and June growth of plants was positive, with a peak of elongation rate occurring in May after which the rate decreased by half. From June onwards the mean growth became significantly negative as a result of the early onset of plant senescence observed at Rhosneigr. As mentioned previously the frequent burial of *S. muticum* plants at Tal y Foel due to the shifting nature of the substratum precluded the successful relocation of tagged individuals. However, between April and May, nine successfully relocated tags provided an estimated elongation rate of 1.72 ± 0.40 $cm.day^{-1}$ (Table 3.2). Comparative growth rate analysis between Tal y Foel, Rhosneigr and Broad Haven from the period between April and May, for which data was available from all three sites revealed

significant differences between locations (One-way ANOVA, $F_{2,35} = 13.533$, $P < 0.001$). A further *post hoc* Tukey test indicated that the growth rate of plants from the Broad Haven rockpools was significantly different ($P < 0.05$) from the other two locations.

When comparing plant growth rates based on the average primary lateral length data, variability in both the timing and magnitude of growth was evident between the three open coast sites (Table 3.3A-C). At Rhosneigr growth rate showed an increase up to the start of spring when rates began to slow coinciding with an increase in reproductive fertility (see Fig. 3.11A). From June onwards growth was negative as plants began to undergo senescence resulting in increased lateral breakage. Several growth rate estimates were comparable to those based on tagged plants (e.g. rates from March to April and May to June). However, the mean growth rate from June to July based upon tagged individuals was considerably larger than the rate derived from the average plant size (Fig. 3.15 and Table 3.3A). Comparisons between the single growth rate measurement based on tagged individuals from Tal y Foel and that based on average plant length data for the same period (April – May) showed a clear difference with the rate based on tagged plants over three times higher than that based on average plant size changes (Tables 3.2 and 3.3B). In fact the mean growth rate of tagged plants was more comparable to the growth rate estimate based on average plant length recorded during the following survey period (May – June). Estimated plant growth rates at Cei Bach showed a similar seasonal trend to those from Tal y Foel with positive growth through spring into early summer followed by a negative elongation rates from July onwards, although with one exception between August and September when surveyed plants had increased in mean size (Table 3.3C).

Table 3.2 Mean ± S.E. of the elongation rate ($cm.day^{-1}$) for different periods of sampling at the Tal y Foel survey site. *N* = number of tagged plants.

Growth Period	*N*	Elongation Rate
28/04/2010 - 27/05/2010	9	1.72 ± 0.40

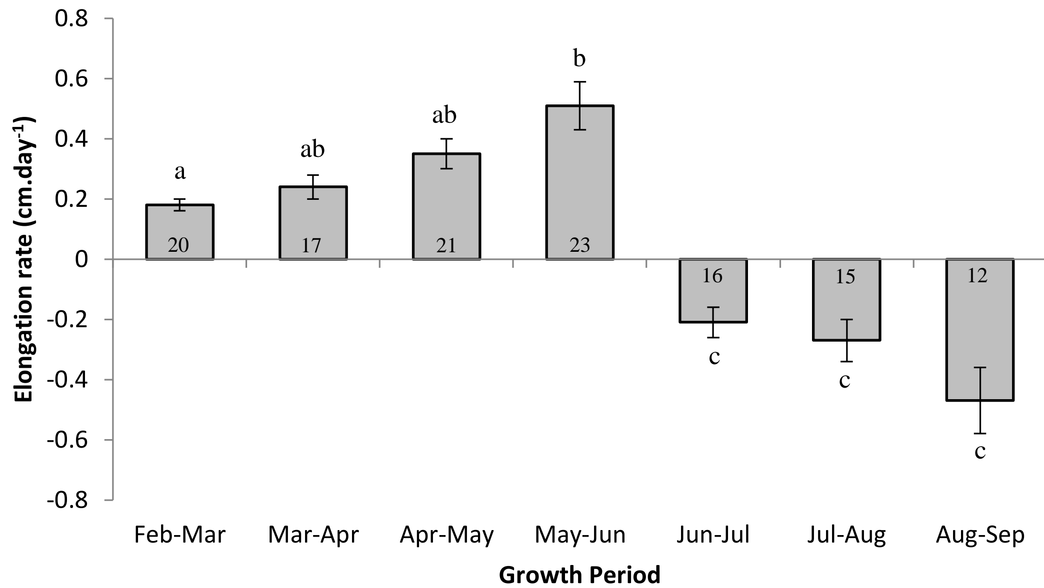


Fig. 3.14 Mean ± S.E. of the elongation rate (cm.day $^{-1}$) for different periods of sampling at the Broad Haven survey site. Numbers within bars indicates *N* value (number of tagged plants). Bars sharing the same letter above are not significantly different from each other as identified by *post hoc* Tukey tests ($P > 0.05$).

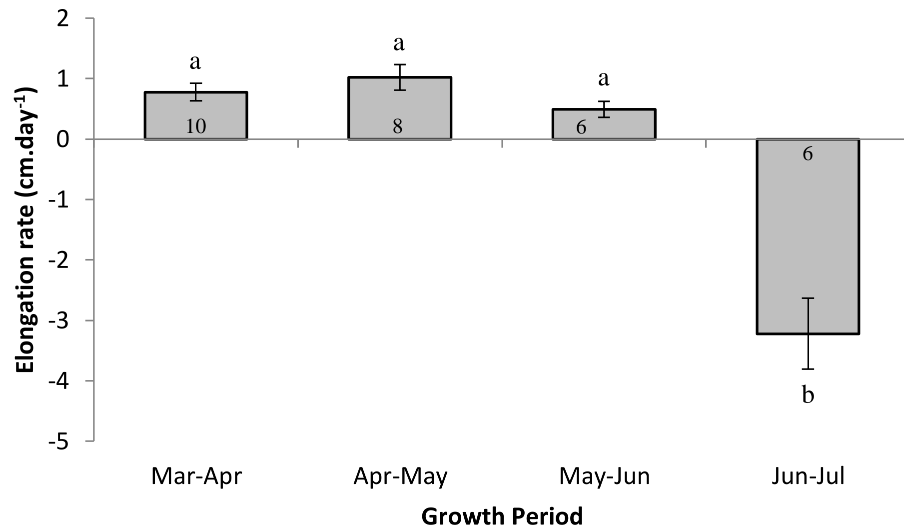


Fig. 3.15 Mean ± S.E. of the elongation rate (cm.day $^{-1}$) for different periods of sampling at the Rhosneigr survey site. Numbers within bars indicates *N* value (number of tagged plants). Bars sharing the same letter above are not significantly different from each other as identified by Mann-Whitney pairwise comparisons followed by Bonferroni correction ($P > 0.008$).

Table 3.3 Growth rate (cm.day $^{-1}$) based upon difference in the average primary lateral length data for different periods of sampling at (A) Rhosneigr, (B) Tal y Foel and (C) Cei Bach.

A

Growth Period	Elongation Rate
29/01/2010 - 27/02/2010	0.25
27/02/2010 - 28/03/2010	0.46
28/03/2010 - 28/04/2010	0.73
28/04/2010 - 27/05/2010	0.68
27/05/2010 - 15/06/2010	0.34
15/06/2010 - 15/07/2010	-0.73
15/07/2010 - 11/08/2010	-0.35
11/08/2010 - 12/09/2010	-1.09
12/09/2010 - 10/10/2010	-0.45
10/10/2010 - 06/11/2010	0.01

B

Growth Period	Elongation Rate
28/02/2010 - 27/03/2010	0.57
27/03/2010 - 28/04/2010	0.74
28/04/2010 - 27/05/2010	0.50
27/05/2010 - 14/06/2010	1.82
14/06/2010 - 16/07/2010	1.01
16/07/2010 - 10/08/2010	-0.98
10/08/2010 - 11/09/2010	-0.95
11/09/2010 - 09/10/2010	-1.20
09/10/2010 - 06/11/2010	-1.03

C

Growth Period	Elongation Rate
30/01/2010 - 26/02/2010	0.02
26/02/2010 - 29/03/2010	0.08
29/03/2010 - 29/04/2010	0.07
29/04/2010 - 26/05/2010	0.71
26/05/2010 - 13/06/2010	0.52
13/06/2010 - 16/07/2010	0.64
16/07/2010 - 09/08/2010	-0.48
09/08/2010 - 08/09/2010	0.30
08/09/2010 - 07/10/2010	-0.69
07/10/2010 - 07/11/2010	-1.04

3.4 DISCUSSION

This study provides a first insight into the seasonal population dynamics of *Sargassum muticum* from coastal habitats within Wales. The study revealed habitat-specific variability in thallus size, growth, the duration and level of reproductive maturity.

3.4.1 Plant size

As seen in previous studies of *S. muticum* (Critchley et al., 1987, Wernberg et al., 2001, Strong, 2003, Plouguerne et al., 2006, Baer and Stengel, 2010), each population was characterised by a period of increasing thallus length during the spring into early summer, followed by a decrease in size into the autumn months as plants began to senesce (see Fig. 3.9). When examining overall *S. muticum* plant lengths there were clear differences between the contrasting habitats surveyed along the Welsh coastline. In particular, the plants at Tal y Foel in the Menai Strait were considerably larger than those from the other Anglesey site at Rhosneigr. This confirms previous observations by Grant (2007) who also recorded larger plants growing within the sheltered, tide swept environments of Anglesey (e.g. Tal y Foel) compared to more wave exposed locations (e.g. Rhosneigr). The maximum plant size recorded by Grant (2007) at Tal y Foel during July 2007 was 5 metres in length. In contrast, the maximum plant size recorded from Tal y Foel during this study was 6 metres during August 2010. In July 2010 only a maximum plant size of 4 metres was observed from this location. Discrepancies between the results of these two investigations may be attributed simply to inter-annual variability and the fact that the population at Tal y Foel is likely to shift considerably due to the instability of the substrate. Despite these inter-annual differences in the maximum plant size recorded, average plant sizes were more similar with an average of ~170 cm recorded from Grant's (2007) study and an average length of 152 cm recorded from this investigation. At the contrasting site of Rhosneigr, the maximum recorded plant size from study by Grant (2007) was 2.75 metres during July 2007. In the current study the overall maximum plant size observed at Rhosneigr was 2.25 metres also recorded during the month of July. In terms of the average size, plants from Grant's (2007) study were approximately 64 cm in length, which again is very similar to the average plant length of 66 cm recorded from this investigation during the

same month. These similar trends in plant length observed between disparate years may be a reflection of the increased stability of the population located at Rhosneigr.

The size differences between these two contrasting sites on Anglesey confirm previous records from the literature that have demonstrated larger size macroalgae from sheltered locations. For example, compared to more exposed areas, *S. muticum* plants growing within the sheltered lagoon of Venice produced thalli which were more well developed (Curiel et al., 1998). In contrast, within Strangford Lough, Northern Ireland, Strong (2003) showed the longest primary laterals of *S. muticum* plants from a sheltered site, being 37% longer and with 50% more main axial shoots than plants from an extremely sheltered site. A similar situation was also observed at Aramar on the north coast of Spain where Andrew and Viejo (1998) found plants at exposed sites were longer than those in sheltered conditions. This may be due to an increased growth rate of the algae from more exposed locations as was reported to explain comparable observations in thalli lateral length of *Sargassum polyceratium* (Engelen et al., 2005). Strong (2003) postulated that this phenomenon of increasing plant length with increasing wave exposure might be particular to *S. muticum* among fucoids, whose thallus size generally decreases with increasing exposure.

It should be noted that the sheltered tide swept habitat at Tal y Foel represents a comparatively specialised habitat, as the strong tidal currents which algae are subjected to are qualitatively different to that of direct wave action, subjecting the algae to less physical stress. Previous measures of the current flow rates within the Menai Strait have demonstrated velocities reaching 8 knots on spring tides in the Swellies area at Menai Bridge and around 5 knots at Penmon Sound and Abermenai Point towards the northern end of the Menai Strait (Birkett and Maggs, 2001). Elsewhere in the Strait, tidal streams do not exceed 3 or 4 knots and there are regions where the current is significantly less. In fact, evidence provided by Harvey (1968) indicates a residual flow to the southwest through the Menai Strait with an average velocity of 15 $cm.s^{-1}$. The strong tidal currents in this region may also provide an increased supply of nutrients and facilitate gas exchange for *S. muticum* and other macroalgae growing within the local area. Although, a study by Kane and Chamberlain (1979) found that *S. muticum*'s growth did not appear to be limited by the availability of nutrients or available nitrogen. Therefore growth conditions for macroalgae are predicted to be elevated within this area allowing the

development of larger sized individuals. Similar observations in growth form have also been documented for *Saccorhiza polyschides* which attains a significantly larger size and a distinct morphology growing within the tidal rapids of Lough Hyne in contrast to individuals from the open coast (Norton, 1969).

Size differences were also observed between *S. muticum* plants from the rockpools located at different tidal heights at Broad Haven with larger individuals more common in the low shore pool (see Fig. 3.9B). This result appears to reflect a previous report by Fernandez (1999) who observed considerably smaller thallus lengths from plants growing in mid-intertidal rockpools compared to those from rockpools at lower intertidal levels on the coast of northern Spain. Variability in the local environmental conditions between rockpools is a likely explanation for these size contrasts. The environment becomes less favourable from the low to mid-intertidal, especially due to increased desiccation stress (Norton, 1977a) or, in the case of rockpools, isolation from the sea (Dethier, 1984). Pools lower down the shore spend less time uncovered by the tide and therefore will have longer access to dissolved nutrient and gases within the water column facilitating plant growth. In contrast, plants from pools located at higher tidal levels are more likely to be growing at the limits of their environmental tolerance range, and as such impacts on growth of these plants are expected. A further likely explanation for the larger plant sizes in the low shore pool could be the increased depth of the pool compared to others higher on the shore. The study by Fernandez (1999) also reported similar findings and it has been suggested that the deeper the rockpool, the more constant the environment conditions which may provide growth benefits (Gustavson, 1972).

Compared to previous reports of an average *S. muticum* plant size of 2-4 metres from British waters (Jephson and Gray, 1977, Critchley et al., 1990) the average plant size recorded from coastal waters in Wales appear somewhat smaller. In fact, at both Rhosneigr and Cei Bach the average summer plant length was closer to that recorded from the native range in Japan (75-150 cm, Yendo, 1907, Rueness, 1989). The largest specimen observed during this study was a 6 metre plant from Tal y Foel which again is considerably shorter than maximum plant sizes recorded from other introduced localities. For example plants up to 10-12 metres have been collected from the Brittany coast of France (Critchley et al., 1990b) and off the Pacific coast of North America the

alga may reach 7 metres in length (Nicholson et al., 1981). As discussed previously (see section 3.1) it is likely that combinations of factors are responsible for the size variations seen in *S. muticum* plants from various introduced populations. For example, the effects of increased seawater temperatures have been demonstrated to enhance growth in *S. muticum* (Norton, 1977a, Hales and Fletcher, 1989) with the potential for overall increases in algal size. The availability of nutrients is another important factor regulating growth and ultimate size of macroalgae (Norton et al., 1981). In northern Spain, Sanchez and Fernandez (2006) observed an enhanced rate of growth in *S. muticum* linked to the occurrence of local nutrient enrichment. The effects of water flow and tidal currents are also likely influential factors in determining overall size of *S. muticum* plants. For example, several reports of larger sized macroalgal specimens have been documented from areas subjected to strong tidal currents attributable to the enhancement of nutrient delivery and gaseous exchange (Norton, 1969, Reynolds, 1971, Norton et al., 1981).

With regards to the number of laterals per plant no clear, striking patterns of seasonality were evident from the results of this study. Previous reports however, have documented an increase in lateral number or frond/plant ratio during late winter into spring, peaking around the start of the summer months (Strong, 2003, Baer and Stengel, 2010). Following this peak, lateral numbers begin to decrease during late summer into the autumn months following the onset of plant senescence, resulting in increased frequency of lateral fragmentation.

In the current study no plants with more than 5 laterals were recorded in Rhosneigr during the month of July. An average of around two laterals was recorded from plants at Rhosneigr during July in this study which is similar to the observations of Grant (2007) during her surveys from the same location in July 2007. Although, in contrast to a maximum of 8 laterals reported from plants at Rhosneigr, during Grant's (2007) study no plants with more than 5 laterals were observed during the month of July from the current investigation. These differences are therefore likely to be the result of simple inter-annual variations in lateral development.

3.4.2 Reproductive maturity

Due to time constraints in this study it was not possible to undertake a more quantitative investigation of fertility in *S. muticum* for example receptacle numbers, receptacle size and the proportion of reproduction biomass. However, variability in the duration and level of reproductive fertility was evident between populations of *S. muticum* from contrasting environments in Welsh coastal waters (Figs 3.11 and 3.12). Across all locations the peak in the level of reproductive maturity typically coincided with a seasonal peak in seawater temperature (Fig. 3.13), a trend also observed from previous studies (e.g. Deysher, 1984, Arenas and Fernandez, 1998, Plouguerne et al., 2006). Differences in the onset and duration of fertility were apparent between the open coast sites, in particular at Cei Bach where reproductive plants appeared two months later in the year in July, compared to the plants from Tal y Foel and Rhosneigr (see Fig. 3.11). This may be explained by the relatively slower plant growth rates at Cei Bach as indicated by the smaller changes in lateral length (see Fig. 3.9A). The other main notable disparity in seasonal fertility between the three open coast sites was the significantly lower levels of fertility observed from the population at Rhosneigr with only a maximum proportion of 52% of plants from the population recorded during the reproductive period (see Fig. 3.11A). Given the earlier onset of senescence observed at Rhosneigr (see Fig. 3.9A) it is likely that maturity may not have been reached at Rhosneigr, although further monitoring of the alga's reproductive phenology is required before reaching a more conclusive statement.

Population differences in the reproductive potential of populations from different sites on Anglesey have previously been identified by Grant (2007) based on comparisons of average receptacle size. Results from this author's study showed differences in the maximum receptacle size of plants from different environments with larger sizes from more sheltered localities. However, no indication by the author was made as to the proportion of plants possessing reproductive structures. Therefore comparisons with the results of this study are not possible at this time.

Comparisons between the duration of reproductive fertility of plants from the rockpool habitats at Broad Haven and those from the open coast sites indicated relatively similar timings (see Figs 3.11 and 3.12). However, the reproductive

phenology of plants from rockpools at Broad Haven contrasts with observations from rockpool plants on the coast of northern Spain (Fernandez, 1999). Fernandez (1999) showed that the reproductive season typically lasted four months between May and August, whereas the reproductive period of rockpool plants from Broad Haven was extended, with fertile plants recorded until October.

It is likely that these differences are attributable to the contrasting environmental conditions between the two geographical locations. Also reported from Fernandez's (1999) study were differences in the level of fertility between plants from different tidal heights, with a greater proportion of fertile individuals from low intertidal rocks compared to those from mid tidal rockpools. A similar observation could be seen from the rockpool plants at Broad Haven in which a general trend of higher levels of fertility was more common from the low shore pool (see Fig. 3.12). This may be a result of the more stable environmental conditions more typical of the low shore rockpool habitats (Gustavson, 1972).

Previous research has documented a correlation between the timing of reproductive development in *S. muticum* and the geographical latitude (Norton and Deysher, 1989; Figure 3.16). The greater the latitude the more marked the seasonality of the *S. muticum* population. Towards the southern end of the alga's introduced range the observed trend is for the fertile period to begin earlier and last longer. For example, at Baja California in Mexico, the most southerly latitude for *S. muticum*, fertile plants can be found at any time of the year (Aguilar-Rosas and Galindo, 1990). Furthermore, studies by Deysher (1984) involving transplants of *S. muticum* between introduced populations spanning the range of the alga on the Pacific coast of N. America confirmed that environmental cues were responsible for the timing of reproduction rather than the occurrence of different ecotypes (Davison, 2009).

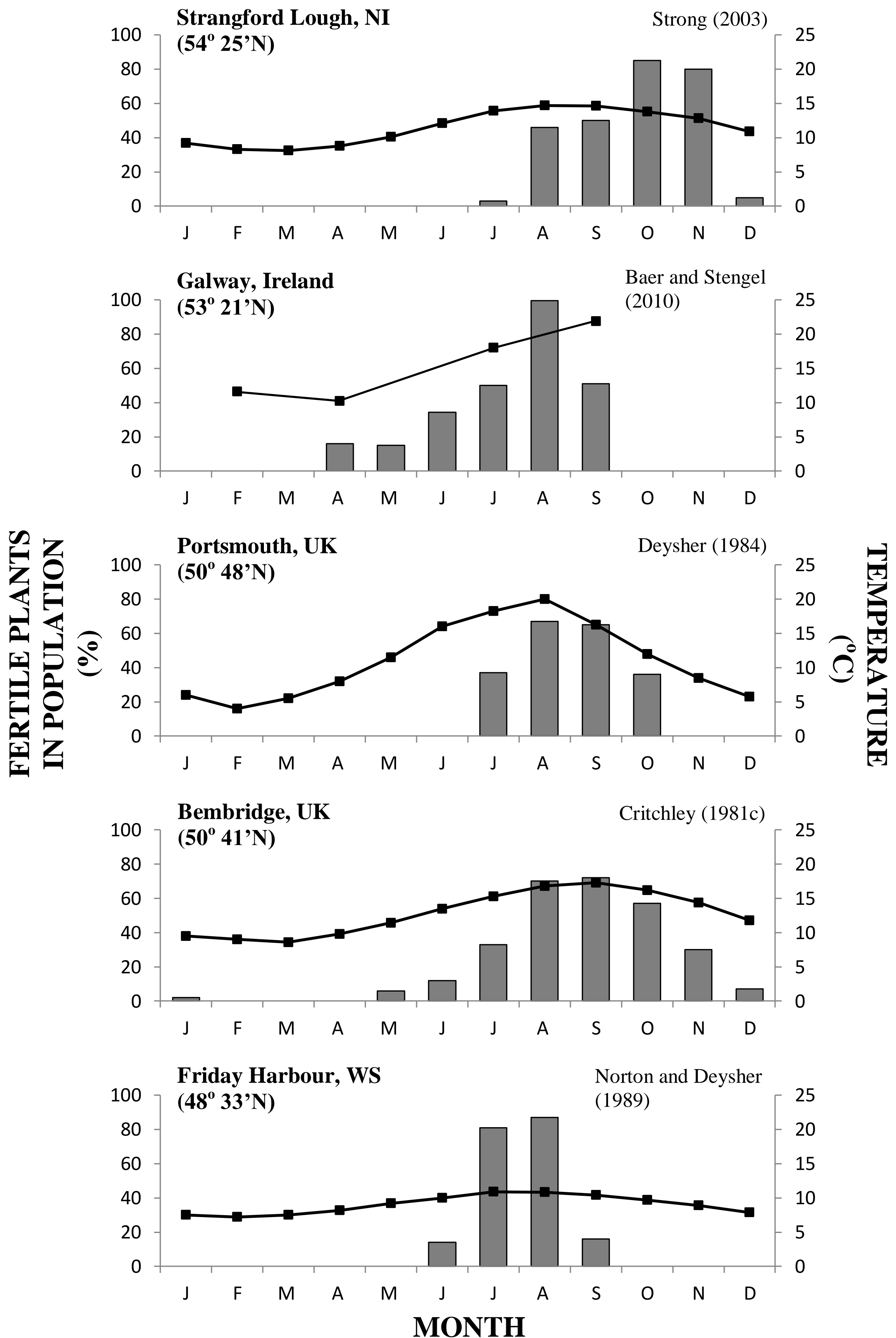


Fig. 3.16 Reproductive phenology (bars) of *S. muticum* and seawater temperatures (line) reported from introduced populations at different latitudes.

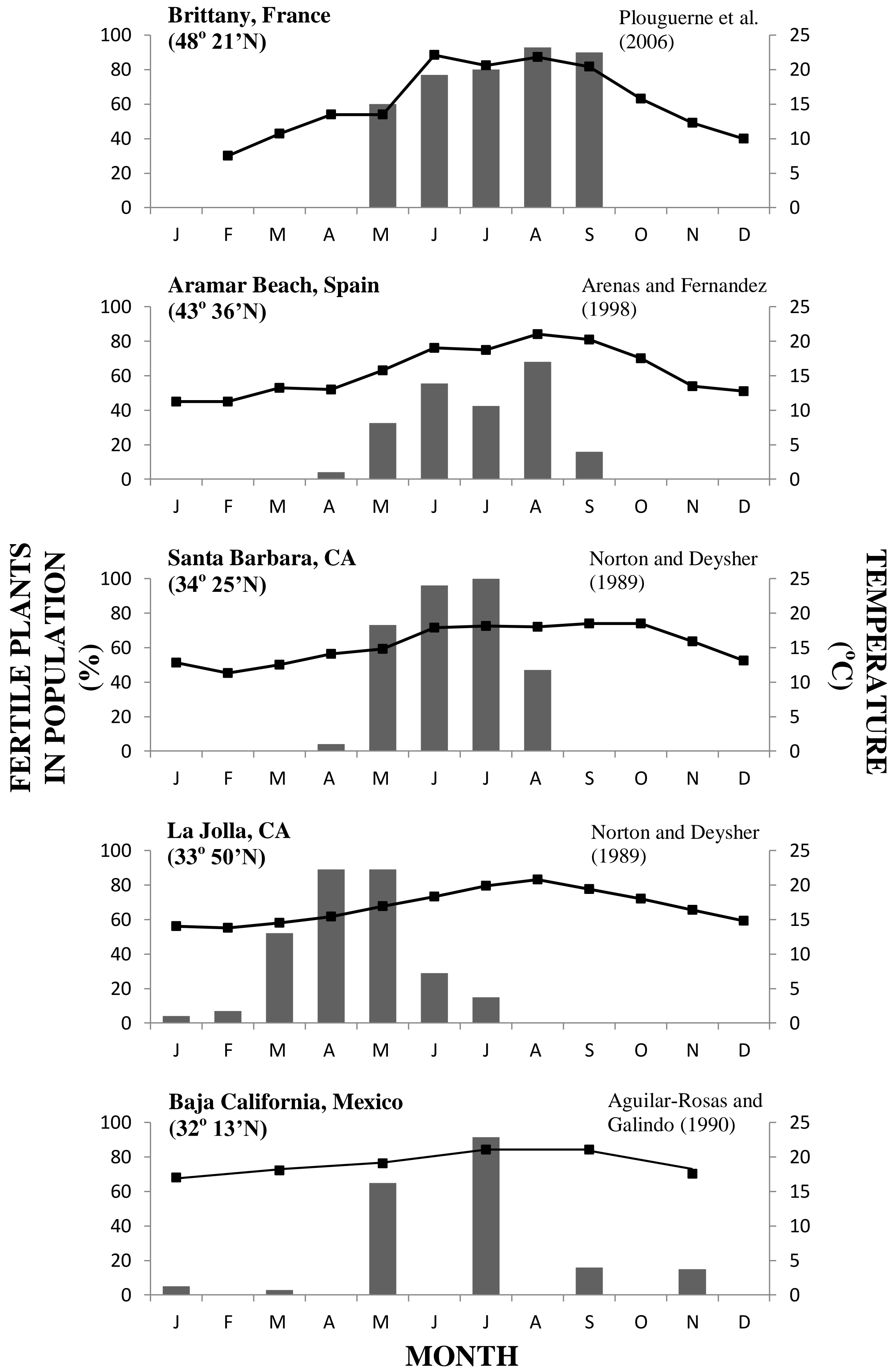


Fig. 3.16 continued Reproductive phenology (bars) of *S. muticum* and seawater temperatures (line) reported from introduced populations at different latitudes.

3.4.3 Plant growth rates

In keeping with previous reports, *Sargassum muticum* from coastal waters in Wales exhibits one distinct period of vegetative growth followed by notable frond size shortening due to the onset of senescence. This study has also highlighted the existence of environmental variability in plant growth rate. Maximum growth rates, provided from both tagged individuals and indirect estimates from monthly plant length data were observed from plants at Tal y Foel (Tables 3.2 and 3.3B). As previously suggested by Grant (2007), this observation may therefore offer an explanation as to the plant size differences observed between the three contrasting open coast sites. In her study, growth rates of *S. muticum* were not successful at Tal y Foel due to the complete loss of tagged plants, so no comparative analyses could be undertaken between other locations. Despite this, growth rate estimates of 0.137 and 0.431 $cm.day^{-1}$ during July 2007 were obtained from plants at the Anglesey sites of Rhosneigr and Porth Cwyfan respectively during Grant's (2007) study. During the month of July in the current investigation, plants from Rhosneigr were already undergoing senescence resulting in an overall negative rate of elongation (Fig. 3.15 and Table 3.3A). Hence inter-annual variability in growth may be considerable at this location but requires further investigation for a more accurate assessment.

The initial increase in growth rates from spring into early summer seen in rockpool plants from Broad Haven (Fig. 3.14) resembles observations of *S. muticum* plants from northern Spain previously reported by Arenas et al. (1995). However, during the onset of plant senescence a reverse trend was apparent from this investigation with the rate of shortening increasing from month to month in contrast to observations by Arenas et al. (1995) where the rate of plant shortening decreased over time. A similar trend of plant shortening seen from Broad Haven was also apparent at Cei Bach, except for an anomaly in August when an increase in plant size was observed (Table 3.3C). Although, estimates from Cei Bach were obtained from differences between consecutive monthly means for frond length, instead of measuring growth rates directly. The most significant rate of plant shortening was observed at Rhosneigr when following an early onset of senescence average plant length decrease was recorded at -3.22 $cm.day^{-1}$ (Fig 3.15). At this time *S. muticum* thalli from this location had become extremely

fragile, requiring extra care when recording length measurements (P Hallas, personal observation).

In terms of previous studies that have examined growth rates in *Sargassum muticum* from its introduced range, many have shown relatively high elongation rates during the middle of the alga's vegetative growth phase. Plants growing in the low-intertidal zone off the coasts of northern Spain display growth rates up to 2.08 cm.day $^{-1}$ during April to May (Arenas et al., 1995). In developed plants, growth rates of 4 cm.day $^{-1}$ have been recorded on the Pacific coast of North America and in the Solent, UK (Critchley, 1981c, Nicholson et al., 1981). Strong (2003) found that *S. muticum* typically grew by 2 cm.day $^{-1}$ during late spring and early summer in Strangford Lough, Northern Ireland. Summer growth rates reported from St Helens and Bembridge on the Isle of Wight were similar at 2.1 cm and 2.4 cm.day $^{-1}$ respectively (Lewey and Farnham, 1981). However, Norton (1977b) recorded a growth rate of 0.43 cm.day $^{-1}$ more similar to the overall average growth rates observed from plants in this study, in particular those from Rhosneigr, Cei Bach and Broad Haven.

High rates of growth have also been observed in other species of *Sargassum*. For example, a maximum growth rate of 3.6 cm.day $^{-1}$ has been recorded during early spring from *S. lapazeanum* off the south-western coast of the Gulf of California, Mexico (Rivera and Scrosati, 2006). Other large brown macroalgae such as the giant kelp, *Macrocystis pyrifera* may grow even faster at around 20.8 cm.day $^{-1}$ (Hernández-Carmona, 1996).

3.4.4 Future work

Results from this investigation have highlighted a number of key areas that would benefit from further work. The first main recommendation is for the continued monitoring of the targeted *S. muticum* populations over a number of consecutive years in order to develop a more accurate picture of the growth and reproductive phenology of the alga within Wales. Replicate surveys will also be necessary for an assessment as to the level of inter-annual variability in growth and reproductive seasonality within and between different locations.

Owing to the high tag losses encountered in the investigations carried out for this study (likely due to a high drag factor of the tags during immersion leading to

increased risk of plant lateral breakage) a more robust method of plant tagging would be required for future growth rate studies. One method which proved successful in the studies of *S. muticum* from Strangford Lough, involved using a labelling gun (Viking Direct, UK) to inset plastic tags through the primary laterals (Strong, 2003). As this approach rarely contributed to broken laterals, tag losses were minimal. Therefore this tagging technique may be an effective approach to consider for the continued monitoring of plants from the wide diversity of colonised marine habitats in Wales. As no native macroalgae were tagged for growth rate measurements, comparative analyses of growth rates between *S. muticum* and native seaweeds were not possible at this time. Results from Arenas et al. (1995) who compared growth of *S. muticum* with that of *Cystoseira nodicaulis* from northern Spain showed marked difference in seasonality of growth of the two species. Therefore in order to gain an accurate picture as to the competitive ability of *S. muticum*, comparative growth rate analyses between the invader and native seaweeds will be necessary. Previous work from Strangford Lough, Northern Ireland has also demonstrated that growth rates of *S. muticum* can be enhanced when present in mixed algal canopies owing to reduced effects of intraspecific competition (Strong, 2003). Therefore comparisons between the seasonal phenology of native macroalgae and that of *S. muticum* may also offer potential explanations for the relative success of the alga from various coastal habitats in Wales.

Whilst no assessment was made as to the growth rates and size structure of native macroalgae for a comparative analysis, previous studies have demonstrated the potential for significant impacts of the *S. muticum*. For example, in heavily invaded rockpool habitats, from northern Spain, the invasive alga induced significant changes in the resident macroalgal assemblages (Viejo, 1997). The most notable difference was a reduction in leathery macrophyte species, in particular *Bifurcaria bifurcate* which has been attributed to the significant shading effects of *S. muticum*. The observed presence of *B. bifurcata* within several heavily invaded low shore pools at Broad Haven (P Hallas, personal observation) therefore presents concern regarding the occurrence of a similar such effect. However, the confirmation of any potential impacts on native macrophytes within this region would require further experimental testing.

As reproduction was only assessed via estimates of the proportion of plants bearing receptacles no quantitative assessment of reproductive output of the alga from

the contrasting locations could be provided. The study by Grant (2007) offered an indirect estimation of reproductive output via a comparison of receptacle length from plants taken from contrasting environments on Anglesey. Her study revealed significant differences in size between locations suggesting the potential for different reproductive capacities between populations from contrasting environments. This may be apparent from the population at Tal y Foel where considerably larger plants were recorded. Norton and Deysher (1989) estimated that a single average receptacle of *S. muticum* could produce between 270 and 378 germlings. Therefore *S. muticum* populations containing larger individuals may contribute disproportionately to the reproductive pool due to their increased numbers of receptacles. Overall, an examination of recruitment patterns from contrasting habitats will provide valuable information regarding the level of population stability and the likelihood for the alga's expansion.

Another recommendation for future monitoring surveys is to measure the seasonal changes in density and percentage cover of both *S. muticum* and the local native macroalgae. Plant densities have been shown to exert a strong influence on the size structure and growth form of *S. muticum* with individual thalli becoming longer, thinner and less branched at high plant densities (Arenas et al., 2002). Furthermore, at higher monospecific densities intraspecific competition within stands of the alga can lead to considerable decreased growth (Arenas et al., 2002, Strong, 2003). Grant provided an estimate of plant density from various locations around Anglesey during the summer of 2007 with values ranging from 0.04 to 9.6 individuals.m^{-2}. A resurvey of these locations including an assessment of plant density may provide valuable information as to the growth and development of *S. muticum* at these sites.

Estimations as to the extent of canopy cover of *S. muticum* will also be important in further investigations. Negative impacts on the lower turf forming algal species as a result of shading effects by the invader have been reported. For example, a study by Viejo (1997) revealed evidence of significant impacts on leathery macrophytes species in rockpool assemblages due to the shading effects by *S. muticum*. In several of the low shore pools at Broad Haven including the one monitored during this investigation, canopy cover of *S. muticum* was approaching 100% during the summer months (see Fig. 3.17). It is likely that comparable effects to those observed by Viejo

(1997) may be occurring at Broad Haven, although further experiments will be required to test this prediction.

Fig. 3.17 Photos showing canopy coverage of *S. muticum* in rockpools at Broad Haven during June 2010.

3.4.5 Conclusions

Despite the fact that *S. muticum* has been present on the coastline of Wales since 1998 and is still currently expanding its distribution, relatively few comprehensive ecological investigations of the alga have been conducted from this region (Grant, 2007). Results from this investigation now provide a first assessment of the seasonality and population dynamics of *S. muticum* from Welsh coastal waters.

The rapid colonisation of *S. muticum* along the Welsh coastline can be explained by: (1) the alga's reproductive and adaptive capacities, (2) its dispersive capabilities and (3) its rapid growth rates. Depending on the area, *S. muticum* appears to have a 6-8 month fertility window within coastal waters of Wales. During this reproductive period each plant bears large numbers of receptacles (although not quantified in this study), each with the capacity to liberate vast numbers of germlings (e.g. a single receptacle can produce up to 324 zygotes, Norton and Deysher, 1989). Furthermore, within three months of arrival at a new site *S. muticum* plants can become fertile. This early onset of maturity provides a significant competitive advantage to *S. muticum* over many native fucoid alga which often require at least two years to develop to reproductive maturity (Norton, 1976). Towards the end of the reproductive period the plants had begun to enter senescence resulting in increased breakage of fronds explaining the observed drop in overall thallus length. The detachment and subsequent drift of fronds still bearing reproductive structures is an important mechanism for the dispersal and establishment of *S. muticum* on a regional scale (Fletcher and Fletcher, 1975, Deysher and Norton, 1982, Critchley et al., 1983).

Further spread of the alga within Wales is likely given the availability of suitable habitats. Water temperature will not be a limiting factor for the alga's expansion in Wales as *S. muticum* requires a temperature of more than 8°C for at least four months to reproduce (Steen and Rueness, 2004), a temperature typically exceeded during the summer months in Welsh coastal waters. Another introduced species, the New Zealand flat oyster (*Tiostrea lutaria*) has recently spread in the Menai Strait, North Wales, in an apparent response to warmer spring temperatures (Morgan, 2007). Increasing seawater temperatures within this area will also likely influence the growth and reproduction of

established populations of *S. muticum*, with consequences for the alga's continued spread within the region.

The present study shows that *S. muticum* is not performing equally at different sites around the coasts of Wales given the observed variability in growth and reproductive levels. As a consequence, the potential impact of the alga is therefore likely to be strongly site-dependent. Eradication is not a viable option given both the duration of the alga's establishment in Welsh coastal waters and the unsuccessful programmes of removal attempted in the past (see Chapter 1, Section 1.2.9). Overall, the recommendations stemming from the results of this study are for the continued monitoring of targeted populations of the alga along the Welsh coastline to determine the levels of inter-annual variability in population dynamics and to assess any likely impacts on native communities.

CHAPTER FOUR EPIPHYTIC ALGAE AND FAUNAL INVESTIGATIONS

4.1 INTRODUCTION

Among the invaders that have the potential to cause the most dramatic impact are those that can cause direct changes to the habitat as such modifications are likely to have cascading effects on other trophic levels of the ecosystem (Crooks, 2002). For example those introduced species that lead to increases in habitat structure and complexity tend to cause elevations in species abundances whereas those that reduce heterogeneity can cause the reverse. For example introductions of Japanese eelgrass, *Zostera japonica*, have led to increases in both density and diversity of mudflat macrofauna compared to the original bare sediment (Posey, 1988). In contrast, grazing by the introduced periwinkle snail decreased structural complexity of the vegetation leading to a reduction in density and diversity of soft-sediment fauna following a comparison of grazed versus ungrazed rocks (Bertness, 1984).

Macroalgae are important components of benthic marine communities with many of the larger species e.g. fucoids and kelps providing important habitats for a wide diversity of other marine organisms (Christie et al., 2009). As space is often a limiting resource within hard bottomed intertidal communities (Dayton, 1971), large macroalgae play an important role in providing additional attachment substrate for both epiphytes and invertebrates (Crooks, 2002, Wikström and Kautsky, 2004, Schmidt and Scheibling, 2006). The surface of many long-lived perennial seaweeds are often heavily colonised by epiphytic organisms that use the host seaweed as an attachment substrate. The host algae may also a habitat and a potential food resource for a wide range of motile fauna. Therefore any reduction in the abundance and/or replacement of a habitat forming algae may have significant consequences on the associated local community.

Several studies have demonstrated a link between morphological complexity of the host algae and the abundance and composition of associated assemblages (Hicks, 1985, Gee and Warwick, 1994, Davenport et al., 1999, Chemello and Milazzo, 2002, Kelaher, 2003, Hauser et al., 2006, Cacabelos et al., 2010). The reasons for this trend relate to the increase in resources such a living space, food sources and protection from

biological and physical stressors (Taylor and Cole, 1994). The overall size of the host algae also has an impact with larger specimens typically harbouring greater numbers of organisms (Gunnill, 1982, Norderhaug et al., 2007). Structural differences between macrophyte communities may influence the composition of associated fauna (Christie et al., 2009). This was suggested as the reason for the epibiont community differences between *Sargasum muticum* and *F. vesiculosus* in the North Sea (Buschbaum et al., 2006). Furthermore, the community similarities between *S. muticum* and *Halidrys siliquosa*, also from the same region, were attributed to their comparable structural morphologies (Buschbaum et al., 2006). In contrast, results from Cacabelos et al. (2010) indicated that structural complexity was only of minor importance for explaining differences in epifaunal assemblages between the invader, *S. muticum* and the native kelp, *Laminaria ochroleuca*. Other factors may therefore be more important in determining community composition of epibiota associated with algal hosts. For example results from a study by Schreider et al. (2003) also indicated a lack of influence of structural complexity on amphipod abundance levels between simple and complex plants. Instead their study placed more emphasis on tidal height as a contributing factor to difference in amphipod abundance between different host macroalgae. Wikstrom and Kautsky (2004) showed clear differences in the epiphytic communities supported by two closely related species of *Fucus*, with the introduced *F. evanescens* displaying consistently lower species richness and biomass of epibiota than compared to native *F. vesiculosus*. However, the mechanisms behind this lower epiphytic colonisation on the invader still remain unresolved. One main suggestion behind the differential fouling on macroalgal species relates to the structural complexity of the host algae. For example, within a soft sediment habitat, an increased species richness of epibiota on the introduced alga, *S. muticum* compared to native *F. vesiculosus* was attributed to the enhancement of substrate availability and habitat heterogeneity provide by the invader (Buschbaum et al., 2006).

The effects of introduced macroalgae on associated epibiota have been conflicting. Several studies have shown a positive enhancement of diversity and abundance of epibionts compared with native conspecifics. For example, a study by Drouin et al. (2011) revealed that the invasion of eelgrass beds by *Codium fragile* ssp. *fragile* contributed to increases in faunal density and diversity. Furthermore, the presence of colonising epiphytic algae on invasive macroalgal hosts may in turn provide

resources for higher trophic levels (Jones and Thornber, 2010). The importance of this relationship may be magnified in situations where the basiphyte host alga is less palatable (Chavanich and Harris, 2002). In contrast, other research has shown that epiphytic communities are relatively similar. Finally, some studies have shown that the species richness and biomass of epibiota are significantly reduced on non-native macroalgae compared to native conspecifics (Wikström and Kautsky, 2004, Janiak and Whitlatch, 2012). However the magnitude of any changes will be governed by the ability of local species to colonise the invading seaweed (Wikström and Kautsky, 2004). Host specificity has been documented in some epiphytic algae e.g. *Polysiphonia lanosa* and *Ascophyllum nodosum* host (Lining and Garbary, 1992). Host specificity within free-living fauna can be more acute as these organisms have the ability to actively choose their associated host macrophyte. However, most studies indicate that motile fauna are relatively unspecific choice of host (Lutz et al., 2010). Hence, the introduction of non-native macroalgae may have contrasting impacts on the associated epibionts depending on their levels of host specificity (Wikström and Kautsky, 2004). Investigations in to the epibiota communities on *S. muticum* have demonstrated that almost all of the species found in association with the alga originated from with the regional species pool of the local environment (Bjaerke and Fredriksen, 2003, Buschbaum et al., 2006). Due to the conflicting results regarding the impacts of non-indigenous macroalgae on associated epibiota, the development of generalisations may be largely inappropriate for investigating this aspect of macroalgal invasions (Lutz et al., 2010).

The invasive macroalgae, *Sargassum muticum* has successfully colonised numerous regions and a wide range of habitats with the northeast Atlantic, northeast Pacific and the Mediterranean since its introduction (Davison, 2009). Within several of these areas it has become a dominant member of the macroalgal community (Britton-Simmons, 2004, Engelen and Santos, 2009). Given the potential for significant ecological changes by this habitat forming invasive seaweed, several studies have examined the alga's impact on associated flora and fauna (Withers et al., 1975, Strong, 2003, Wernberg et al., 2004, Buschbaum et al., 2006, Harries et al., 2007b, Cacabelos et al., 2010). Most of those studies have highlighted relatively few adverse effect of the alga's introduction attributable to a low degree of habitat specificity among epibiota and

the enhancement of habitat heterogeneity created by the morphology of the alga (Wernberg et al., 2004, Buschbaum et al., 2006).

The pseudo-perennial life history of *S. muticum* in temperate waters means that the associated communities are typically lost following shedding and plant senescence (Norton and Benson, 1983). As a host macrophyte, *S. muticum* can be considered a relatively unstable habitat with obvious consequences for the abundance and diversity of its associated species communities (Buschbaum et al., 2006). Therefore annual recolonisation of the alga must take place as only a few species typically remain attached to the perennial basal stem and holdfast (Norton and Benson, 1983). Fauna and flora replacement is suggested to occur from the regional species pool of the respective environment (Bjaerke and Fredriksen, 2003, Buschbaum et al., 2006). Evidence of this trait was demonstrated by the likeness of the epibiotic assemblages associated with *S. muticum* and a structurally similar native alga (Buschbaum et al., 2006).

Differences in the distribution of colonising epibiota have also been demonstrated in *Sargassum muticum*. Some studies have shown that the perennial sections of the alga (i.e. basal stem and holdfast) support a greater diversity and abundance of epiphytic species. In contrast a number of studies have shown no such spatial segregation on the alga (Bjaerke and Fredriksen, 2003). One of the reasons put forward in explanation of the reduced numbers of species colonising the annual laterals is due to the presence of chemical inhibitors. *Sargassum muticum* is known to produce tannin-like substances which have been shown to display antifouling properties (Conover and McN. Sieburth, 1964, Sieburth and Conover, 1965, Withers et al., 1975, Jephson and Gray, 1977). These compounds may also act as a grazing deterrent, which has been suggested as one of mechanisms contributing to the introduction success of the alga (Monteiro et al., 2009a, White, 2010, Engelen et al., 2011).

The aim of this study was to investigate how the invasion of *Sargassum muticum* affects the associated littoral community within an invaded area, by comparing the associated biota of the invader with that of a native conspecific alga. As previous research has demonstrated contrasting effects of the algae on epibiota assemblages between differing invaded habitats (Buschbaum et al., 2006), two divergent colonised areas were included in the investigation. The specific questions addressed were: 1) Does the invader *S. muticum* support a reduced assemblage of epibionts in terms of biomass,

species richness and diversity, than compared with the native alga? 2) Are there differences between the epibiont communities supported by the invader from contrasting environments along the Welsh coast?

4.2 MATERIALS AND METHODS

4.2.1 Study sites

The study was carried out at two contrasting locations on the Isle of Anglesey, North Wales, both of which encompassed the typical types of habitat colonised by *Sargassum muticum* along the Welsh coastline. These two locations where macroalgae sampling took place were at Tal y Foel and Rhosneigr. Samples at both sites were collected from within the low subtidal zone during low water spring tides (0.8m LWT), during the month of June 2010.

Tal y Foel is located in a sheltered position at the south western end of the Menai Strait (SH 474 645). The shore experiences a strong tidal cycle throughout much of the tidal cycle. For a more complete description of the site characteristics see Chapter 3, Section 3.2.1.2. All samples were taken from the main *S. muticum* bed located within the centre of a belt at grid reference SH 4748 6448.

Rhosneigr is located on the west coast of Anglesey and has a south-westerly facing aspect (SH 314 762). The area is subjected to moderate levels of wave exposure but no significant tidal currents. For a more complete description of the site characteristics see Chapter 3, Section 3.2.1.1. The site where the macroalgal samples were collected was located within one of the rock-protected lagoons with the centre of the area at grid reference SH 3133 7270.

4.2.2 Collection of algae

At each of the two survey locations eight thalli of the invasive *S. muticum* and a native conspecific macroalga were sampled from the same area and water depth. At Tal y Foel the only other habitat-providing native alga growing within the same tidal zone was *Fucus vesiculosus*, therefore this alga was selected as the comparator against *S. muticum* for this study. At Rhosneigr the native alga chosen for comparison against *S.*

muticum was the structurally similar *Cystoseira* spp. (most likely *C. nodicaulis*) due to its comparable level of abundance with the *S. muticum* survey area. All seaweed collections were made during June 2010, when the abundance of associated organisms are approaching their peak (Withers et al., 1975, Wernberg et al., 2004, Gestoso et al., 2010) using the same random collection protocol as detailed in section 3.2.2 (Chapter 3). One-way ANOVA revealed all algae sampled were of a similar size (average fresh weights (± S.E.): *S. muticum* (Rhos) = 126.9 ± 17.3g; *S. muticum* (Tal) = 79.7 ± 15.4g; *Cystoseira* spp. = 89.6 ± 23.8g; *F. vesiculosus* = 112.9 ± 15.2g, (One-way ANOVA, $F_{3,28} = 1.38$, $p = 0.268$). A large plastic bag was carefully placed over the top of the sampled thalli of each species in order to minimise the dislodgement of mobile faunal and the plant holdfast prised off the substrate using a paint scraper. Bags containing the sampled thalli and small amount of the neighbouring seawater were then sealed and labelled. Plants were kept on ice upon return to the laboratory where weighing (both epiphytic algae and basiphyte biomass) and identification of the epibiota were undertaken. In addition to the taxonomic identification, the location of each algal epiphyte and epifaunal species on the basiphyte was recorded.

4.2.3 Laboratory analysis

In the laboratory, samples were transferred into large buckets containing distilled water and shaken up and down to dislodge motile species. This process was then repeated in a second water filled bucket. The water from the two buckets was then sieved through 500 µM sieve to collect the associated organisms. The collection bags were also washed through into the sieve to gather any epiphytic and motile fauna that that had become detached during transport. The basiphyte thallus was laid out stretched in a white sorting tray where all fronds of the thallus were checked for the presence of other mobile fauna and both epiphytic and epifaunal species, which were subsequently removed from the basiphyte thallus. All collected organisms were then identified to the lowest possible taxonomic level using dissecting and light microscopes, counted and preserved in 70% ethanol. In the case of fragmented animals (such as Polychaeta), only heads were counted. Basiphyte plants were thoroughly checked under a dissecting microscope and all epiphytic macroalgal species were removed from the basiphyte plant. In the case of colonial faunal taxa (e.g. Bryozoa), only the numbers of distinct patches of the species occurring on the basiphyte were recorded as a measure of

abundance. Only presence/absence of the different epiphytic algal taxa identified was recorded. For faunal taxa, identifications were made using the Handbook of the Marine Fauna of North-West Europe (Hayward and Ryland, 1995). For the epiphytic algae, Seaweeds of the British Isles (Irvine, 1983, Maggs and Hommersand, 2001, Dixon and Irvine, 2005), a Field Key to the British Brown Seaweeds (Hiscock, 1979) and Green Seaweeds of Britain and Ireland (Brodie et al., 2007) were used. Fresh weight of the basiphyte species and epiphytic algae were measured after blotting dry with blue roll to remove excess water. The dry weight of epiphytes and basiphyte plant were calculated after drying them for 48 hours at 70°C.

4.2.4 Feeding habits of the epifauna

It was not possible to revisit archived samples of fauna due to degradation of samples preserved in alcohol. For example, most amphipod specimens had become fragmented during storage making accurate size measurements impractical. Instead a comparison of the feeding behaviours of the mobile epifauna associated with each macroalgal host was examined. The feeding habits of the mobile epifauna were determined using literature searches. Taxa were classified as either herbivores, predators, detritivores or omnivores. For those species or taxa where feeding habits were absent from the literature a category of unknown was used to group the data. From the resulting grouped data together with the average number of each taxa per host macroalga, the percentage of each feeding guild making up the associated mobile epifauna with each macroalga was determined. Details of the feeding habits of the epifaunal taxa examined plus the bibliographic references as to where the information was sourced are provided in Appendix C.3.

4.2.5 Epiphytic algal functional grouping

As a further investigation into the composition of the epiphytic algae associated with the host macroalgae the each species identified was assigned to an appropriate functional group based on the model described by Steneck and Dethier (1994). Five groups were considered: 1) filamentous species, 2) foliose species, 3) corticated macrophytes, 4) leathery macrophytes and 5) calcareous crustose forms. From the resulting grouped data the percentage of each functional group in the composition of the epiflora of each of the four macroalgal host was determined.

As an additional analysis, the epiphytic algae were also classified in terms of their maximum size (determined from searches of the literature). Due the absence of growth rate data for many of the epifloral taxa identified in this study, maximum size was used as an indirect measure for algal growth rate. Four categories of size were used: 1) small (3-10 cm), 2) medium (11-20 cm), 3) medium-large (21-50 cm) and 4) large (>50 cm). As some algal species fell into more than one category the decision was made to place such species placing it the maximum size category recorded. Only species for which size data could be sourced were included in the overall analysis.

4.2.6 Statistical Analyses

4.2.6.1 Univariate analyses

The epibiota dataset used in the final analyses is presented in Appendices C.1 and C.2. As the data collected consisted of both quantitative abundance measures (e.g. mobile epifauna) and records of presence/absence of a species (e.g. algal epiphytes) a range of different statistical analyses were performed on various groupings of the raw data. Changes in the number of individuals (*N*), the number of species (*S*) and diversity indices were calculated and analysed using standard one-way ANOVA followed by Tukey *post hoc* tests. Homogeneity of variances was examined using the Levene's test. Data sets not showing homogeneity of variance were transformed and the Levene's test repeated to confirm homogeneity of variance. As quantitative abundance values had only been recorded for species of mobile epifauna, diversity indices were only computed for this data set. The following diversity measures were computed:

Shannon Wiener: this statistic is a measure of species diversity that incorporates the abundance of each species, thereby combining aspects of community equitability. The Shannon Wiener statistic ($\boldsymbol{H'}$) was calculated as follows:

$$\boldsymbol{H' = -\Sigma Pi\, log(Pi)}$$

Where $\boldsymbol{Pi}$ is the proportion of individuals belonging to the $\boldsymbol{i}$th species in the data set.

Pielou's evenness: this statistic represents how evenly distributed species abundance levels are across the sample. Values close to 1 are indicative of a very even distribution of abundances amongst species, whereas values closer to 0 are more

characteristic of samples with a very uneven distribution of abundance among species. It is very rare that all species within a sample will occur at the same magnitude. Some maybe better competitors for example, and therefore may occur in larger numbers. Generally speaking increasing evenness within an ecosystem increases the diversity of the system, hence such systems are often considered to be healthier. In contrast, pollution or presence of an invasive can drastically reduce species evenness and diversity measures of a habitat.

The Pielou's evenness statistic ($\boldsymbol{J'}$) was calculated as follows:

$$\boldsymbol{J' = \frac{H'}{lnS}}$$

Where $\boldsymbol{H'}$ is the number derived from the Shannon Wiener diversity index and $\boldsymbol{S}$ is the number of species within the sample.

Margalef index (species richness): this statistic incorporates the total number of individuals and is a measure of the number of species present for a given number of sampled individuals. The greater the value of the index, the greater the diversity. The Margalef's index was calculated as follows:

$$\boldsymbol{D = \frac{S-1}{lnN}}$$

Where $\boldsymbol{S}$ is the number of species within the sample and $\boldsymbol{N}$ is the total number of individuals from the sample.

Wet weight of epiphytic algal biomass was standardised to a value per gram dry weight of the basiphyte. Comparisons of both epiphytic algal biomass and basiphyte biomass were made using standard one-way ANOVA followed by *post hoc* Tukey tests. Chi-square tests were employed in the comparative analyses of the mobile epifauna feeding habits and functional grouping of the epiphytic algae. All univariate analyses were undertaken in SPSS (SPSS for Windows, Version 18.0) and Microsoft Excel.

4.2.6.2 Multivariate Analyses

In order to test for differences in epibiota communities between the various basiphyte species, a routine ANOSIM (analysis of similarity) which employs a standard

one-way ANOVA was performed. ANOSIM allows for the test of the null hypotheses that there is no assemblage difference between *a priori* groupings of samples specified by the levels of a single factor. ANOSIM results are primarily reported as a global *R* statistic, along with a p value which is determined by the number of permutations that result in an *R* value greater than or equal to the global *R* statistic. When the global *R* statistic is much larger than any of the default 999 permutated values calculated, it results in a significance level of $p < 0.001$ (Clarke and Gorley, 2006). To examine the overall similarity of the communities associated with the different basiphyte groupings, cluster analysis and a multidimensional scaling (MDS) were undertaken (Clarke 1993). Non-parametric multidimensional scaling (nMDS) ordination plots of the samples were constructed based on Bray-Curtis similarity generated from the data set. MDS analysis was displayed using 2-dimensional ordination. Dendrograms were constructed to display the results of cluster analysis, using hierarchical agglomerative clustering with group averaging. Using the dendrogram output by the cluster analysis, the degree to which clusters of samples are similar or dissimilar can be determined. Similarly MDS plots provide a representation of the sample data in two-dimensional space. Points that are close together represent samples of similar composition and points further apart correspond to samples that are more different in composition. MDS plots where the associated stress values were > 0.2 were not considered useful for interpretation (Clarke and Gorley, 2006). Similarity percentage (SIMPER) analysis was used to identify the species that contributed significantly to dissimilarities between the observed groups within the dataset; species that contributed to at least 5% of host similarity were considered characteristic species of that assemblage (Clarke and Warwick, 2001). All the multivariate analyses were undertaken using the PRIMER (Plymouth Routines in Multivariate Ecological Research) software package of the Plymouth Marine Laboratory (Clarke and Gorley, 2006).

For the epiphytic algae, sessile fauna and complete epibiota data sets analyses were conducted based upon presence/absence records. With regards to the mobile epifaunal data, a number of different analyses were conducted: the first with epifaunal abundance transformed into presence/absence data; the second with a square root transformation of the epifaunal abundance data to reduce the influence of highly abundant species, as values ranged over two orders of magnitude; and thirdly with the

epifaunal abundance data corrected to the biomass of the basiphyte species without epiphytes (i.e. abundance per gram wet weight eight of basiphyte host).

4.3 RESULTS

Despite considerable variation within host basiphyte groupings (i.e. groups based on macroalgal host species and location), no significant differences in basiphyte wet weight standing biomass were detected between the four basiphyte groupings (One-way ANOVA, $F_{3,28} = 1.384$, $P = 0.268$ (Table 4.1)). In contrast, the dry weight standing biomass of *Fucus vesiculosus* was significantly greater than the other three basiphyte groupings (One-way ANOVA, $F_{3,28} = 9.572$, $P < 0.001$, (Table 4.1)).

Table 4.1 Basiphyte biomass of the host macroalgae collected from the two different locations on Anglesey, North Wales in June 2010. Means sharing the same superscript letter are not significantly different from each other as identified by *post hoc* Tukey tests.

Basiphyte species and Location	Mean basiphyte wet weight biomass in grams (±S.D., $n = 8$)	Mean basiphyte dry weight biomass in grams (±S.D., $n = 8$)
Tal y Foel		
Sargassum muticum	79.70 (±43.69)[a]	7.54 (±3.66)[a]
Fucus vesiculosus	112.97 (±42.92)[a]	23.67 (±8.75)[b]
Rhosneigr		
Sargassum muticum	126.85 (±49.01)[a]	11.95 (±5.05)[a]
Cystoseira spp.	89.63 (±67.40)[a]	9.74 (±7.59)[a]

4.3.1 Epiphytic algae on native and invasive basiphyte hosts

Overall a total of 57 species of epiphytic algae were recorded from the host basiphytes collected from both habitats (see Appendix C.2). The species richness of associated epiphytic algae was greater from Rhosneigr (47 species) compared with Tal y

Foel (34 species). When all samples from each basiphyte grouping were combined, *Cystoseira* spp. displayed the greatest overall species richness (42 species) compared to the other three basiphyte groupings (*S. muticum*, Rhosneigr = 27 species; *F. vesiculosus*, Tal y Foel = 20 species; *S. muticum*, Tal y Foel = 24 species). A similar result was detected when the mean number of epiphyte species per host basiphyte were compared (Fig. 4.1). The mean number of epiphyte species per basiphyte was significantly larger on *Cystoseira* spp. when compared to all other sampled basiphytes with typically twice the number of epiphytic algae species per thallus than those basiphytes collected from Tal y Foel. Individuals of *Sargassum muticum* from Rhosneigr possessed an intermediate level of epiphyte diversity per thallus.

When the epiphyte biomass (wet weight in grams) was standardised against basiphyte biomass (dry weight in grams), the epiphytic fouling on *Cystoseira* spp. was larger compared to that of the other basiphyte species (Fig. 4.2). However, due to high variability within the data sets only significant differences were evident between *F. vesiculosus* from Tal y Foel and *S. muticum* from Rhosneigr. Epiphytic algal fouling on *Fucus vesiculosus* was consistently the lowest out of the three basiphyte species sampled over the two localities.

For the purposes of the epiphytic algal community analysis data were converted to presence/absence format as only a semi-quantitative measure of epiphytic algae had been recorded. The results of the ANOSIM indicated that the composition of the epiphytic algal assemblages on all four basiphyte groupings differed significantly (Global R-value = 0.660, $P < 0.001$; Table 4.2). A two dimensional MDS plot displayed some degree of segregation, particularly evident in the *Cystoseira* spp. and *S. muticum* samples from Rhosneigr (Fig. 4.3). Although, due to the relatively high stress value (Stress = 0.22) of the MDS ordination plot (Fig. 4.3) this suggests that not too much confidence should be placed on the distribution patterns displayed in the plot (Clarke and Gorley, 2006).

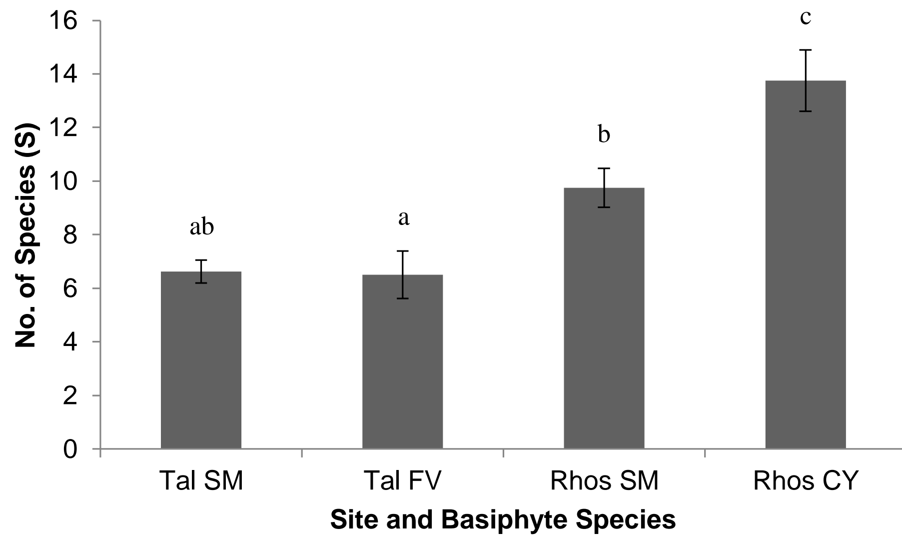


Fig. 4.1 Mean (±1 S.E.) number of epiflora species per host basiphyte sampled from the two differing habitat locations. One-way ANOVA, $F_{3,28} = 16.620$, $P < 0.001$. Bars sharing the same letter above are not significantly different from each other as identified by *post hoc* Tukey tests.

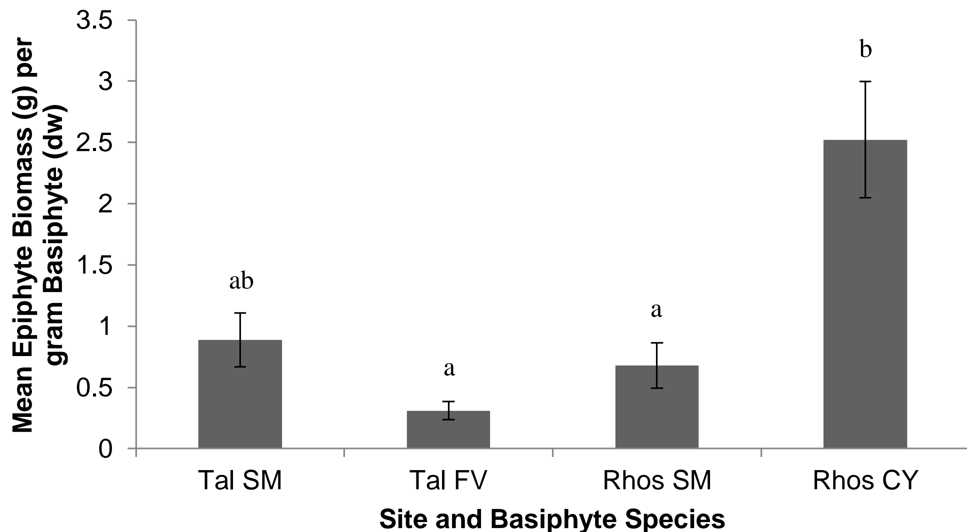


Fig. 4.2 Mean (±1 S.E.) epiphytic algal biomass (weight weight) per gram basiphyte (dry weight) sampled from the two differing habitat locations. ANOVA (log $_{10}$ transformed data) was used to test for differences in epiphytic algal biomass between basiphyte species (One-way ANOVA, $F_{3,28} = 8.604$, $P < 0.001$). Bars sharing the same letter above are not significantly different from each other as identified by *post hoc* Tukey tests.

Results from the SIMPER analysis revealed that no one species of epiphytic algae was present on all basiphyte species sampled (Table 4.3). An epiphytic alga of the genus *Elachista* was a taxon that clearly distinguished *Cystoseira* spp. from the other basiphyte species. *Elachista* spp. was the dominant epiphyte on *Cystoseira* spp. basiphyte samples contributing on average 75% of the total epiphytic algal biomass. Epiphytic growths of ectocarpoid species were the main characteristic species of *Sargassum muticum* basiphytes sampled from the two locations and also *F. vesiculosus*. Epiphyte species representative of *S. muticum* collected from Rhosneigr also included *Dictyota dichotoma*. Distinction between invasive and native basiphytes from Tal y Foel was attributed to two main epiphyte species, with *Polysiphonia* spp. only found on *S. muticum* and epiphytic algae of the genus *Cladophora* more common on *F. vesiculosus*.

Table. 4.2 Pairwise ANOSIM comparisons of epiphytic algae composition on the various basiphyte species collected from the two contrasting habitats on Anglesey. Due to the occurrence of multiple hypothesis testing, a *P* value of 0.008 has been used for the global significance threshold. Species and site codes: TS = *S. muticum*, Tal y Foel; TF = *F. vesiculosus*, Tal y Foel; RS = *S. muticum*, Rhosneigr; RC = *Cystoseira* spp., Rhosneigr.

	***R* Value from one-way ANOSIM**		
	TF	RS	RC
TS	0.483 $P = 0.004$	0.416 $P = 0.004$	0.919 $P = 0.001$
TF		0.707 $P = 0.001$	0.935 $P = 0.001$
RS			0.432 $P = 0.001$

ANOSIM, one-way, sample statistic (Global R): 0.660, $P < 0.001$.

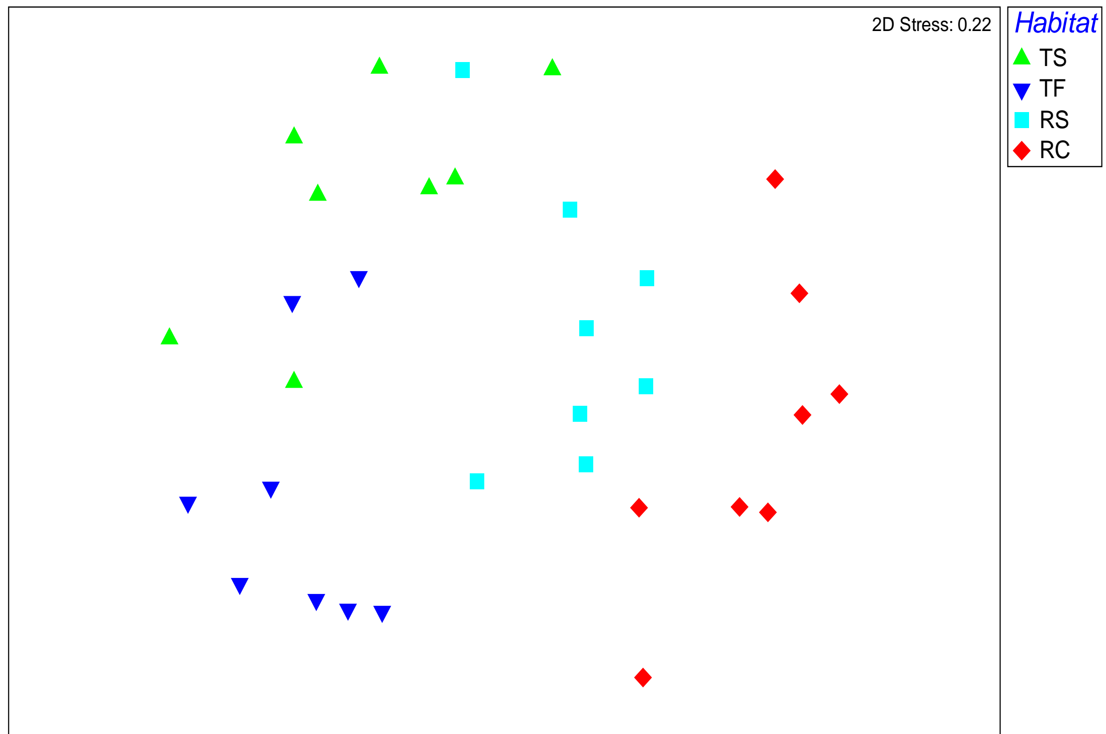


Fig. 4.3 Multidimensional scaling plot (PRIMER) of the epiphytic algal assemblages on the different basiphyte species collected from the two locations on Anglesey. Analysis is based upon the presence/absence data of species and on Bray-Curtis similarities. Samples are identified by location and host basiphyte: TS = *S. muticum*, Tal y Foel; TF = *F. vesiculosus*, Tal y Foel; RS = *S. muticum*, Rhosneigr; RC = *Cystoseira* spp., Rhosneigr.

Table. 4.3 Similarity percentage (SIMPER) analysis of the epiphytic algal communities on the different basiphyte host species collected from the two contrasting habitats on Anglesey. Average epiflora community similarities among replicate samples in percent and the relative contribution of dominant species (to cumulative 50%) in replicate samples. Analyses based on presence/absence data. TS = *S. muticum*, Tal y Foel; TF = *F. vesiculosus*, Tal y Foel; RS = *S. muticum*, Rhosneigr; RC = *Cystoseira* spp., Rhosneigr.

Host basiphyte (average similarity %)	**TS 40.74%**	**% contribution**	**TF 47.77%**	**% contribution**	**RS 42.36%**	**% contribution**	**RC 46.69%**	**% contribution**
Species dominating epiflora	Ectocarpoid spp.	37.52	Ectocarpoid spp.	34.12	Ectocarpoid spp.	24.64	*Elachista* spp.	15.97
	Polysiphonia spp. 1	20.48	*Cladophora* spp.	16.39	*Ceramium* spp.	18.30	*Boergesniella fruticulosa*	11.77
					Dictyota dichotoma	8.00	*Ceramium* spp.	11.63
							Gelidium spp.	8.76
							Stilophora spp.	8.31
Cumulative percentage > 50%	Σ	58.00	Σ	50.50	Σ	50.94	Σ	56.45

An examination of the associated epiphytic algal taxa classified into functional groups revealed no significant association between the proportional abundance of functional grouping with the different host macroalgal species (Chi-square, $\chi^2 = 10.127$, df = 12, $P = 0.450$, see Fig. 4.4). A similar non-significant relationship was also apparent when the epiphytic algae were grouped on the basis of maximum size (Chi-square, $\chi^2 = 5.694$, df = 9, $P = 0.770$, see Fig. 4.5).

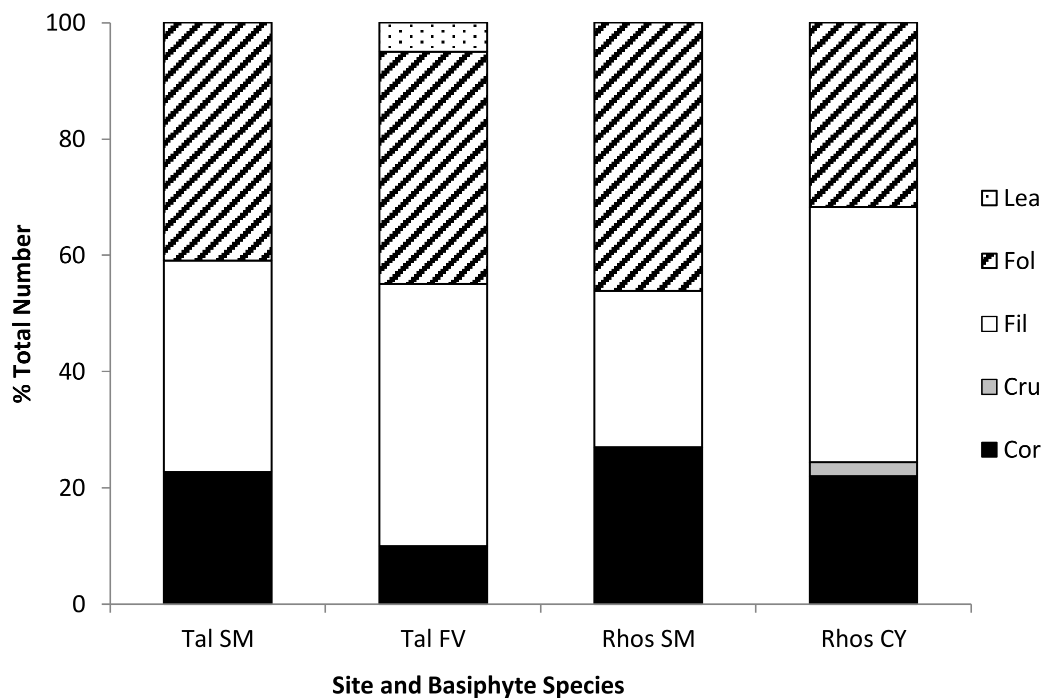


Fig. 4.4 Composition of epiphytic algal functional groups associated with the different macroalgal host seaweeds examined in this study. Data represent the average % composition extrapolated over all sample replicates from each macroalgal host. Lea = leathery, Fol = foliose, Fil = filamentous, Cru = crustose and Cor = corticated.

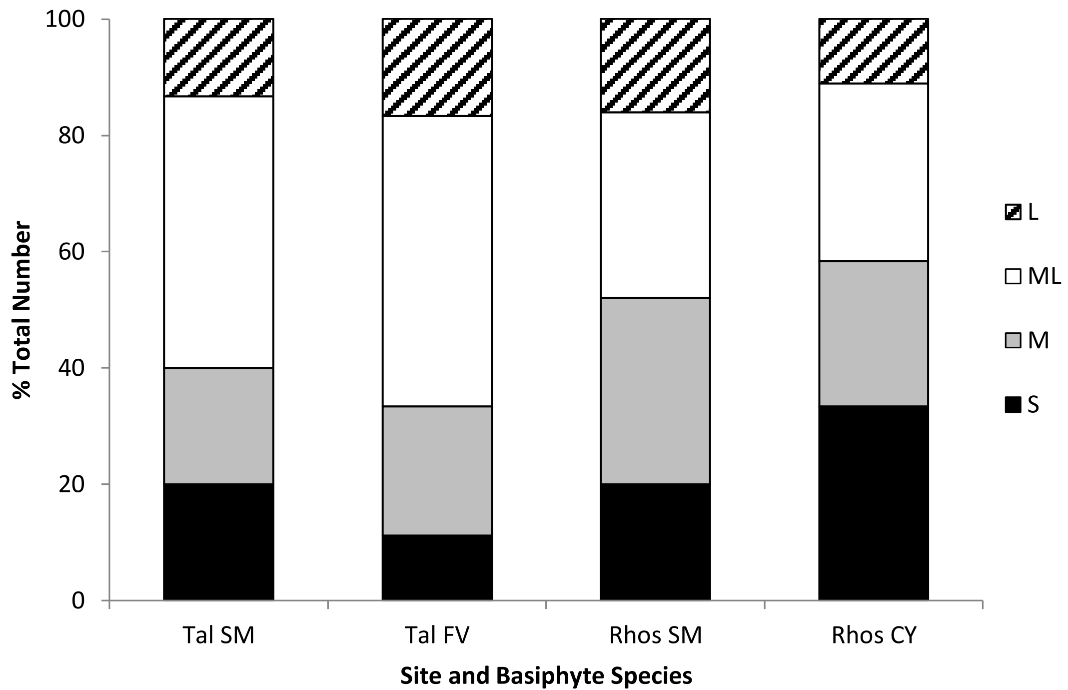


Fig. 4.5 Size composition of epiphytic algal taxa associated with the different macroalgal host seaweeds examined in this study. Data represent the average % composition extrapolated over all sample replicates from each macroalgal host. S = small (3-10 cm), M = medium (11-20 cm), ML = medium-large (21-50 cm) and L = large (>50 cm).

4.3.2 Sessile epiphytic fauna on native and invasive basiphyte hosts

Due to the disparities in abundance measurements of several of the sessile fauna (e.g. all individual *Spirorbis* spp. tubes were counted whereas only the number of discrete patches of species such as *Alyconidium* spp. were counted), only the presence or absence of a species was used in the analysis of the results. Overall, a total of 17 sessile epifauna were identified from all four basiphyte groupings. The mean number of sessile epiphytic faunal species per host basiphyte is presented in Fig. 4.6.

The basiphyte *Fucus vesiculosus* had the greatest species richness of sessile epiphytic fauna than compared with the other basiphyte species, with up to 17x more species per basiphyte than found on samples of *S. muticum* from the same location. This difference may be attributed to the structural difference between the two host basiphytes, with the large, flatter fronds of *F. vesiculosus* providing more suitable space for colonisation by sessile fauna such as tube worms, bryozoans and hydroids.

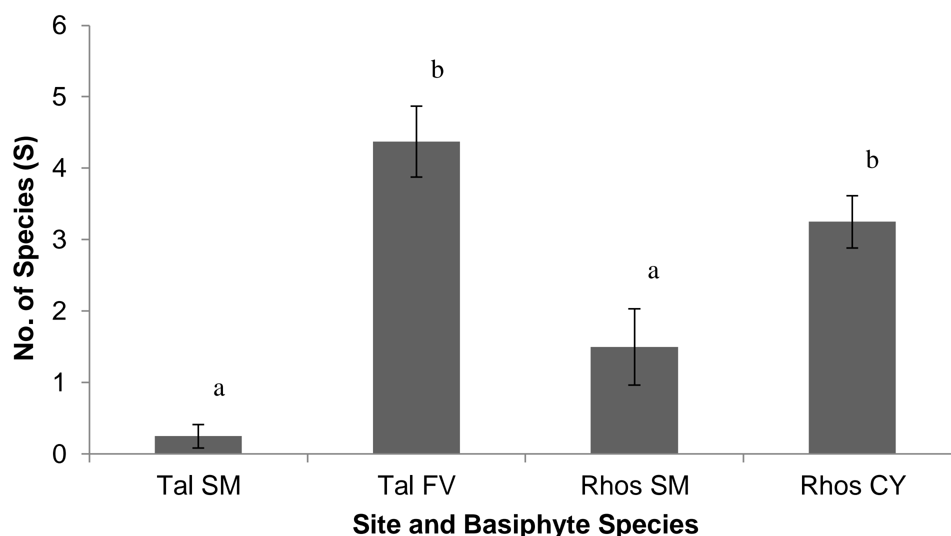


Fig. 4.6 Mean (±1 S.E.) number of species of sessile epifauna per host basiphyte sampled from the two differing habitat locations. One-way ANOVA, $F_{3,28}$ = 19.289, P < 0.001. Bars sharing the same letter above are not significantly different from each other as identified by *post hoc* Tukey tests.

ANOSIM analysis within PRIMER generated a significant overall *R*-value (Global *R*-value = 0.523, P < 0.001). However, pairwise community comparisons revealed no significant differences between *S. muticum* basiphytes from the two sampled locations (Table 4.4). Furthermore, no community differences were detected between the two basiphyte species from Rhosneigr. However, in each location the native basiphyte had a significantly greater number of sessile epifauna per thallus than in comparison to the invasive *S. muticum*. With regard to the individual species present in the assemblages, several individuals of *S. muticum* from Tal y Foel had the most distinct community. This can be attributed to the lack relative lack of sessile fauna (hence lack of data) colonising *S. muticum* from Tal y Foel. In fact, only two basiphyte specimens (TS3 and TS8) collected had been colonised by a single sessile species of epifauna. Furthermore, the results of a CLUSTER analysis in PRIMER (data not shown) demonstrated a relatively clear segregation of the communities associated with *F. vesiculosus* than compared with the other basiphytes.

SIMPER analysis showed that the sessile epifauna on *F. vesiculosus* was characterised predominantly by *Spirorbis* spp. and hydroids of the genus *Obelia* (Table 4.5). In contrast, the sessile epiphytic fauna of other three basiphyte groupings were

dominated by juvenile bivalves (Mytilidae spp.). The colonial ascidian, *Diplosoma* spp. also constituted a significant component of the epiphytic community of *Cystoseira* spp. thalli. Samples of *S. muticum* from Tal y Foel displayed a low overall average similarity (7.69%) due to the absence of sessile fauna from the majority of sampled basiphytes (i.e. sessile fauna was only identified from two of the 8 samples collected; see Appendix C.1).

Table. 4.4 Pairwise ANOSIM comparisons of sessile epiphytic faunal composition on the various basiphyte species collected from the two contrasting habitats on Anglesey. Due to the occurrence of multiple hypothesis testing, a *P* value of 0.008 has been used for the global significance threshold. Species and site codes: TS = *S. muticum*, Tal y Foel; TF = *F. vesiculosus*, Tal y Foel; RS = *S. muticum*, Rhosneigr; RC = *Cystoseira* spp., Rhosneigr.

	***R* Value from one-way ANOSIM**		
	TF	RS	RC
TS	0.707 $P = 0.001$	0.207 $P = 0.032$	0.464 $P = 0.001$
TF		0.800 $P = 0.002$	0.855 $P = 0.001$
RS			0.040 $P = 0.235$

ANOSIM, one-way, sample statistic (Global R): 0.523, $P < 0.001$

Table. 4.5 Similarity percentage (SIMPER) analysis of the sessile epiphytic faunal communities on the different basiphyte host species collected from the two contrasting habitats on Anglesey. Average epifaunal community similarities among replicate samples in percent and the relative contribution of dominant species (to cumulative 50%) in replicate samples. Analyses based on presence/absence data. TS = *S. muticum*, Tal y Foel; TF = *F. vesiculosus*, Tal y Foel; RS = *S. muticum*, Rhosneigr; RC = *Cystoseira* spp., Rhosneigr.

Host basiphyte (average similarity %)	**TS 7.69%**	**% contribution**	**TF 60.15%**	**% contribution**	**RS 41.33%**	**% contribution**	**RC 47.81%**	**% contribution**
Species dominating epifauna	Mytilidae spp.	100.00	*Spirorbis* spp.	39.70	Mytilidae spp.	88.89	Mytilidae spp.	49.87
			Obelia spp.	21.68			*Diplosoma* spp.	36.09
Cumulative percentage > 50%	Σ	100.00	Σ	61.38	Σ	88.89	Σ	85.95

4.3.3 Mobile epiphytic fauna on native and invasive basiphyte hosts

All univariate analyses were conducted on the raw abundance data set (Appendix C.1). In contrast, the multivariate community analysis of the associated mobile epiphytic fauna was performed on several manipulations of the raw data set before constructing a Bray-Curtis similarity matrix. A total of 6416 individuals of mobile fauna belonging to 52 different taxa were identified from host basiphytes from both sampled locations (Appendix C.1). Crustaceans, specifically amphipods were the most abundant group of organisms from all basiphyte species sampled, with gastropod mollusc species constituting the majority of the remaining faunal abundance. In terms of the mean species number per host basiphyte, the only significant differences occurred between the two contrasting locations with significantly more species being recorded from basiphyte samples collected from Rhosneigr (Table 4.6). In contrast, no significant differences were detected in the total number of individuals present on each basiphyte sample, although samples of *F. vesiculosus* did have fewer numbers of individuals than compared to all the other basiphytes. In a similar trend to species number, the species richness of basiphyte samples from Rhosneigr were significantly larger than those from Tal y Foel, but within a location species richness levels were comparable between invasive and native basiphytes (Table 4.6). The only significant differences in Shannon diversity index per thallus occurred between *S. muticum* samples from Tal y Foel which were considerably lower than the other three basiphyte groupings (Table 4.6); this can in part be explained by the significantly lower evenness value for *S. muticum* samples from Tal y Foel, which resulted from the high community abundance not being distributed evenly among the resident species in these samples (on average 85% of the individuals were one species; see Appendix C.1). Finally, the overall abundance of mobile epifauna was positively correlated with both the biomass of associated epiphytic algae (Spearman correlation $R = 0.526$, $P = 0.002$) and the species richness of epiphytes (Pearson correlation $R = 0.623$, $P < 0.001$).

4.3.3.1 Epifauna abundance data transformed into presence/absence

Results of the ANOSIM analysis (PRIMER) of the mobile epifaunal communities indicated that all comparisons were significantly different between the basiphyte groupings (Global R-value: 0.817, $P < 0.001$; Table 4.7). The epiphytic faunal

communities associated with *F. vesiculosus* and *S. muticum* from Tal y Foel showed a clear distinction. In contrast, a degree of overlap in community similarity between the two basiphytes from Rhosneigr was evident from the results of a CLUSTER analysis (Fig. 4.7). The significant differences detected from the results of the one-way ANOSIM were confirmed from a MDS sample plot based on community similarities (Fig. 4.8). All basiphyte groupings showed discrete clustering on the plot with samples of *F. vesiculosus* from Tal y Foel forming a tighter cluster due to their higher community similarity as confirmed by the results of the CLUSTER analysis (Fig. 4.7).

The results of a SIMPER analysis indicated that species of the amphipod *Gammarus* appear to be characteristic of mobile epifaunal assemblages associated with basiphyte thalli growing within the Tal y Foel area of the Menai Strait on Anglesey (Table 4.8). In contrast, the amphipods *Dexamine spinosa* and *Ampithoe* spp. were species characteristic of the mobile epifaunal community associated with basiphyte thalli from Rhosneigr. The presence of the amphipod *Caprella acanthifera* distinguished *Sargassum muticum* thalli sampled from both locations from all other host basiphyte species. However, irrespective of the basiphyte species and location, crab megalopae larvae were a common component of the associated communities of epiphytic flora and fauna (Table 4.8).

Table. 4.6 Univariate descriptive statistics for the mobile epiphytic faunal diversity per thallus on the different macroalgal basiphytes species collected from Tal y Foel and Rhosneigr on Anglesey in June 2010.

Species	**Tal y Foel**		**Rhosneigr**	
	S. muticum	*F. vesiculosus*	*S. muticum*	*Cystoseira* spp.
No. of replicate basiphyte thalli	8	8	8	8
No. of species (±S.D.)	8.13 (±1.81)[a]	8.25 (±1.58)[a]	12.25 (±2.60)[b]	11.75 (±2.76)[b]
No. of individuals (±S.D.)	223.0 (±136.04)[a]	124.0 (±70.86)[a]	227.13 (±100.14)[a]	227.88 (±173.41)[a]
Shannon diversity index (±S.D.)	0.601 (±0.344)[a]	1.308 (±0.226)[b]	1.400 (±0.254)[b]	1.436 (±0.270)[b]
Margalef's index (±S.D.)	1.373 (±0.312)[a]	1.532 (±0.253)[a]	2.113 (±0.435)[b]	2.085 (±0.440)[b]
Pielou's eveness index (±S.D.)	0.299 (±0.195)[a]	0.631 (±0.134)[b]	0.565 (±0.101)[b]	0.590 (±0.108)[b]

ANOVA analyses: No. of species: One-way ANOVA, $F_{3,28} = 7.747$, $P < 0.001$; No. of individuals: One-way ANOVA, $F_{3,28} = 1.310$, $P = 0.291$; Shannon diversity: One-way ANOVA, $F_{3,28} = 16.529$, $P < 0.001$; Margalef's index: One-way ANOVA, $F_{3,28} = 8.445$, $P < 0.001$; Pielou's eveness: One-way ANOVA, $F_{3,28} = 9.345$, $P < 0.001$. Indices sharing the same superscript letter are not significantly different from each other as identified by *post hoc* Tukey tests.

Table. 4.7 Pairwise ANOSIM comparisons of mobile epiphytic faunal composition on the various basiphyte species collected from the two contrasting habitats on Anglesey. Due to the occurrence of multiple hypothesis testing, a *P* value of 0.008 has been used for the global significance threshold. Analyses based on presence/absence data. Species and site codes: TS = *S. muticum*, Tal y Foel; TF = *F. vesiculosus*, Tal y Foel; RS = *S. muticum*, Rhosneigr; RC = *Cystoseira* spp., Rhosneigr.

	***R* Value from one-way ANOSIM**		
	TF	RS	RC
TS	0.685 $P = 0.001$	0.624 $P = 0.001$	0.864 $P = 0.001$
TF		1.000 $P = 0.001$	0.999 $P = 0.001$
RS			0.593 $P = 0.001$

ANOSIM, one-way, sample statistic (Global R): 0.819, $P < 0.001$

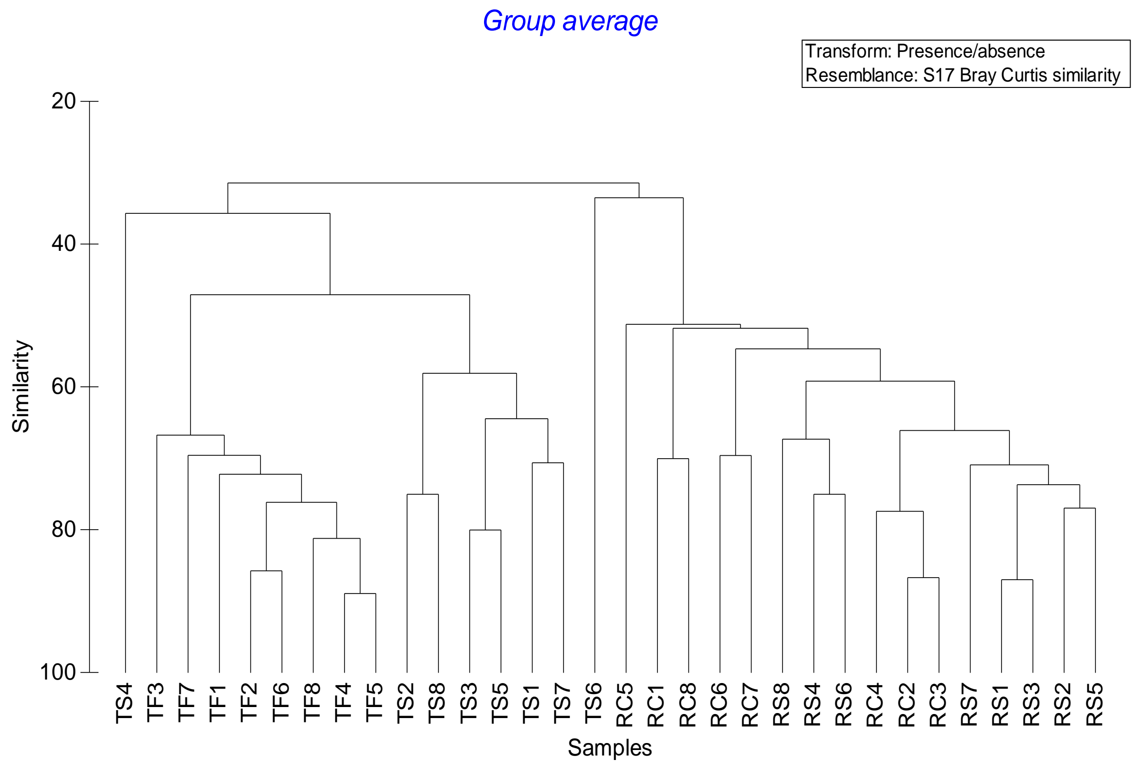


Fig. 4.7 A similarity dendrogram (CLUSTER analysis) of the mobile epiphytic faunal assemblages on the samples of the four different basiphytes collected from the two locations on Anglesey. Analysis is based upon the presence/absence data of species and on Bray-Curtis similarities. Species and site codes: TS = *S. muticum*, Tal y Foel; TF = *F. vesiculosus*, Tal y Foel; RS = *S. muticum*, Rhosneigr; RC = *Cystoseira* spp., Rhosneigr.

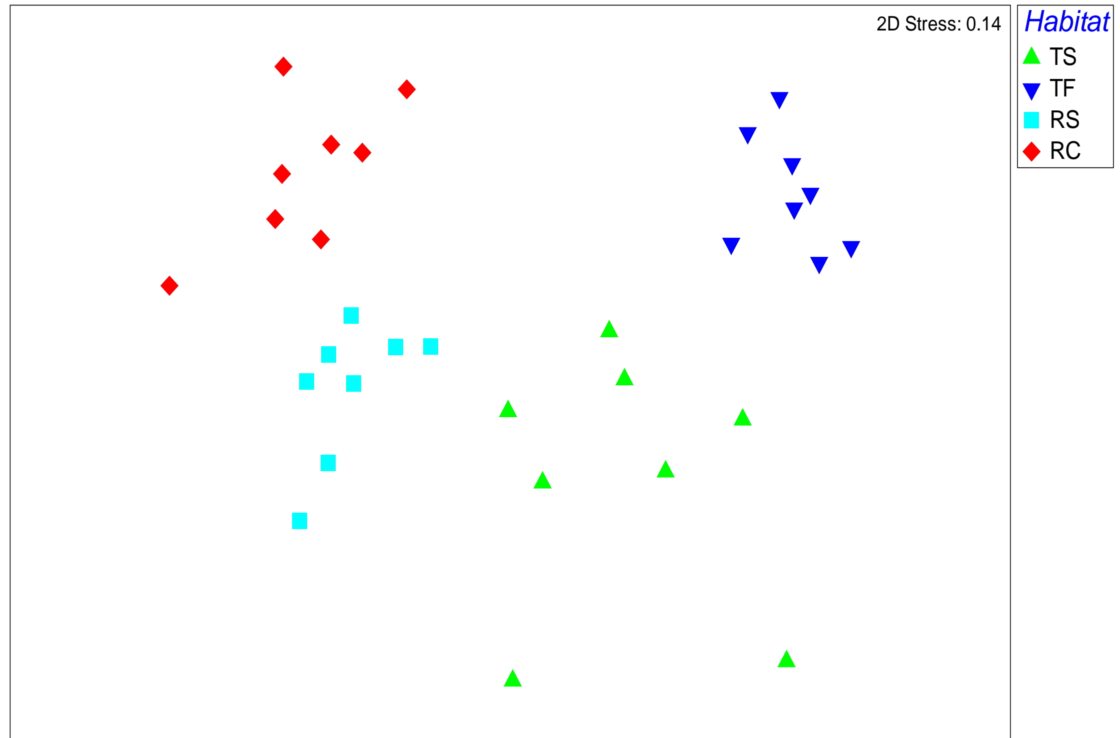


Fig. 4.8 Multidimensional scaling plot (PRIMER) of the mobile epiphytic faunal assemblages on the different basiphyte species collected from the two locations on Anglesey. Analysis is based upon the presence/absence data of species and on Bray-Curtis similarities. Samples are identified by location and host basiphyte: TS = *S. muticum*, Tal y Foel; TF = *F. vesiculosus*, Tal y Foel; RS = *S. muticum*, Rhosneigr; RC = *Cystoseira* spp., Rhosneigr.

Table 4.8. Similarity percentage (SIMPER) analysis of the mobile epiphytic faunal communities on the different basiphyte host species collected from the two contrasting habitats on Anglesey. Average epifaunal community similarities among replicate samples in percent and the relative contribution of dominant species (to cumulative 50%) in replicate samples. Analyses based on presence/absence data. TS = *S. muticum*, Tal y Foel; TF = *F. vesiculosus*, Tal y Foel; RS = *S. muticum*, Rhosneigr; RC = *Cystoseira* spp., Rhosneigr.

Host basiphyte (average similarity %)	**TS 52.78%**	**% contribution**	**TF 72.82%**	**% contribution**	**RS 68.52%**	**% contribution**	**RC 61.31%**	**% contribution**
Species dominating epifauna	*Gammarus* spp.	23.78	*Gammarus locusta*	16.87	*Dexamine spinosa*	7.38	*Ampithoe* spp.	14.16
	Crab megalopae	23.78	*Gammarus* spp.	16.87	*Ampithoe* spp.	7.38	*Dexamine spinosa*	14.16
	Caprella acanthifera	16.74	Crab megalopae	16.87	*Caprella acanthifera*	7.38	Crab megalopae	14.16
			Littorina mariae	16.87	*Dynamene bidentata*	7.38	*Rissoa parva*	14.16
					Crab megalopae	7.38	Nereidae spp. 1	10.41
Cumulative percentage > 50%	Σ	64.30	Σ	67.49	Σ	60.60	Σ	67.06

4.3.3.2 Epifauna abundance data square-root transformed

Square-root transformation of the raw abundance data was applied to remove effects of highly abundant species within the mobile epifauna community before construction of the Bray-Curtis similarity matrix. The results of ANOSIM analysis of transformed mobile epifauna abundance also showed significant differences in epifaunal community structure between the four basiphyte groupings (Global *R*-value: 0.864, $P < 0.001$; Table 4.9). Of the pairwise community comparisons the two basiphytes from Rhosneigr showed the weakest *R*-value although the magnitude of the difference was still significant. (*R*-value = 0.497, P = 0.001).

Table. 4.9 Pairwise ANOSIM comparisons of mobile epiphytic faunal composition on the various basiphyte species collected from the two contrasting habitats on Anglesey. Due to the occurrence of multiple hypothesis testing, a *P* value of 0.008 has been used for the global significance threshold. Analyses based on square-root transformed abundance data. Species and site codes: TS = *S. muticum*, Tal y Foel; TF = *F. vesiculosus*, Tal y Foel; RS = *S. muticum*, Rhosneigr; RC = *Cystoseira* spp., Rhosneigr.

	***R* Value from one-way ANOSIM**		
	TF	RS	RC
TS	0.768 *P* = 0.001	0.994 *P* = 0.001	0.997 *P* = 0.001
TF		1.000 *P* = 0.001	1.000 *P* = 0.001
RS			0.497 *P* = 0.001

ANOSIM, one-way, sample statistic (Global R): 0.864, $P < 0.001$

CLUSTER analysis showed a clear segregation in the mobile epifaunal community similarity between the basiphyte species from the two sampled locations on Anglesey (Fig. 4.9). Further sub-clustering of the samples based on their respective basiphyte grouping was also evident with an average Bray-Curtis similarity value of ~60%. A two-dimensional mNDS ordination plot based on community similarities also showed discrete clustering of the samples based on their basiphyte groupings (Fig. 4.10). Samples from the two basiphyte species collected from Rhosneigr exhibited a degree of overlap based on the epifaunal community similarity; a result reflected by the lower *R*-value obtained from the pairwise ANOSIM results (Table 4.9).

SIMPER analysis identified four taxa as important contributing in discriminating between the different basiphyte groupings (Table 4.10). Comparable to the SIMPER analysis based on presence/absence transformed data, species of the amphipod *Gammarus* were characteristic of mobile epifaunal assemblages associated with the two basiphyte species sampled from Tal y Foel. On the other hand, the amphipods *Dexamine spinosa* and *Ampithoe* spp. were species characteristic of the mobile epifaunal community associated with basiphyte thalli from Rhosneigr.

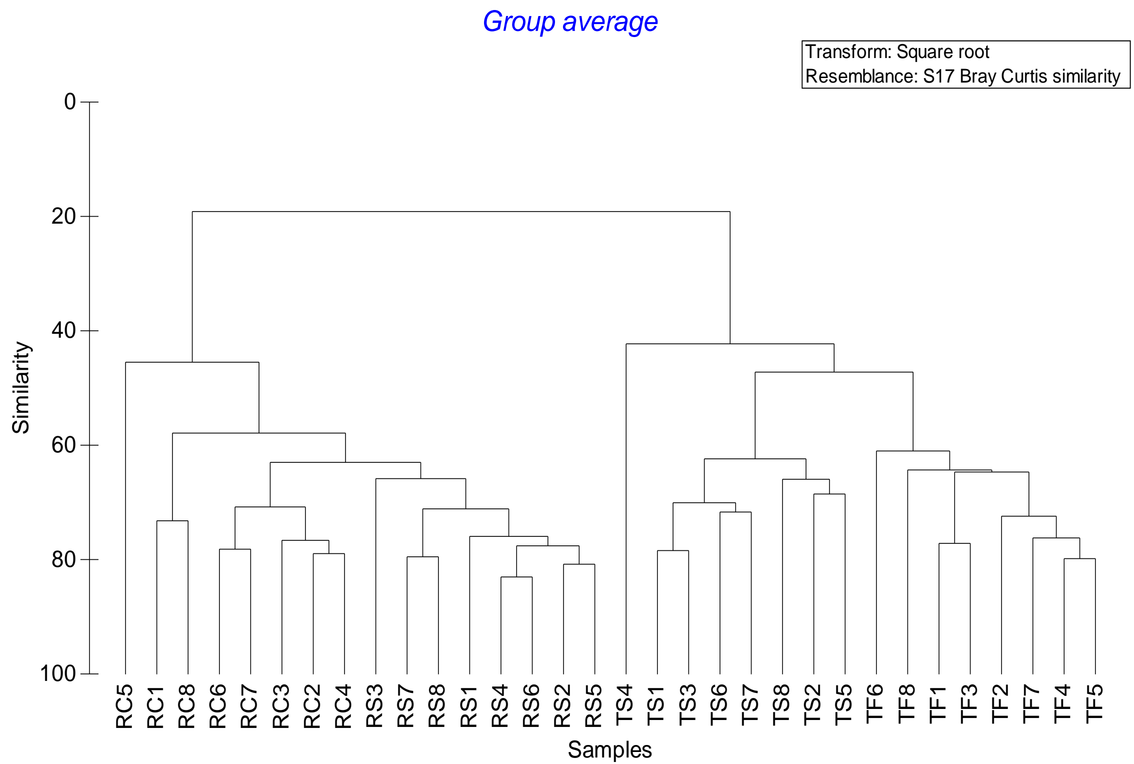


Fig. 4.9 A similarity dendrogram (CLUSTER analysis) of the mobile epiphytic faunal assemblages on the samples of the four different basiphytes collected from the two locations on Anglesey. Analysis is based upon the square-root transformation of abundance data and on Bray-Curtis similarities. Species and site codes: TS = *S. muticum*, Tal y Foel; TF = *F. vesiculosus*, Tal y Foel; RS = *S. muticum*, Rhosneigr; RC = *Cystoseira* spp., Rhosneigr.

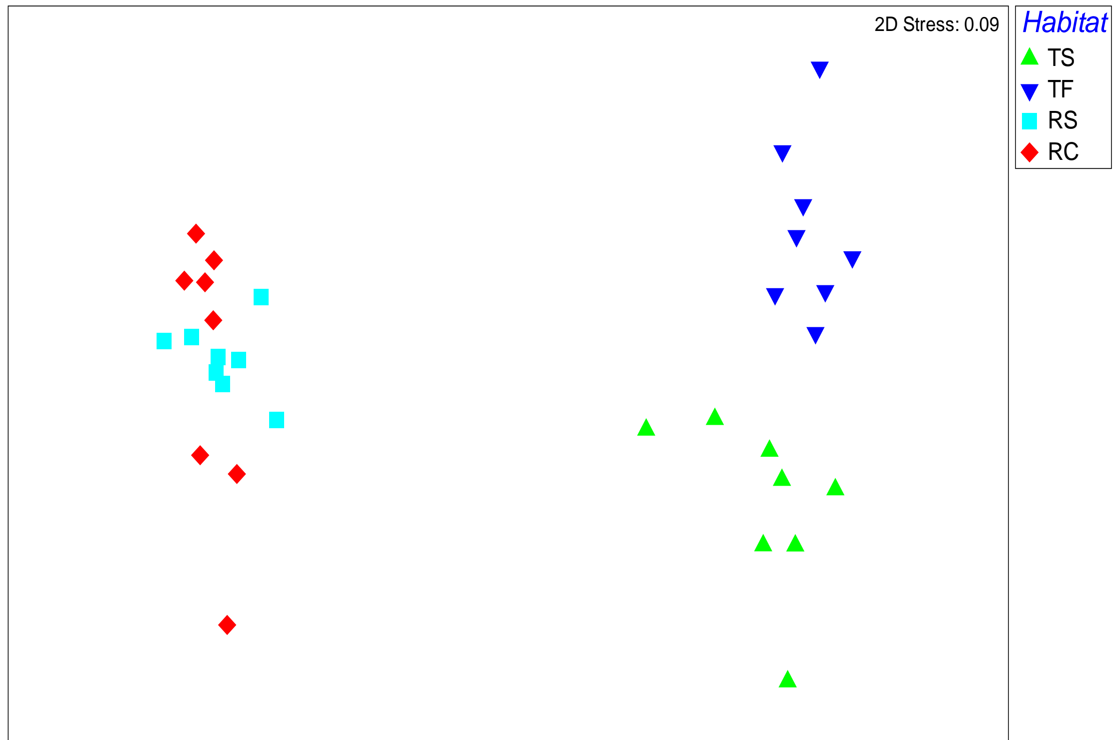


Fig. 4.10 Multidimensional scaling plot (PRIMER) of the mobile epiphytic faunal assemblages on the different basiphyte species collected from the two locations on Anglesey. Analysis is based upon the square-root transformation of abundance data and on Bray-Curtis similarities. Samples are identified by location and host basiphyte: TS = *S. muticum*, Tal y Foel; TF = *F. vesiculosus*, Tal y Foel; RS = *S. muticum*, Rhosneigr; RC = *Cystoseira* spp., Rhosneigr.

Table. 4.10 Similarity percentage (SIMPER) analysis of the mobile epiphytic faunal communities on the different basiphyte host species collected from the two contrasting habitats on Anglesey. Average epifaunal community similarities among replicate samples in percent and the relative contribution of dominant species (to cumulative 50%) in replicate samples. Analyses based on square root transformed data. TS = *S. muticum*, Tal y Foel; TF = *F. vesiculosus*, Tal y Foel; RS = *S. muticum*, Rhosneigr; RC = *Cystoseira* spp., Rhosneigr.

Host basiphyte (average similarity %)	**TS 60.34%**	**% contribution**	**TF 66.27%**	**% contribution**	**RS 72.43%**	**% contribution**	**RC 61.81%**	**% contribution**
Species dominating epiflora	*Gammarus* spp.	67.72	*Gammarus locusta*	36.00	*Dexamine spinosa*	31.38	*Dexamine spinosa*	31.73
			Gammarus spp.	18.78	*Ampithoe* spp.	25.05	*Ampithoe* spp.	27.27
Cumulative percentage > 50%	Σ	67.72	Σ	54.79	Σ	56.43	Σ	59.00

4.3.3.3 Epifauna abundance data corrected to biomass (dry weight) of host basiphyte

When the raw abundance data was standardised against the biomass of the host basiphyte sample a large range, on the order to magnitude of abundance was still evident between the values for several samples. Therefore a further square-root transformation of the data was undertaken before constructing the similarity matrix and running the multivariate analyses. All of the analyses as performed with the previous version of the data resulted in a similar set of results. ANOSIM testing indicated that all comparisons were significantly different between different basiphyte groupings (Global R-value: 0.885, $P < 0.001$). Furthermore both the CLUSTER analysis (not shown) and the nMDS plot yielded similar patterns in terms of mobile epifaunal community similarly among basiphyte samples (Fig. 4.11). SIMPER analysis also yielded similar results, again with the discrete distinction between the mobile faunal communities from the two sampled locations based upon the dominance of specific amphipod species (results not shown).

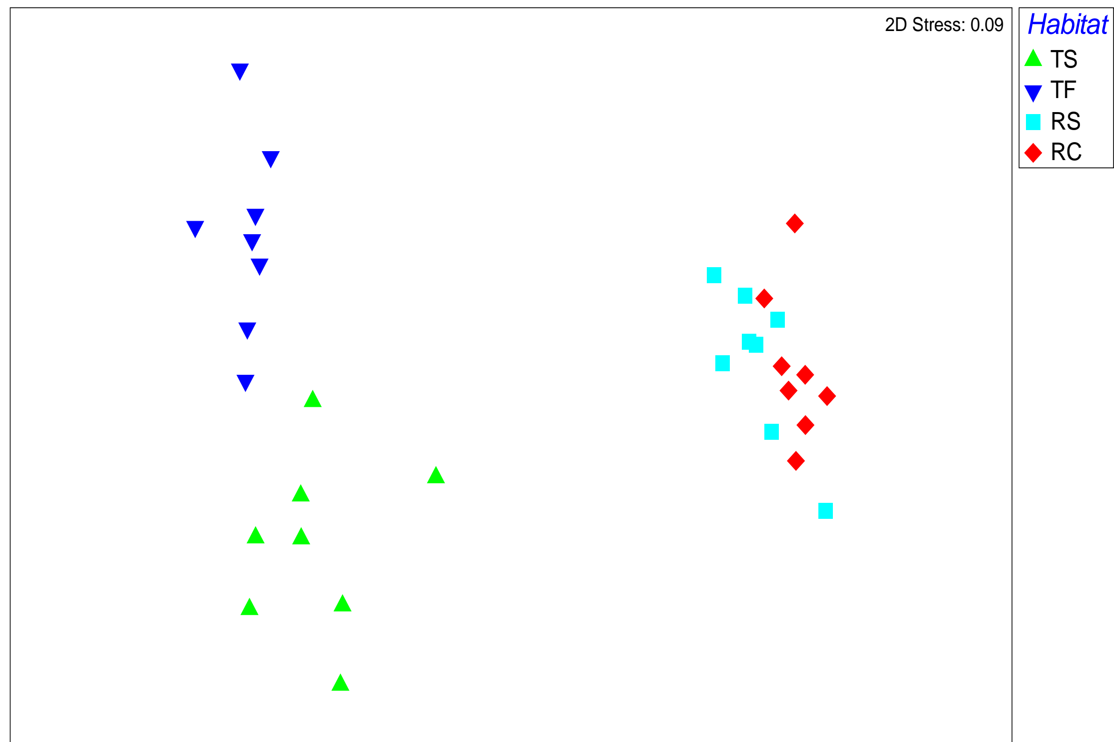


Fig. 4.11 Multidimensional scaling plot (PRIMER) of the mobile epiphytic faunal assemblages on the different basiphyte species collected from the two locations on Anglesey. Analysis is based upon the square-root transformation of abundance data corrected to biomass of host basiphyte and on Bray-Curtis similarities. Samples are identified by location and host basiphyte: TS = *S. muticum*, Tal y Foel; TF = *F. vesiculosus*, Tal y Foel; RS = *S. muticum*, Rhosneigr; RC = *Cystoseira* spp., Rhosneigr.

The trophic status of around 85% of the mobile epifauna was characterised on all four basiphyte seaweed hosts combined. A significant contrast between the seaweeds from the two differing habitats on Anglesey with regards to the predominant feeding mode of the associated mobile epifauna was noted from an examination of feeding habits (Chi-square, $\chi^2 = 76.250$, df = 12, $P < 0.001$). The two host basiphytes from Tal y Foel in the Menai Strait were characterised by a majority of omnivores (Fig 4.15). In contrast, at Rhosneigr herbivorous species constituted the bulk of the associated mobile epifauna.

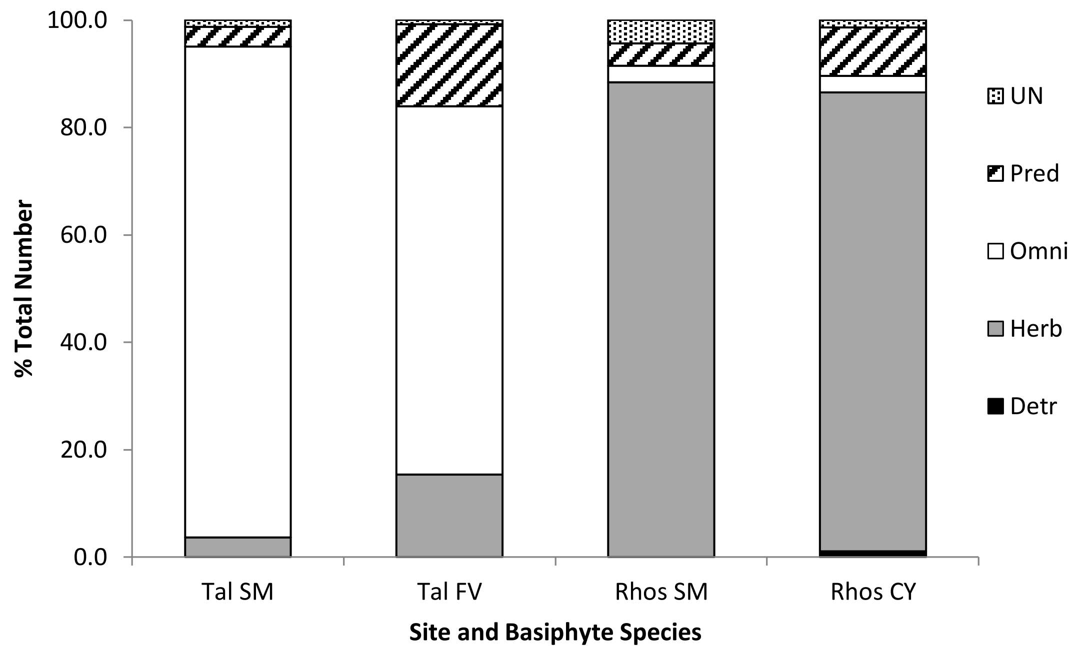


Fig. 4.12 Feeding type composition of typical mobile epifauna associated with the different macroalgal host seaweeds examined in this study. Data represent the average % composition extrapolated over all sample replicates from each macroalgal host. Detr = Detritivore, Herb = Herbivore, Omni = Omnivore, Pred = Predator and UN = unknown.

4.3.4 Total epifauna on native and invasive basiphyte hosts

Due to the differing abundance measurements between several species of the epifauna (e.g. some recorded as the number of total individuals, whereas others recorded as the number of discrete patches/colonies) analysis of total epifauna was conducted on presence/absence transformed data. Overall a total of 69 different faunal taxa were identified from host basiphytes from both sampled locations (Appendix C). The mean number of epifauna per individual basiphyte is shown in Fig. 4.13. The mean number of epifaunal species per plant was significantly lower on *Sargassum muticum* thalli sampled from Tal y Foel than on any of the other basiphyte groupings.

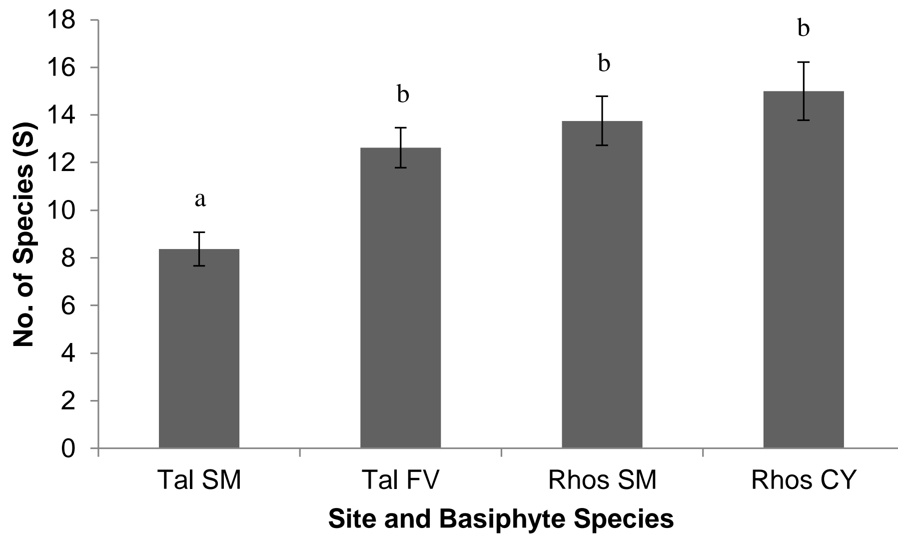


Fig. 4.13 Mean (±1 S.E.) number of species of epifauna per host basiphyte sampled from the two differing habitat locations. One-way ANOVA, $F_{3,28} = 8.859$, $P < 0.001$. Bars sharing the same letter above are not significantly different from each other as identified by *post hoc* Tukey tests.

Results from an ANOSIM analysis revealed that epifaunal communities on the four different basiphyte groupings were all significantly different in composition (Global *R*-value = 0.848, $P < 0.001$; Table 4.11). With regard to the individual species present in the epifaunal assemblages, samples of *Fucus vesiculosus* had the most distinct community, as evidenced from the branching pattern of the CLUSTER analysis (Fig. 4.14). Samples of *Sargassum muticum* from Tal y Foel also displayed a clear segregation from the other basiphyte groupings, although levels of within sample community similarity were lower for this species. These results were confirmed from the two-dimensional nMDS ordination plot of epifaunal community similarity among basiphyte samples (Fig. 4.15)

Table. 4.11 Pairwise ANOSIM comparisons of total epifauna composition on the various basiphyte species collected from the two contrasting habitats on Anglesey. Due to the occurrence of multiple hypothesis testing, a *P* value of 0.008 has been used for the global significance threshold. Analyses based on presence/absence transformed data. Species and site codes: TS = *S. muticum*, Tal y Foel; TF = *F. vesiculosus*, Tal y Foel; RS = *S. muticum*, Rhosneigr; RC = *Cystoseira* spp., Rhosneigr.

	***R* Value from one-way ANOSIM**		
	TF	RS	RC
TS	0.842 $P = 0.001$	0.631 $P = 0.001$	0.894 $P = 0.001$
TF		1.000 $P = 0.001$	1.000 $P = 0.001$
RS			0.608 $P = 0.001$

ANOSIM, one-way, sample statistic (Global R): 0.848, $P < 0.001$

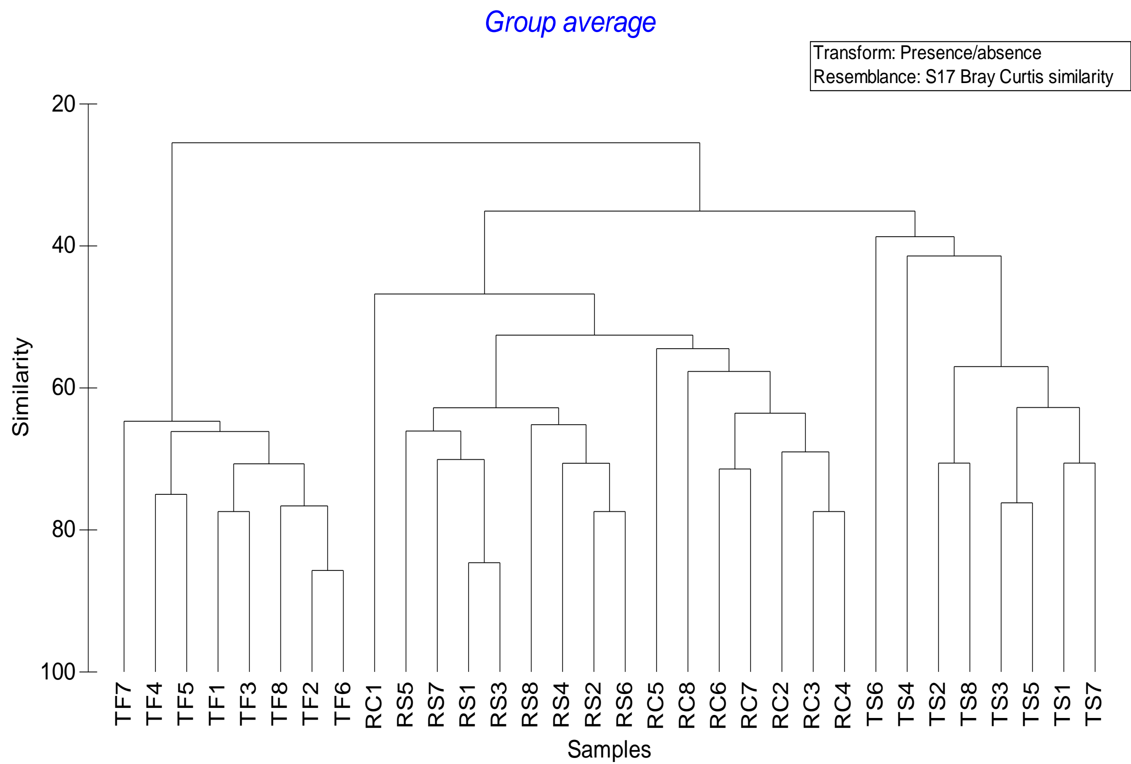


Fig. 4.14 A similarity dendrogram (CLUSTER analysis) of the total epiphytic faunal assemblages on the samples of the four different basiphytes collected from the two locations on Anglesey. Analysis is based upon the presence/absence transformation of abundance data and on Bray-Curtis similarities. Species and site codes: TS = *S. muticum*, Tal y Foel; TF = *F. vesiculosus*, Tal y Foel; RS = *S. muticum*, Rhosneigr; RC = *Cystoseira* spp., Rhosneigr.

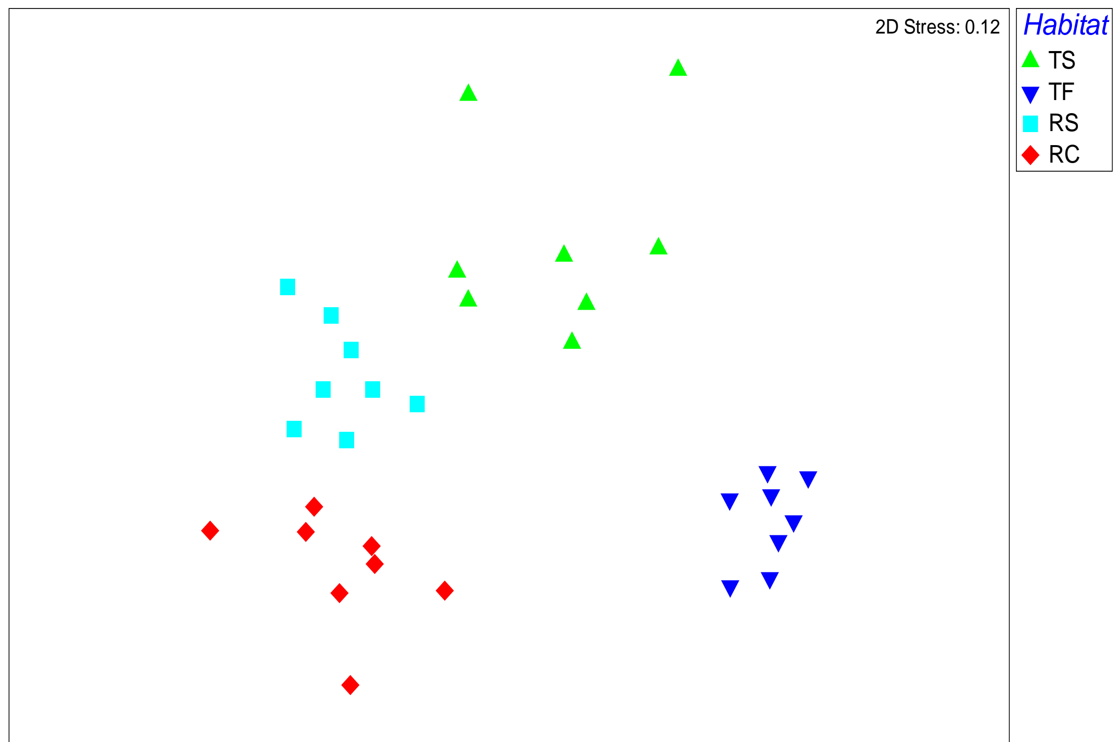


Fig. 4.15 Multidimensional scaling plot (PRIMER) of the total epiphytic faunal assemblages on the different basiphyte species collected from the two locations on Anglesey. Analysis is based upon the presence/absence transformation of abundance data and on Bray-Curtis similarities. Samples are identified by location and host basiphyte: TS = *S. muticum*, Tal y Foel; TF = *F. vesiculosus*, Tal y Foel; RS = *S. muticum*, Rhosneigr; RC = *Cystoseira* spp., Rhosneigr.

Results of the SIMPER analysis revealed similar trends in terms of characteristic species to those obtained from the SIMPER analysis of mobile epifauna presence/absence data. The only addition was the inclusion of *Spirorbis* spp. in the species list characteristic of the epifaunal assemblages associated with *Fucus vesiculosus* (Table 4.12). In terms of the epifauna, the most common groups of taxa were the amphipods with *Dexamine spinosa* and *Gammarus* spp. recorded in high numbers. The only taxon recorded from all samples was that of crab megalopae larvae (Table 4.12). Therefore it is likely that the host algae must play a significant role in the provision of shelter for new recruits of crustaceans. A distinction in the identity of the dominant gastropod species recorded between the two native basiphytes was also evident, with *Littorina mariae* and *Rissoa parva* characteristic of the epifaunal communities on *Fucus vesiculosus* and *Cystoseira* spp. respectively (Table 4.12). Over all basiphyte samples the most commonly recorded species of gastropod was that of *Littorina mariae* (Appendix C.1), with many of the individuals associated with the basiphyte *Fucus vesiculosus* larger in size than compared to those from the other basiphyte species sampled (P Hallas, personal observation).

Table. 4.12 Similarity percentage (SIMPER) analysis of the total epifaunal assemblages associated with the different basiphyte host species collected from the two contrasting habitats on Anglesey. Average epifauna community similarities among replicate samples in percent and the relative contribution of dominant species (to cumulative 50%) in replicate samples. Analyses based on presence/absence data. TS = *S. muticum*, Tal y Foel; TF = *F. vesiculosus*, Tal y Foel; RS = *S. muticum*, Rhosneigr; RC = *Cystoseira* spp., Rhosneigr.

Host basiphyte (average similarity %)	**TS 51.56%**	**% contribution**	**TF 68.92%**	**% contribution**	**RS 65.79%**	**% contribution**	**RC 58.35%**	**% contribution**
Species dominating epifauna	*Gammarus* spp.	23.69	*Spirorbis* spp.	11.65	*Dexamine spinosa*	11.25	*Ampithoe* spp.	11.64
	Crab megalopae	23.69	*Gammarus locusta*	11.65	*Ampithoe* spp.	11.25	*Dexamine spinosa*	11.64
	Caprella acanthifera	16.61	*Gammarus* spp.	11.65	*Caprella acanthifera*	11.25	Crab megalopae	11.64
			Crab megalopae	11.65	*Dynamene bidentata*	11.25	*Rissoa parva*	11.64
			Littorina mariae	11.65	Crab megalopae	11.25	Nereidae spp. 1	8.66
Cumulative percentage > 50%	Σ	63.99	Σ	58.23	Σ	56.27	Σ	55.24

4.3.5 Total epibiota on native and invasive basiphyte hosts

Similar to the analysis of total epifauna, the raw data for the complete epibiota was transformed into presence/absence before performing the various multivariate analyses. Overall a total of 126 different taxa were identified from the basiphytes samples collected from the two locations on Anglesey (Appendices C.1 and C.2). The total number of associated species encountered across replicate samples of the four basiphyte groupings ranged from 46 (*S. muticum* from Tal y Foel) to 82 (*Cystoseira* spp. from Rhosneigr) (Table 4.13). The average species richness of epibiota per sample differed significantly between basiphyte groupings (One-way ANOVA, $F_{3,28} = 15.771$, $P < 0.001$) with basiphyte samples of *Cystoseira* spp. possessing the greatest species richness, nearly double that found associated with *Sargassum muticum* thalli from Tal y Foel (Table 4.13).

Table. 4.13 Species richness (S) of epibiota on basiphyte algae collected from Tal y Foel and Rhosneigr on Anglesey. Means sharing the same superscript letter are not significantly different from each other as identified by *post hoc* Tukey tests.

	Overall S (all samples)			**Mean S per thallus**	
	Flora	**Fauna**	**Total**	**Mean (±SD)**	**n**
Tal y Foel					
Sargassum muticum	24	22	46	15.00 (2.78)^{a}	8
Fucus vesiculosus	20	27	47	19.13 (4.12)^{ab}	8
Rhosneigr					
Sargassum muticum	27	32	59	23.50 (4.47)^{bc}	8
Cystoseira spp.	42	40	82	28.75 (5.09)^{c}	8

Comparing the species communities of epibiota between the different basiphyte groupings, significant differences were revealed between all pairwise comparisons (Table 4.14). The ANOSIM procedure revealed an overall *R*-statistic of 0.888 ($P < 0.001$) for differences among epibiont communities from the four groups of samples. Significant differences in the assemblages of the associated epibionts were displayed between all pairwise grouping comparisons (significant at $P < 0.002$ with *R*-values between 0.667 and 1). The two groupings displaying the most similarity in terms of the associated epibiota were of the *Sargassum muticum* host basiphytes from the two sampled locations on Anglesey, although their pairwise *R*-value was still large and significant (*R*-value 0.667, $P = 0.001$).

Table. 4.14 Pairwise ANOSIM comparisons of total epibiota composition on the various basiphyte species collected from the two contrasting habitats on Anglesey. Due to the occurrence of multiple hypothesis testing, a *P* value of 0.008 has been used for the global significance threshold. Analyses based on presence/absence transformed data. Species and site codes: TS = *S. muticum*, Tal y Foel; TF = *F. vesiculosus*, Tal y Foel; RS = *S. muticum*, Rhosneigr; RC = *Cystoseira* spp., Rhosneigr.

	***R* Value from one-way ANOSIM**		
	TF	RS	RC
TS	0.870 $P = 0.001$	0.667 $P = 0.001$	0.974 $P = 0.001$
TF		1.000 $P = 0.001$	1.000 $P = 0.001$
RS			0.746 $P = 0.001$

ANOSIM, one-way, sample statistic (Global R): 0.888, $P < 0.001$

Results of a CLUSTER analysis indicated discrete clustering of samples based on their respective basiphyte grouping based on an average cut-off Bray-Curtis similarity value of around 40% (Fig. 4.16). Sample of the basiphyte species *Sargassum muticum*, from Rhosneigr and *Fucus vesiculosus* showed particularly high within sample community similarities forming discrete clusters in a two-dimensional nMDS ordination plot (Fig. 4.14). In addition to the segregation between the different basiphyte groupings, a clear separation on community similarity was evident from the two locations on Anglesey where the basiphytes samples were collected (Figs. 4.16 and 4.17).

Finally, by means of a SIMPER analysis, the main species responsible for similarities within and dissimilarities between the different basiphyte groupings were identified (Table 4.15). The average similarity within each vegetation type varied between 46.94% and 61.94% (Table 4.15), with the lowest similarity value observed in the *Sargassum muticum* from Tal y Foel (46.94%). In contrast, *Fucus vesiculosus* basiphyte samples collected from the same location displayed the highest level of community similarity (61.94%).Characteristic species of both epiphytic fauna and flora were similar as identified in the previous analysis of the separate data sets. Again a clear distinction in the dominant amphipod taxa present was evident between the basiphytes species from the two contrasting locations. The amphipod, *Caprella acanthifera* was characteristic of the epiphytic communities on *S. muticum* from both sampled locations on Anglesey (Table 4.15). With regards to the epiphytic algae the most notable distinction between the basiphyte groupings was the replacement of the dominant brown alga, Ectocarpoid spp. by *Elachista* spp. in samples of *Cystoseira* spp. from Rhosneigr (Table 4.15).

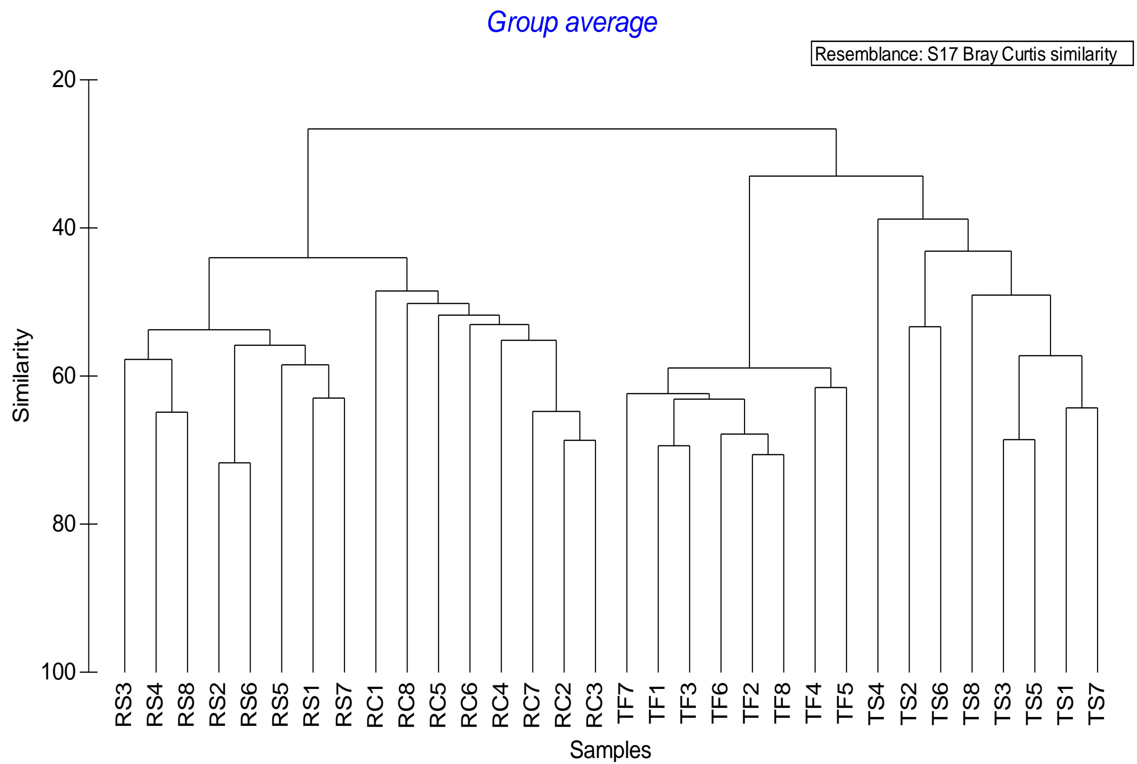


Fig. 4.16 A similarity dendrogram (CLUSTER analysis) of the total epibiota assemblages on the samples of the four different basiphytes collected from the two locations on Anglesey. Analysis is based upon the presence/absence transformation of abundance data and on Bray-Curtis similarities. Species and site codes: TS = *S. muticum*, Tal y Foel; TF = *F. vesiculosus*, Tal y Foel; RS = *S. muticum*, Rhosneigr; RC = *Cystoseira* spp., Rhosneigr.

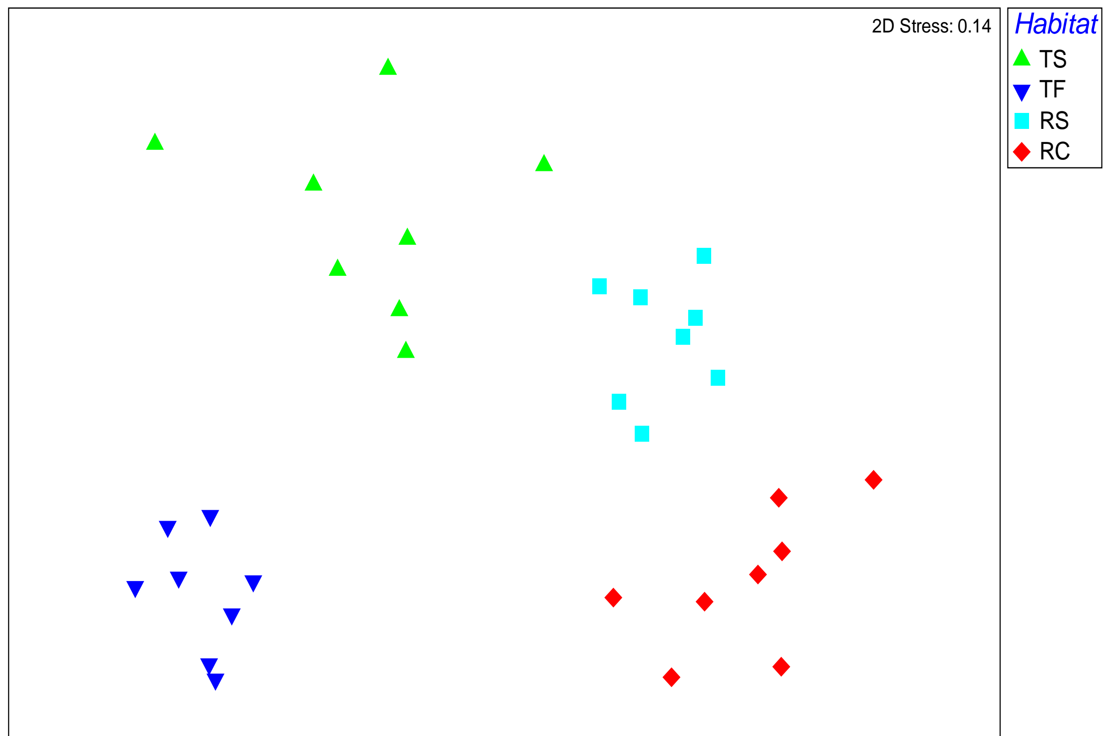


Fig. 4.17 Multidimensional scaling plot (PRIMER) of the total epibiota assemblages on the different basiphyte species collected from the two locations on Anglesey. Analysis is based upon the presence/absence transformation of abundance data and on Bray-Curtis similarities. Samples are identified by location and host basiphyte: TS = *S. muticum*, Tal y Foel; TF = *F. vesiculosus*, Tal y Foel; RS = *S. muticum*, Rhosneigr; RC = *Cystoseira* spp., Rhosneigr.

Table. 4.15 Similarity percentage (SIMPER) analysis of the total epibiota associated with the different basiphyte host species collected from the two contrasting habitats on Anglesey. Average epiflora community similarities among replicate samples in percent and the relative contribution of dominant species (to cumulative 50%) in replicate samples. Analyses based on presence/absence data. TS = *S. muticum*, Tal y Foel; TF = *F. vesiculosus*, Tal y Foel; RS = *S. muticum*, Rhosneigr; RC = *Cystoseira* spp., Rhosneigr.

Host basiphyte (average similarity %)	**TS 46.94%**	**% contribution**	**TF 61.94%**	**% contribution**	**RS 56.18%**	**% contribution**	**RC 52.69%**	**% contribution**
Species dominating epibiota	*Gammarus* spp.	14.40	*Spirorbis* spp.	8.59	*Dexamine spinosa*	7.68	*Dexamine spinosa*	6.68
	Crab megalopae	14.40	*Gammarus locusta*	8.59	*Ampithoe* spp.	7.68	*Ampithoe* spp.	6.68
	Ectocarpoid spp.[a]	14.40	*Gammarus* spp.	8.59	*Caprella acanthifera*	7.68	Crab megalopae	6.68
	Caprella acanthifera	10.23	Crab megalopae	8.59	*Dynamene bidentata*	7.68	*Rissoa parva*	6.68
			Littorina mariae	8.59	Ectocarpoid spp.[a]	7.68	*Elachista* spp.[a]	6.68
			Ectocarpoid spp.[a]	8.59	Crab megalopae	7.68	Nereidae spp. 1	4.99
					Mysidacea spp.	7.68	Mytilidae spp.	4.94
							Boergesniella fruticulosa[a]	4.94
							Ceramium spp.[a]	4.86
Cumulative percentage > 50%	Σ	53.43	Σ	51.53	Σ	53.79	Σ	53.11

[a] Epiphytic algal species

4.4 DISCUSSION

4.4.1 Epibiota assemblages from different environments

Analyses based on the variously grouped data confirmed the clear distinction of community assemblages between the two locations surveyed, although the magnitude of division was less marked based solely on the epiphytic algal communities of the host macrophytes (Fig. 4.3). Therefore the most likely attributable cause for the differences seen in epibiota community similarity was due to the local environmental conditions. Buschbaum et al. (2006) documented a comparatively similar scenario when comparing the epibiota of *S. muticum* sampled from contrasting environments on two islands in the North Sea However, results from Gestoso et al. (2010) indicated that community assemblages associated with invasive *S. muticum* were similar over a range of spatial scales from metres to kilometres indicating the preferential importance of biological factors rather than the physical environment in structuring the epifaunal community. This was somewhat unexpected, given that the sampling locations in the author's study varied in terms of wave exposure, a factor that has previously been shown to influence the structure of coast communities (Fenwick, 1976, Gibbons, 1988). In particular, tidal height has been demonstrated to have significant influence on the community structure of epifaunal assemblages of macroalgae (Benedetti-Cecchi, 2001, Schreider et al., 2003), with those species found higher on the shore more dependent on physical constraints such as aerial exposure and desiccation (Lilley and Schiel, 2006). Such effects have also been demonstrated in *S. muticum* with low shore plants displaying an increased number of taxa and individuals (Viejo, 1999, Cacabelos et al., 2010).

Similar to the findings presented here, a previous small scale investigation into the associated epibiota of *S. muticum* from two contrasting sites on Anglesey also demonstrated distinctions in the assemblage composition (Grant, 2007). In her study a collective total of 29 species of epibionts were identified from the two sites, significantly less than compared with the 80 different species of epibiota identified from *S. muticum* thalli in this investigation. Furthermore, at Tal y Foel, which was also used as one of the author's sample locations, only 7 species were identified from Grant's (2007) survey compared to the 46 different taxa identified in this study. Given the methodology adopted by Grant (2007) which involved targeting *S. muticum* thalli

showing particularly heavy epibiont fouling, the contrasts with the results of my study appear surprising. However, the limitation to only identify epibiota > 2mm, and the different timing of sample collection (August 2007) in this previous investigation may provide a reasonable explanation regarding the discrepancies in the results of the two studies.

A total of 40 species of epifauna and 40 species of epiphytic algae were identified from *S. muticum* basiphytes in this study. However, from the south coast of the UK Withers et al. (1975) observed a considerably higher species richness of epibiota in association with *S. muticum* with a total of 80 recorded epifaunal taxa and 52 different species of epiphytic algae. Such differences may be a simple reflection of the environmental differences between the two regions which would influence species composition the local epifaunal community able to colonise macroalgal hosts. Furthermore, during this study a number of taxa could not be identified down to species level due to fragmented/damaged specimens and a lack of taxonomic expertise, hence species richness may be underestimated.

In keeping with previous studies, herbivorous amphipods were consistently the most abundant taxa from the assemblages of associated epifauna present on macroalgal hosts from the two surveyed locations. The influence of environmental locality on the structural composition of associated epifauna was also reflected by changes in the dominant amphipod taxa recorded. At Tal y Foel, species of *Gammarus* were most numerous whereas at Rhosneigr, the amphipods, *Dexamine spinosa* and *Ampithoe* spp. dominated the community in terms of numbers. The consistency of these differences within both the native and invasive algae points towards the importance of local environmental conditions in determining the structure and composition of associated macroalgal epifauna confirming previous reports (Viejo, 1999, Buschbaum et al., 2006). Investigations into the epiphytic faunal community of *S. muticum* from Strangford Lough also revealed a predominance of the amphipod *Dexamine spinosa* (Strong et al., 2009). However, the number of individuals recorded per thalli were significantly higher (>700 individuals) than from this investigation (~125 individuals) which may be a reflection of further environmental and community differences within each region.

Unlike many native macroalgal species, in temperate waters *S. muticum* possesses pseudo-perennial life history. During late summer/early autumn towards the

end of the reproductive period, the alga enters a period of senescence resulting shedding and release of the annual fronds leaving behind the perennial basal stipe and holdfast from which branches regenerate in the following spring (Fletcher and Fletcher, 1975, Jephson and Gray, 1977, Critchley, 1981c). This pseudo-perennial life history of *S. muticum* is therefore likely to have a significant impact on the associated epiphytic algal and faunal communities. As a result, several studies have documented seasonal variations in the associated epibiota of *S. muticum* with reductions in both abundance and species richness coinciding with the period of senescence (Norton and Benson, 1983, Wernberg et al., 2004). Despite this factor, it is likely that the recolonisation of *S. muticum* is relatively rapid as previous studies have demonstrated rapid colonisation of defaunated substrata by epifaunal populations (Gunnill, 1982, Virstein and Curran, 1986, Martin-Smith, 1994). The associated epibiont community of *S. muticum* must therefore re-establish itself on a seasonal basis, with recruitment originating from the local species pool of the respective environment (Norton and Benson, 1983, Bjaerke and Fredriksen, 2003, Buschbaum et al., 2006). Such a scenario of species recruitment and colonisation is therefore likely to further enhance differentiation in the structure and composition of epibiota assemblages of *S. muticum* between contrasting coastal habitats.

4.4.2 Epibiota assemblages associated with native versus invasive algae

Changes in the habitat structure of vegetated areas as a result of macroalgal introductions have been shown to have direct consequences on the associated epibenthic flora and fauna (Wikström and Kautsky, 2004). Previous studies have demonstrated clear distinctions between the faunal assemblages associated with native and non-native macroalgae (e.g. Wikström and Kautsky, 2004, Schmidt and Scheibling, 2006, Prado and Thibaut, 2008, Janiak and Whitlatch, 2012). The results from this investigation also appear to show distinct community assemblages between *S. muticum* and native macroalgae. In contrast to the clear differentiation between epifaunal communities of invasive and native algae from Tal y Foel, at Rhosneigr the invasive *S. muticum* presented an epifaunal assemblage close to that found on the native *Cystoseira* spp. Both macrophytes from Rhosneigr were characterised by the occurrence of three dominant taxa: *Dexamine spinosa*, *Ampithoe* spp. and crab megalopae (Appendix C.1 ; Table 4.15). Results from a similar study by Viejo (1999) also showed that amphipods

from the family Ampithoidae and the genus *Dexamine* spp. dominated the mobile epifauna of both *S. muticum* and *Cystoseira nodicaulis* in terms of abundance. A striking similarity in the segregation of the mobile epifaunal assemblages associated with the different macroalgae was also revealed between this study and that taken from Viejo (1999). Both studies showed a clear distinction between the assemblages of *S. muticum* and *F. vesiculosus* sampled from the same area, whereas those from *S. muticum* and C. *nodicaulis* exhibited greater similarity (Fig. 4.18). Comparisons of the results of for the total number of mobile taxa identified on each basiphyte from this investigation and that of Viejo's (1999) study also revealed striking similarities, with *F. vesiculosus* harbouring the fewest and *C. nodicaulis* to largest. In terms of the epifaunal assemblages associated with *S. muticum* and *F. vesiculosus* the gammarid amphipods (specifically *Gammarus* spp.) was the most abundant taxa recorded (see Appendix C.1). In contrast, Viejo's (1999) examination of the same two species of host macroalga revealed isopods to be the most abundant taxa. These conflicting patterns are most likely explained by the contrasting environments of the two studies from which the seaweed samples were gathered. In Viejo's (1999) study *S. muticum* was collected from tidepools and *F. vesiculosus* from the main zone of this alga on the rocky shore. In this study however, *S. muticum* and *F. vesiculosus* were sampled from the sub-tidal fringe of a sandy sediment shore in the Menai Strait.

Viejo's (1999) study also measured the qualitative composition of algal epiphytes on both *S. muticum* and *C. nodicaulis* collected from the low intertidal. Several of the epiphytic algal species recorded in the author's study (e.g. *Leathesia difformis*, *Sphacelaria* spp., *Ceramium* spp. and Ulvaceae) were also identified from *S. muticum* and *Cystoseira* spp. in this investigation (Appendix C.2). However, in contrast to the results of Viejo (1999) which showed a similar qualitative composition of algal epiphytes between the two basiphyte species, this study identified a number of algal epiphytes distinguishing the assemblages associated with *S. muticum* and *Cystoseira* spp. thalli. The most notable of these contrasting algal epiphytes was that of *Elachista* spp. found exclusively on thalli of *Cystoseira* spp. (Appendix C.2). Jephson and Gray (1977) working on the Isle of Wight, also found that the epibiont communities on *S. muticum* were similar to those of the brown alga *Cystoseira nodicaulis*.

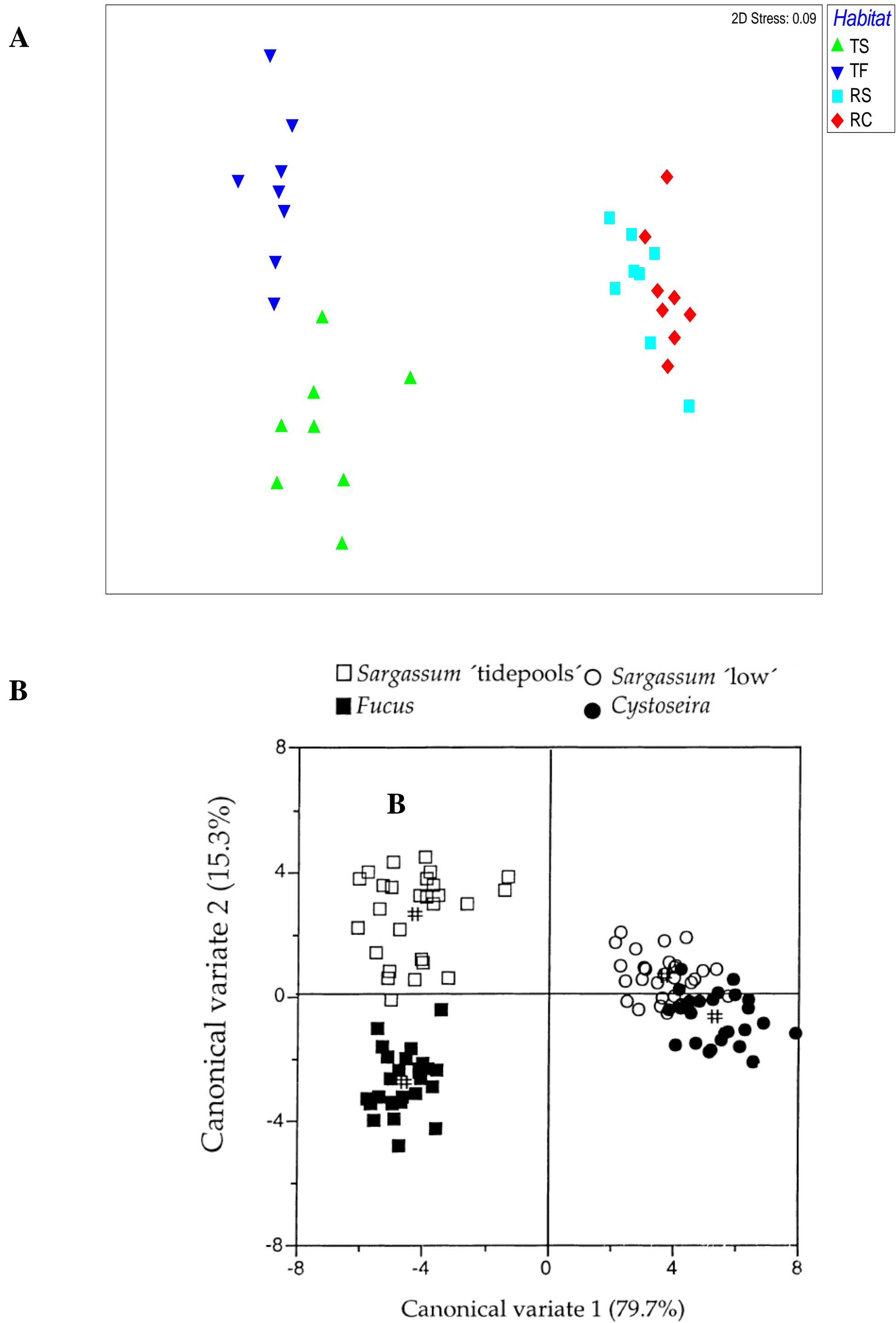


Fig. 4.18 Comparative results of mobile epifaunal assemblages from this study (A) and Viejo, 1999 (B). This study: Multidimensional scaling plot (PRIMER) of the mobile epiphytic faunal assemblages on the different basiphyte species collected from the two locations on Anglesey. Analysis is based upon the square-root transformation of abundance data corrected to biomass of host basiphyte and on Bray-Curtis similarities. Samples are identified by location and host basiphyte: TS = *S. muticum*, Tal y Foel; TF = *F. vesiculosus*, Tal y Foel; RS = *S. muticum*, Rhosneigr; RC = *Cystoseira* spp., Rhosneigr. Viejo's study: Canonical discriminant analysis (CDA) plot of the first 2 canonical variates summarising trends in densities of the 18 commonest epifaunal taxa on *Fucus*, *Sargassum* 'tidepools', *Sargassum* 'low' and *Cystoseira*. Symbol # represent the centroids for each group (seaweed).

It is known that habitat heterogeneity and complexity play an important role in determining the assemblage structure of epibenthic marine communities (Gunnill, 1982, Edgar, 1983, Holmlund et al., 1990, Taylor and Cole, 1994, Hull, 1997, Sueiro et al., 2011). The morphological structure of the macroalgal habitats may affect both biological and physical aspects of the local environment with inevitable likely impacts on the associated epibiotic assemblages (Williams and Seed 1982). Furthermore several studies have shown that the morphological complexity of macroalgae is often positively correlated to the species richness and abundance of associated epibiota (Hacker and Steneck, 1990, Gee and Warwick, 1994, Taylor and Cole, 1994). Macroalgae that are more structurally complex may provide features such as greater habitable space, an increased level of available food resources, and increased refuges from predation or physical disturbance (Hicks, 1980, Hicks, 1985, Jenkins et al., 2002). In contrast, some studies have documented a negative relationship between habitat complexity (as measured by frond density) and the abundance and richness of invertebrate fauna (e.g. gastropod molluscs) indicating that generalised trends may be context specific (Kelaher, 2003). For example, Russo (1990) showed that algal complexity was not a consistent predictor of the abundance and species number of amphipods. In terms of introduced macroalgae, this relationship between morphological complexity and species assemblages may be vital in determining the impacts of the invader. Functional equivalents present with the local vicinity may mean that little differences in the associated epibiota are observed between invasive and native macroalgae (e.g. seen between *S. muticum* and *H. siliquosa* in a study by Buschbaum et al. 2006). Positive impacts following the macroalgal introduction may also be realised. In a study by (Irigoyen et al., 2011b) the abundance and species richness of macrofauna to be elevated in the invasive *Undaria pinnatifida* compared to the local native seaweeds. The authors attributed this to the provision of new habitat structure by *U. pinnatifida*, a larger and structurally more complex species than the local native seaweeds. In contrast, some introduced macroalgae may be structurally less complex and may be of lower nutritional value resulting in reduced abundance and species richness of associated fauna. (e.g. Gollan and Wright, 2006, Janiak and Whitlatch, 2012).

Both *S. muticum* and the native *Cystoseira* spp. examined at Rhosneigr show strong similarities in terms of their overall morphology. Both algae display characteristic arboreal thallus morphology with typically high levels of structural

complexity. This may be a likely explanation for the overlap in similarities between their epifaunal (mobile epifauna in particular) assemblages observed in this study (Figs. 4.8 and 4.9). Viejo (1999) provided a similar theory to explain a comparable set of results when examining the associated mobile epifauna of *S. muticum* and *C. nodicaulis* collected from a low intertidal area of the El Truhet Inlet in northern Spain (see Fig. 4.18). The likeness of the epibiotic assemblages from the native and invasive algae also suggests that most of the associated species are non-selective with regards to their host plant, an observation confirmed by several studies of marine macroalgae (Hay and Fenical, 1988, Taylor and Cole, 1994, Wernberg et al., 2004). A higher overall species richness of epibiota associated with *Cystoseira* spp. shown in this study may be somewhat attributable to an enhanced morphological complexity compared with *S. muticum*. A similar trend was also demonstrated in a study by Tena et al. (1998) which showed a higher level of polychaete faunal diversity in areas colonised by *Cystoseira tamariscifolia*, a result attributed to the higher degree of structural complexity afforded by this species in comparison to macroalgae belonging to crustose, calcareous rhodophyta.

With regards to both the average species richness and number of individuals of mobile epifauna, no significant differences were observed between native and invasive algae from either location (Table 4.6). This is interesting given the distinct contrasts in morphology between *S. muticum* and the native *F. vesiculosus* from Tal y Foel. It appears the algal identity rather than complexity may be more influential in the structuring of epifaunal assemblages within this region. This result contrasts with the general findings of Gestoso et al. (2010) who documented that abundance rather than species composition of epifaunal assemblages was more important in distinguishing between native and invasive macroalgae. On the other hand, morphological differences between *S. muticum* and *F. vesiculosus* may offer some explanation as to the recorded differences in species richness of sessile epifauna (10 identified from *F. vesiculosus* and only 1 from *S. muticum*) between the two algae. In contrast to the branched structure of *S. muticum*, the large, flatter fronds of *F. vesiculosus* may provide more suitable space for colonisation by sessile fauna such as tube worms, bryozoans and hydroids. Several authors have reported the concentration of epiphytic species from the perennial stem and holdfast portions of *S. muticum* (Withers et al., 1975, Aguilar-Rosas and Galindo, 1990, Strong, 2003). Therefore a further explanation to the observed differences in

sessile fauna may be the lack of suitable space available for colonisation on the perennial sections of the *S. muticum* plants, due to their frequent burial within the soft sediment substrate at Tal y Foel.

Despite clearly distinct epibiota assemblages between *S. muticum* and *F. vesiculosus* (Fig. 4.17), the total species richness of epibiota associated with the two algae were comparatively similar (Table 4.13). This results conflict with the findings of Buschbaum et al. (2006) who documented a significantly lower species richness of associated organisms on *F. vesiculosus* (38 species) compared to *S. muticum* (64 species).

Low specificity of epifauna to the host plant is commonly reported in marine ecosystems dominated by macroalgae (Edgar, 1983, Russo, 1990, Wikström and Kautsky, 2004, Prado and Thibaut, 2008). However, a number of species specific community differences between host macroalgae were evident from this study. Firstly, species of caprellid amphipod were exclusively found in *S. muticum* habitats in contrast to the native macroalgae (Appendix C.1). Studies of the associated epifauna of a variety of *Sargassum* species including *S. muticum* from Japanese waters have also highlighted the importance of caprellid taxa in contributing to significant differences in associated epifauna between sites (Engelen, 2007). This pattern appears to be mirrored within the alga's introduced range. Secondly the tube dwelling polychaete, *Spirorbis* spp. was exclusively associated to native basiphytes, being especially abundant on *F. vesiculosus*. Comparable to the findings of Gestoso et al. (2010) the isopod, *Dynamena bidentata* was particularly characteristic of *S. muticum* from Rhosneigr in terms its overall abundance. The composition of epifauna on *F. vesiculosus* from this investigation also appears to contrast with that from several previous studies which have reported a predominance of harpacticoid copepods within the assemblages (Colman, 1940, Ohm, 1964, Johnson and Scheibling, 1987). However the reason for their lack in samples from this study can be largely attributed to the retention of fauna to > 0.5 mm whereas in the studies previously mentioned epifauna were retained down to 0.063 mm.

Epiphytic algal biomass has been implicated as a significant factor in determining the structure of epifaunal assemblages, with several studies showing a positive correlation between epiphyte biomass and abundance of mobile epifauna (Viejo, 1999, Parker et al., 2001, Wikström and Kautsky, 2004, Cacabelos et al., 2010,

Gestoso et al., 2010).The biomass of associated epiphytic algae has been previously suggested to be a factor in determining the structural and abundance of epifaunal assemblages (Parker et al., 2001, Wikström and Kautsky, 2004, Gestoso et al., 2010). In this study, the basiphyte *Cystoseira* spp. had a significantly higher loading of epiphytes in terms of overall biomass per host basiphyte (Fig. 4.2). However, when compared to the *Sargassum muticum* basiphyte samples the abundance of mobile epifauna did not differ significantly (Table 4.6). In fact, despite a significant difference in terms of epiphytic biomass between *S. muticum* and *Cystoseira* spp. basiphytes collected from Rhosneigr the average number of mobile epifauna per host thallus was almost identical (Table 4.6). The reduced level of epiphytic algal biomass on *S. muticum* may in part be explained by the chemical physiology of the alga itself. Previous research has demonstrated the existence of a chemical defence mechanism in *S. muticum* which is in operation before the onset to the reproductive period of the alga (Plouguerne et al., 2006). Presence of these antifouling compounds in thalli of *S. muticum* may therefore provide a possible explanation for the reduced epiphytic algal biomass associated with the alga. Furthermore, this scenario has also been put forward following observations of heavy epiphytic fouling of *S. muticum* around the time of senescence when concentrations of phenolic compounds are noted to decrease (Jephson and Gray, 1977, Gorham and Lewey, 1984).

4.4.3 Consequences of the introduction by *S. muticum* to the area

Impacts of macroalgal introductions on native communities appear dependent on a variety of factors including invader identity (Wikström and Kautsky, 2004), host-plant specificity (Schmidt and Scheibling, 2006), and characteristics of the invaded habitat (Buschbaum et al., 2006). Both negative and positive impacts may be realised following the introduction of non-native macroalgae. For example, the invasion of *Sargassum muticum* in northern Spain caused long-term algal changes that resulted in a decrease of the dominant red alga *Gelidium spinosum* and an increase in algal species richness and diversity (Sanchez et al., 2005). Pacios et al. (2011) showed that the non-native alga *Asparagopsis armata* was able to support a crustacean community of comparable diversity to that found on adjacent native macroalgal substrate and in some instance enhancing diversity by promoting the appearance of new crustacean species. Introduced algae may even have direct impacts on fauna from higher trophic levels in coastal

systems. For example, the presence of *Undaria pinnatifida* off the coast of Argentina has been shown to result in transitory habitat loss for reef fishes inhabiting low-relief reefs, leading to a marked decrease in their overall abundance (Irigoyen et al., 2011a). Species specific changes may also occur following the colonisation of native macroalgal communities by introduced seaweeds. This has be seen in a number of studies where a shift in the dominant species of amphipod with the epifaunal assemblages has resulted attributed to resource preferences of different species (Vazquez-Luis et al., 2012).

Overall the results of the study suggest that the impact of *S. muticum* invasion on the associated epibiota assemblages is dependent upon similarities between the invasive alga and native seaweeds, and upon the cohort of organisms inhabiting the local environment. At the two locations surveyed on Anglesey *S. muticum* appears to offer a suitable habitat for a wide variety of epiphytic organisms, particularly amphipods. The high numbers of amphipod crustaceans recorded from *S. muticum* in both locations (Appendix C.1) may also translate to increased prey availability for predatory species such as fish. Therefore the presence of *S. muticum* may have an indirect effect on the flow of matter and energy from invertebrates to higher trophic levels, a theory that has been previously suggested for introduced macroalgae (Crooks, 2002). Further research will be required in order to fully understand the consequences of the invasion by *S. muticum*, specifically with regards to the impacts on species from higher trophic levels.

The consequences of the introduction of *S. muticum* to Rhosneigr are of particular concern due to the status of this site as a Site of Special Scientific Interest (SSSI). One of five SSSI around the coast of Anglesey, Rhosneigr was selected based on the high diversity of littoral and shallow sublittoral algae in pools and extensive lagoon, including less common or rare species such as the red algae *Laurencia obtusa* (CCW, 1992). Even though mobile epifaunal community composition of the native and invasive alga at this site showed strong similarities, thalli of the invasive alga were associated with an overall lower species richness of epibiota (Table 4.13). Therefore an increase in abundance of *S. muticum* and replacement of native species such as *Cystoseira* spp. may lead to significant reductions in local biodiversity which therefore presents concerns about the integrity of the site as a SSSI. However, as yet no studies have assessed whether the introduction to and subsequent population expansion of *S. muticum* at Rhosneigr and other locations around Wales has led to the replacement of

native marine species. Although a number of previous studies have documented the displacement of native macrophytes through competition with the invader (Ambrose and Nelson, 1982, De Wreede, 1983, Stæhr et al., 2000, Britton-Simmons, 2004). Conseuently the hypothesis of species replacement by *S. muticum* in Welsh waters remains to be tested and as such should ideally be included in future work examining the ecological effects of the alga on native marine communities in Wales.

Often, introduced species may act as vectors and habitat for the further introductions of non-native species (Buschbaum et al., 2006). For example, *Sargassum muticum* introduced into native seagrass beds of British Columbia, Canada, facilitated the incursion two other the non-native species (the ascidians, *Styela clava* and *Botrylloides violaceous*) into the eelgrass bed through habitat provisioning (White and Orr, 2011). However in this study, no further invader associated with *S. muticum* has been detected from either of the two locations on Anglesey. On the other hand, two individuals of the invasive sea squirt *Corella euymota* were recorded from one specimen of *F. vesiculosus* sampled from the Menai Strait (Appendix C.1). To my knowledge this discovery represented the first recorded observation of this non-native ascidian from this region. Furthermore, following a return visit to the area as part of the seasonal phenology surveys of *S. muticum* (chapter three) further observations of *C. eumyota* were recorded from both native fucoids and attached to several large boulders located within the intertidal/subtidal fringe (see Fig. 4.19). As a confirmation of species identity, several samples were collected and sequenced for a fragment of the mitochondrial COI gene (regarded as a standard DNA barcoding gene (Hebert et al., 2003)). Blast search comparisons (Altschul et al., 1990) reported a 99% sequence identity with *C. eumyota* COI sequences deposited on the GenBank database, thus providing species confirmation.

Fig. 4.19 Photographs of *Corella eumyota* discovered at Tal y Foel during August 2010. (A) Individuals attached to fronds of fucoid algae. (B) Individuals attached to underside of boulder within shallow subtidal.

4.4.4 Directions for future investigations

Without a thorough understanding of the impacts of invasive species on natural ecosystems, the development of appropriate management strategies will be problematic. This investigation has offered further insights on the effects of *S. muticum* on recipient communities from two contrasting habitats as well as providing a first preliminary analysis as to the current state of the ecosystem inhabited by the alga within Welsh waters. However, the study has also led to the generation of several new avenues of investigation that should be addressed in future research.

As the results of this study were based on a single collection of the native and invasive basiphytes, they may not represent the complete list of epibiota found in association with *S. muticum* from the two study locations. In order to increase the validity of the findings the epibiota community structure and abundance on native and invasive algal hosts need to be examined over a seasonal period. In that way a complete species list of associated epibiota can be accurately provided, plus an assessment of the influence of the life history differences of algae. Based on previous studies, expectations are for a much more marked seasonality in epibiota abundance on *S. muticum* and other species that have a pseudo-perennial life cycle. For example, study by Thomsen et al (2006) demonstrated a peak in species richness coinciding with the period of maximum size in *S. muticum*. However a study by Tweedley (2006) showed no significant increase in species number of epifauna associated with S. muticum over a growth season, although a seasonal increase in abundance of the epifauna was observed. Furthermore, higher levels of epiphytic fouling on *S. muticum* have also been observed during the time that the plants begin to undergo senescence (Jephson and Gray, 1977, Wernberg et al., 2004). A suggested reason for this relates to the decrease in level of antifouling phenolic compounds produced by the thallus during this time period (Gorham and Lewey, 1984). Findings of a recent study by Bulleri et al. (2010) even suggest that exotic seaweeds can alter the structure of native communities even when they are found at relatively low abundances and when characterized by a seasonal resting phase. This observation provides further evidence in support of a continuation study examining the seasonality of epibiota associated with *S. muticum* and native macroalgae.

Given the diversity of habitats on the Welsh coast where *S. muticum* has now become established (see chapter 1, Table 1.1), future studies on the associated epibiota should ideally incorporate plants from a wide selection of these invaded environments. The alga is known from several rockpool habitats on the Welsh coastline, but as yet no investigations as to the impact of the alga on native communities within these systems in Wales has been evaluated.

It could also be interesting to examine in detail the interaction between mesograzers and the different native versus invasive macroalgae as previous research on this topic has produced conflicting results. For instance some studies have demonstrated negative effects of invasive seaweed on grazing activity and feeding habits of herbivores such as gastropods and amphipods (Gollan and Wright, 2006, Wikstrom et al., 2006, Monteiro et al., 2009a, Engelen et al., 2011). *Dynamena bidentata* was more common on *S. muticum* than native *Cystoseira* spp. from Rhosneigr. This isopod has previously been shown to graze on the host species that it inhabits (Viejo and Arrontes 1992) and has been observed to graze on *S. muticum* (Viejo 1999) under experimental laboratory conditions. Strong et al.'s (2009) study indicated that *Dexamine spinosa* exhibited a preference for grazing on *S. muticum* over native algae in grazing choice laboratory experiments. As this amphipod species was present in high numbers from *S. muticum* at Rhosneigr, it is likely that grazing pressure on the invasive alga could be equivalent to that experienced by native macroalgae. However, without a set of comparative grazing experiments using samples from this region, this hypothesis remains to be tested.

4.4.5 Conclusions

Generally, the effects of *S. muticum* on the associated epibiota assemblages appeared heavily dependent upon the invaded location. However, the limited sample numbers in the study and the lack of spatial replication mean the results should be treated with some degree of caution. Based on the community differences, *S. muticum* at Tal y Foel appears to provide a novel habitat for the colonisation of epibenthic organisms. The introduction and continued spread of *S. muticum* along the Menai Strait may therefore provide additional habitat structure available for colonisation by a diverse community of native epibionts that may otherwise by absent or rare. However, further

sampling from other areas with the Menai Strait will be required before a more generalised assessment as to the invasive alga's impacts can be determined. In contrast at Rhosneigr, morphological similarities between *S. muticum* and the native algae result in the occurrence of comparatively similar communities of associated epibiota. However, total epibiota species richness of the invasive seaweed was reduced and hence may present future problems in the event of native species replacement. Results from this study should be taken into consideration regarding any future management and monitoring programmes to control this invasive seaweed in Welsh waters.

Overall, the expected interactions between climate change impacts and alien species will present complex challenges for the management of biodiversity and ecosystem function (Stachowicz et al., 2002, Occhipinti-Ambrogi, 2007). Consequently, management practices with regard to the introduction of exotic species will require a critical evaluation of changing environmental conditions, and will likely necessitate a case by case review. Occhipinti-Ambrogi and Savini (2003) state that stressed environments contribute significantly to an increased likelihood of introduction by exotics. For that reason, environmental conservation practices will be fundamental in the prevention of the introduction and spread of non-native marine species.

CHAPTER FIVE GENERAL DISCUSSION

The overall aim of this study was to examine the genetics and ecology of *S. muticum* will a focus on the invasive alga within Wales, UK. Here the main findings of each chapter are summarised, including some discussion of the issues faced while undertaking this project. The main findings are examined within the wider context and avenues for future research are discussed.

5.1 GENETICS OF *S. MUTICUM*

Given the dramatic and sometimes irreversible effects on invaded communities, the prevention of biological invasions has become a high priority for governmental bodies worldwide (Hewitt et al., 2009). Knowledge of sources and pathways of species introductions are vital prerequisites for stemming the flow of new invaders (Le Roux and Wieczorek, 2009). However, such information can be difficult to accurately determine using traditional understanding of the biology and ecology of a species (Holland, 2000). For this reason, genetic methods have become increasing popular in invasion biology studies for their ability to provide answers to questions, including: the true taxonomic identity of invaders (e.g. Geller et al., 1997, McIvor et al., 2001), the source population(s) of an invader (e.g. Downie, 2002, Marston and Villalard-Bohnsack, 2002), the pathway(s) of an invader's introduction (e.g. Hanfling et al., 2002, Voisin et al., 2005) and the likely number of founding individuals involved in the invasion (e.g. Zardus and Hadfield, 2005, Ficetola et al., 2008). In addition, knowledge of the level of genetic diversity may provide an indication as to the capacity for evolutionary adaptation within introduced populations (Lee, 2002, Roman and Darling, 2007).

Based on the results from this study (Chapter 2) in combination with previous findings by Cheang et al. (2010c), populations of *S. muticum* appear genetically homogenous. This is particularly so for all invasive populations examined to date which are monomorphic for all DNA sequencing markers currently investigated. Of the genetic markers successfully employed in populations from both the native and invasive range, only those from the mitochondrial genome (i.e. *Trn*W_I spacer and *cox*3) have shown any evidence of variability. However the lack of any detectable phylogeographic

structure within the native range precludes any inferences as to the precise location of source populations responsible for the primary introductions of this seaweed. Similar problems have also been encountered in investigations of the invasion history of the Japanese skeleton shrimp, *Caprella mutica* (Ashton et al., 2008). Results from this investigation are in contrast to the findings of Zhao et al. (2008) who demonstrated significant genetic differentiation among native populations of *S. muticum* from China. Although, this discrepancy between their study and this present investigation is most likely attributable to the different genetic markers employed, a notion also adopted by Cheang et al. (2010c). The study by Zhao et al. (2008) utilised both RAPD and inter simple spacer repeat (ISSR) markers which are typically more variable than DNA sequencing markers due to their faster rate of evolution (Sunnucks, 2000).

A vital prerequisite for any study attempting to identify sources of invasive populations is an adequate sampling of individuals and coverage of source populations to reveal true phylogeographic patterns (Downie, 2002, Muirhead et al., 2008). The identification of an additional mitochondrial haplotype (*Trn*W_I spacer, Hap C) from the alga's native range (not previously detected in the study by Cheang et al. 2010c) clearly provides further support for a comprehensive sampling approach in studies of the invasion genetics of introduced species. Therefore, future investigations into the genetics of *S. muticum* should ideally include material from populations spanning a wide distribution of the alga's native range, particularly in Japan. Given the occurrence of Trn*W_I*, Hap B from a population (Yura, Chapter 2) near the entrance to the Seto Inland Sea (the region where plants of Hap A are more commonly found), additional sampling may also provide further clarification as to the region of the boundary between the two main *Trn*W_I spacer haplotypes in Japan.

Of the samples that successful AFLP profiles could be generated from, a further lack of variability was demonstrated. Furthermore, as only samples from introduced populations were among those that produced clear AFLP fingerprints, the extent of variability within native population samples remains to be characterised using this technique. However, based on the previous findings by Zhao et al (2008), it is expected that an AFLP analysis of native populations should reveal variability at both the intra and interpopulation level.

The lack of genetic variability precludes any identification or resolution of the pathways of spread of the alga within its introduced range at both a regional and local spatial level. Furthermore, the low genetic variability exhibited in *S. muticum* means that it is not currently possible to determine the number of potential founders involved in the initial introduction, and whether repeated introductions have occurred over time. However, *S. muticum* is relatively unique among marine invaders, as a relatively detailed invasion history of the alga has been compiled since its introduction to the two main geographical regions of North America and Europe (Critchley et al., 1990b, Harries et al., 2007a, Davison, 2009). These records have enabled the generation of robust hypotheses regarding the probable vectors of introduction and pathways of the alga's secondary spread. In terms of the situation in Wales it appears most likely that natural dispersal via drift material has been responsible for alga's expansion along the coastline.

Drifting of detached fertile fronds has long been suggested as an important mechanism for long distance dispersal of *S. muticum* (Fletcher and Fletcher, 1975, Norton, 1981b, Deysher and Norton, 1982, Critchley et al., 1983, Harries et al., 2007a). However, it is not clear as to whether this mode of spread represents a significant role in the connectivity between populations. The occurrence of significant isolation by distance in native populations of *S. muticum* from China revealed from a previous study by Zhao et al. (2008) suggests limited connectivity and gene flow between populations of the alga. This confirms expectations based upon the life-history of the alga, given that reported dispersal distances of germlings are typically limited to within a few metres of the parental plant (Deysher and Norton, 1982, Kendrick and Walker, 1995).

Interestingly, no drift material has been included in any prior genetic studies on *S. muticum,* including those from this investigation (Chapter 2). However, given the inherent lack of variability in this alga (even from a recent microsatellite analysis of introduced populations, Marinexus, 2010) it is doubtful whether the inclusion of such sample material will contribute any further insight into connectivity and dispersal of this species within its introduced range. In contrast genetic investigations of the kelp, *Macrocystis pyrifera* have shown that dispersal via reproductive drift material is a significant mechanism in maintaining connectivity among populations (Macaya, 2010).

It is often suggested that low levels of genetic diversity reduce the ability of a species to deal with stressors such as pathogens or environmental change, ultimately increasing the species' risk of extinction (Frankham et al., 2002). However, despite the lack of genetic variation uncovered in *S. muticum*, the alga still manages to thrive within its introduced range. This lack of genetic diversity within introduced populations of *S. muticum* infers that the invasive success of the alga is more likely attributable to other aspects of its biology and life history. Traits that have been suggested as important in determining the invasive success of *S. muticum* are presented in Table 5.1. Indeed, where other invasions proceed with very low amounts of genetic variation, their success has been understood to rely not on adaptation but instead a high degree of plasticity (e.g. Meimberg et al., 2006, Geng et al., 2007).

Table 5.1 Characteristics that may have contributed to the introduction success of *S. muticum*.

Trait	Explanation
Broad environmental tolerance	Survival at temperatures from 3 – 30°C and salinities from 6.8 – 34.0 ppt.
Broad habitat preference	Hard substrate required for attachment, but capable of surviving attached to stones and shell fragments buried in soft sediment.
High fecundity	Up to 500,000 eggs can be released in first season. Well-developed germlings enhances survival. Reproductive output is amplified with age as result of increased number of laterals.
Rapid growth rate	Maximum growth rates may reach 2 – 4 cm.day^{-1} in the field.
Early maturity	Fertile plants can appear within 3 months of colonisation of new area.
Monoecious and self-fertile	Detached drifting material can continue to reproduce on route enabling inoculation of new regions.
Pseudoperennial, flexible growth cycle	Perennial holdfast secures continued existence of the plant. Annual cycle of lateral growth is flexible depending on water temperature.
Multiple dispersal strategies	Dispersal achieved via germlings, stone walking plants, drifting laterals and anthropogenic vectors (e.g. oyster transfers, hull fouling).

As genetic variability within populations of *S. muticum* has only been examined using a small selection of molecular markers, we can only presently infer that genetic variation is low, rather than non-existent. It would be interesting to examine the levels of genetic variability within introduced populations using the RAPD and ISSR markers previously employed in the genetic analysis of *S. muticum* by Zhao et al. (2008). Although, given the lack of variability revealed by AFLP markers (Chapter 2) and microsatellites (Marinexus, 2010), we may expect a similar scenario of low genetic diversity.

5.2 POPULATION DYNAMICS OF *S. MUTICUM* WITHIN WALES

An understanding of the basics of the biology and ecology of invasive species together with their impacts on invaded ecosystems are vital prerequisites for the development and application of appropriate management policies (Ricciardi and Rasmussen, 1998, Sakai et al., 2001, Allendorf and Lundquist, 2003). For example during attempts to control the spread of *Undaria pinnatifida* in a Tasmanian marine reserve, Hewitt et al. (2005) highlighted need for increased frequency of removal efforts through the late season due to the earlier development of reproductive structure in smaller plants. Therefore with regards to any future potential management strategies of *S. muticum* in Wales, the aim of chapter three was to establish some basic information regarding the population dynamics of the alga within welsh waters.

Results showed that as with most introduced populations, *S. muticum* in Wales exhibited a distinct period of growth and reproduction during spring/summer followed by a period of plant senescence in the autumn (Chapter 3). One of the key findings from the investigations was the significantly longer thallus lengths attained by *S. muticum* individuals from the sheltered, tide swept environment at Tal y Foel in the Menai Strait. This comparative difference in plant morphology between the site at Tal y Foel and the other two open coast sites (Rhosneigr and Cei Bach) examined during the study is likely attributed to the differing environmental conditions, with the strong tidal currents present in the Menai Strait subjecting the algae to less physical stress, but providing abundant nutrients and facilitating gas exchange resulting in increased plant growth (Norton et al., 1981). In addition, the faster rates of plant growth seen at Tal y Foel compared to the other sites may be explained by similar such reasoning. Although maximum summer growth rates observed during this investigation were relatively small

in comparison to values previously recorded from south coast of England (Critchley, 1981c). Again it is likely that variations in environmental conditions are responsible for these discrepancies.

The other main finding from this study was the occurrence of variability in the timing and duration of the fertile period of *S. muticum* from contrasting habitats in Wales. In particular, an earlier onset of plant senescence coupled with a reduced proportion of fertile plants was noted from the site at Rhosneigr (Figure 3.11A). Total reproductive output from the population at Rhosneigr is therefore likely to be reduced compared to the other open coast populations examined. This may be significant in reducing the impact and spread of the *S. muticum* population at Rhosneigr. However, as the results were based on a survey of the plants over a single growth and reproductive season, the extent of inter-annual variability in plant fertility remains unknown. Hence, future work should include monitoring of the targeted *S. muticum* populations over a number of consecutive years in order to develop a more accurate picture of the growth and reproductive phenology of the alga within Wales.

At Broad Haven *S. muticum* was observed to be the dominant macroalgal species in many of the rockpools at the site (Paul Hallas, personal observation). Furthermore, observations of the canopy cover of *S. muticum* within several rockpools were seen to approach ~100% during the summer months. This raises concern regarding the potential negative impacts particularly on the understorey algal species caused by shading effects of *S. muticum*, as has previously been documented from rockpool habitats (Viejo, 1997). Therefore, further investigations at this site into the impacts of invader density and abundance on native species are recommended.

In European waters, few attached macroalgae are associated with soft sediment environments (Strong et al., 2006). However, due to the ability of *S. muticum* to colonise both hard and soft substrata, the total area of habitat open to occupation by the alga is vast. In Wales this presents a particular concern as soft sediment shores constitute the main proportion of coastal habitats (Webb et al., 2010). Therefore cumulative habitat modification could be significant given further expansion of the alga in welsh coastal waters. Furthermore as up to 75% of the Welsh intertidal zone falls within SSSI designated areas (Brazier et al., 2007) expansion of this alga may significantly compromise the conservation status of such sites.

S. muticum has now been present in Welsh waters for more than fourteen years, and it appears to have become a permanent member of the macroalgal flora. Given the population size of several stands of the alga, there is likely to be many more opportunities for recruitment and population spread through drifting individuals.

5.3 EPIBIOTA ASSEMBLAGES

Many invasive species provoke strong impacts on invaded communities through the modification of ecosystem properties (Richardson et al., 2000, Crooks, 2002). Physical resources that may be affected by the introduction of ecosystem engineer species include living space or habitat, light, temperature, sediment and water to name but a few (Wallentinus and Nyberg, 2007). Due to the role of marine macroalgae as habitat providers, changes associated with the introduction and establishment of foreign species may have significant impacts on the associated biota (Buschbaum et al., 2006, Schmidt and Scheibling, 2006, Janiak and Whitlatch, 2012). Replacement of dominant macroalgae by introduced species may result in shifts of community structure with cascading effects on multiple organisms within local food webs (Williams and Smith, 2007, Irigoyen et al., 2011, Vazquez-Luis et al., 2012). The magnitude of these impacts in part depends on whether the introduced seaweed are suitable hosts for colonisation by epibiota, compared to native seaweeds that may be replaced during the invasion. For example, a study by Wikstrom and Kautsky (2004) revealed a lower abundance and diversity of epibiota on the non-native alga *Fucus evanescens* compared to the native species of *F. vesiculosus* and *Ascophyllum nodosum* suggesting a less favourable habitat provided by the invasive seaweed.

The fronds of *Sargassum muticum* provide an ideal habitat for epiphytic macrofaunal communities (Viejo, 1999, Wernberg et al., 2004, Buschbaum et al., 2006, Gestoso et al., 2010) and research has indicated that the presence of the seaweed may contribute to localise increases in biodiversity within certain coastal systems (Withers et al., 1975, Viejo, 1999, Buschbaum et al., 2006). Results obtained in this thesis (Chapter 4) show that *S. muticum* growing in coastal waters off Anglesey still maintains a diverse and abundant epibiota assemblage. In habitats similar to those at Tal y Foel in the Menai Strait, with a low initial cover of macroalgae, the presence of *S. muticum* may supply a new and additional habitat for the local epibiota. Furthermore, the invertebrates able to successfully colonise the invader may in turn become the prey of fishes and

decapod crustaceans, which ultimately may result in an increase in the secondary production in the local benthic system (Viejo, 1999). Overall the main observed community differences occurred between the two contrasting locations on Anglesey, a trend also seen in previous studies of *S. muticum* (e.g. Lewey and Farnham, 1981, Buschbaum et al., 2006, Cacabelos et al., 2010).

With regards to the mobile epifauna, again distinct community assemblages were observed on native and invasive macroalgae with the sampling location contributing to the main observed effect. However, at Rhosneigr the associated epifaunal community on *S. muticum* did not appear to differ significantly from that found on the native macroalgae, *Cystoseira* spp. An almost identical observation to this was reported by Viejo's (1999) comparative study of the mobile epifauna of *S. muticum* and the native macroalga, *Cystoseira nodicaulis*. Results from these two studies combined therefore provide support of the existence of a labile association between macroalgae and the invertebrates inhabiting them.

On the other hand, in terms of overall epibiota, compared to the native alga, *Cystoseira* spp. from Rhosneigr, *S. muticum* supports a lower species richness community of epibionts (Table 4.13). This may be attributable to a reduction in habitat quality and a possible decrease in resources necessary for the species inhabiting the non-native seaweed. However, further investigation is required to assess the potential role of *S. muticum* as both habitat and food for invertebrate grazers within native macroalgal communities on the welsh coast.

One of the most ubiquitous and abundant invertebrate groups in marine macroalgae dominated habitats are the amphipods, whose densities can often reach thousands per metre square (Duffy, 1990, Duffy and Hay, 2000, Vazquez-Luis, 2011). Results from this investigation demonstrated that the amphipod assemblage were significant in defining the associated epibiota from the two contrasting survey locations (Chapter 4). The amphipod, *Dexamine spinosa* was characteristic of assemblages associated with macrophytes from Rhosneigr whereas *Gammarus* spp. was more characteristic of the assemblages associated with macrophytes at Tal y Foel. The abundance of *D. spinosa* at Rhosneigr suggests that the habitat may be similar to that from Strangford Lough as an abundance of the amphipod has also been recorded from *S. muticum* communities there (Strong et al., 2009).

The specific association of caprellid crustaceans from *S. muticum* macrophytes observed from the epibiota studies (Chapter 4) suggests the potential suitability of the alga for the non-native skeleton shrimp *Caprella mutica*. In Scotland where *C. mutica* is becoming increasingly common, numerous observations of this species' association with *S. muticum* have been documented (Oakley, 2006, Johnston, 2007). Furthermore within both species' native range of Japan *C. mutica* has been found attached to floating specimens of *S. muticum* (Sano et al., 2003). As *C. mutica* species has been reported from Holyhead Harbour on Anglesey (Ashton et al., 2007), it is likely that further spread of *S. muticum* around the coast may inadvertently facilitate the expansion of *Caprella mutica* along the Welsh coast presenting a further threat to native marine species within Wales. *Caprella mutica* has also been recently recorded from Milford Haven marina in 2009 (Judith Oakley, personal communication, 2009).

As a consequence of habitat provisioning, the introduction of *S. muticum* may potentially facilitate the introduction of other non-native marine species. Research on *S. muticum* found in Portsmouth, U.K identified two associated species of spirorbid worm, *Janua brasiliensis* and *Pileolaria rosepigmentata*, both native to Japan, that had not previously been recorded in Europe (Critchley et al., 1997). It is thought that both species were introduced to Portsmouth via *S. muticum* (Eno and Clark, 1995). There now exists a growing body of evidence demonstrating the facilitative interactions between invasive species resulting in what has been termed as an "invasional meltdown" of ecosystems (Simberloff and Von Holle, 1999, Rodriguez, 2006).

One of the other findings during the investigations in this project was the discovery of *Corella eumyota* from around the area of Tal y Foel in the Menai Strait during June 2010. This species, assumed to be native to regions of the southern hemisphere was first recorded in UK waters in 2004 and according to published reports has been confined several isolated regions along the south coast of England. Its discovery from the Menai Strait in north Wales should be of particular concern due to the shellfish (mussel and oyster) fisheries that currently operate within areas of the Strait. In fact, *Corella eumyota* was included in a list of eight non-native species examined for their potential to be introduced to north Wales via imports of mussel seed (Sewell et al., 2008). The report concluded that colonisation of mussel seed beds would be likely and that impacts of invasion by *C. eumyota* locally would be competition for

spatial resources and smothering of already established species. A further prediction was that large populations may reduce levels of suspended organic matter in the water column, competing with existing suspension feeders in the region (Sewell et al., 2008). This non-native ascidian was also recorded from Neyland marina in Milford Haven in 2008 (Anne Bunker, personal communication).

Overall, the results from the epibiota surveys in this study indicate that *S. muticum* appears to support, for at least part of the year, a relatively diverse species assemblage. However, due to the sampling of associated epibiota during a single time period, the complete species assemblage associated with *S. muticum* in Welsh waters remains to be fully characterised. Furthermore, due to the pseudo-perennial nature of the alga, with the shedding of the annual laterals means that any potential benefits of increased habitat architecture gained by the presence of *S. muticum*, will likely fluctuate on a seasonal basis. Therefore it is recommended that future epibiota investigations should include a seasonal aspect of monitoring to determine what effects this annual senescence might have on the associated communities of epibionts.

5.4 FINAL CONCLUSIONS

With regards to our understanding of the impacts of invasive seaweeds, research is still limited in comparison to other marine invaders (Grosholz, 2002, Inderjit et al., 2006, Johnson and Chapman, 2007), although notable exceptions do occur, including species such as *S. muticum*, *Undaria pinnatifida* and *Caulerpa taxifolia* and *Codium fragile* ssp. *tomentosoides* (Schaffelke and Hewitt, 2007). Furthermore, little is known of the economic impacts posed by seaweed invasions (Johnson and Chapman, 2007), a surprising result given the potential for significant ecological changes, especially by habitat-forming alien macroalgae. The importance of a multi-faceted approach to macroalgal invasion research cannot be understated. Only by combining data from taxonomic, genetic, population biology and invasion ecology studies can we begin to understand marine macroalgal invasion in totality (Trowbridge, 2007).

It is clear from the present study that there is still much to learn regarding the seasonal dynamics of *S. muticum* in Wales and the alga's effects on native marine communities within Welsh coastal waters. Throughout Wales the wide availability of suitable substrate within the environmental tolerance ranges of *S. muticum* emphasises

the importance of identifying the mechanisms contributing to the alga's successful establishment and which habitats are at greatest risk from future invasion. The findings of this study therefore will provide relevant information that can feed into managerial options for any future mitigation of impacts of this invasive alga within Wales. The information presented in this thesis has made a contribution to furthering our understanding of the invasion biology of one of the most globally successful marine invaders.

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APPENDIX A AFLP ADAPTER PREPARATION PROTOCOL

EcoRI Adapter

3.4 µl of EcoRI-F oligonucleotide (1 µg / µl)

3.0 µl of EcoRI-R oligonucleotide (1 µg / µl)

6.0 µl of 10X NEB Buffer #4

107.6 µl of ddH_2O

This gives a final concentration of 5 µM

MseI Adapter

32 µl of MseI-F oligonucleotide (1 µg / µl)

28 µl of MseI-R oligonucleotide (1 µg / µl)

6.0 µl of 10X NEB Buffer #4

54.0 µl of ddH_2O

This gives a final concentration of 50 µM

Reaction mixtures are placed at 95oC for 5 minutes and then left to cool slowly to room temperature.

APPENDIX B AFLP ELECTROPHEROGRAMS

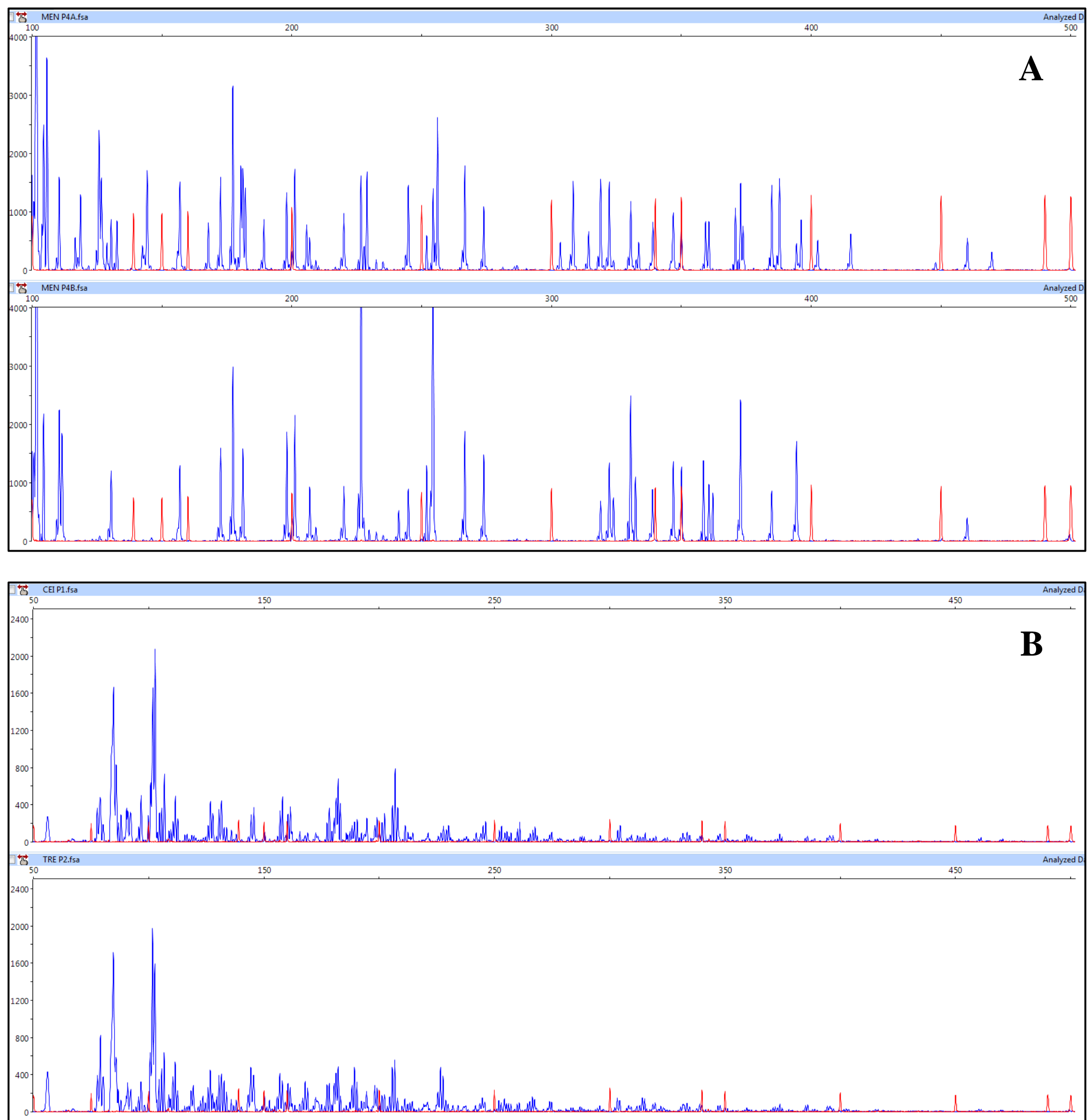

Fig. B.1 (**A**) Electropherograms showing poorly reproducible AFLP data using primer pair AG/CAA. Each panel represent data for the same individual collected from Tal y Foel, Menai Strait (MEN P4). All steps of the process from DNA extraction and AFLP procedures were run on different days. (**B**) Examples of AFLP Electropherograms profiles generated from failed AFLP reactions, using primer pair AG/CAA. Note the general absence and low intensity of fragments > 300 bp. Horizontal scaling indicates fragment sizing (base pairs); vertical scaling indicates fluorescence signal intensity of amplified fragments (rfu). Blue peaks represent sample fragments; red peaks indicate internal size standard (ROX-500) added to the samples.

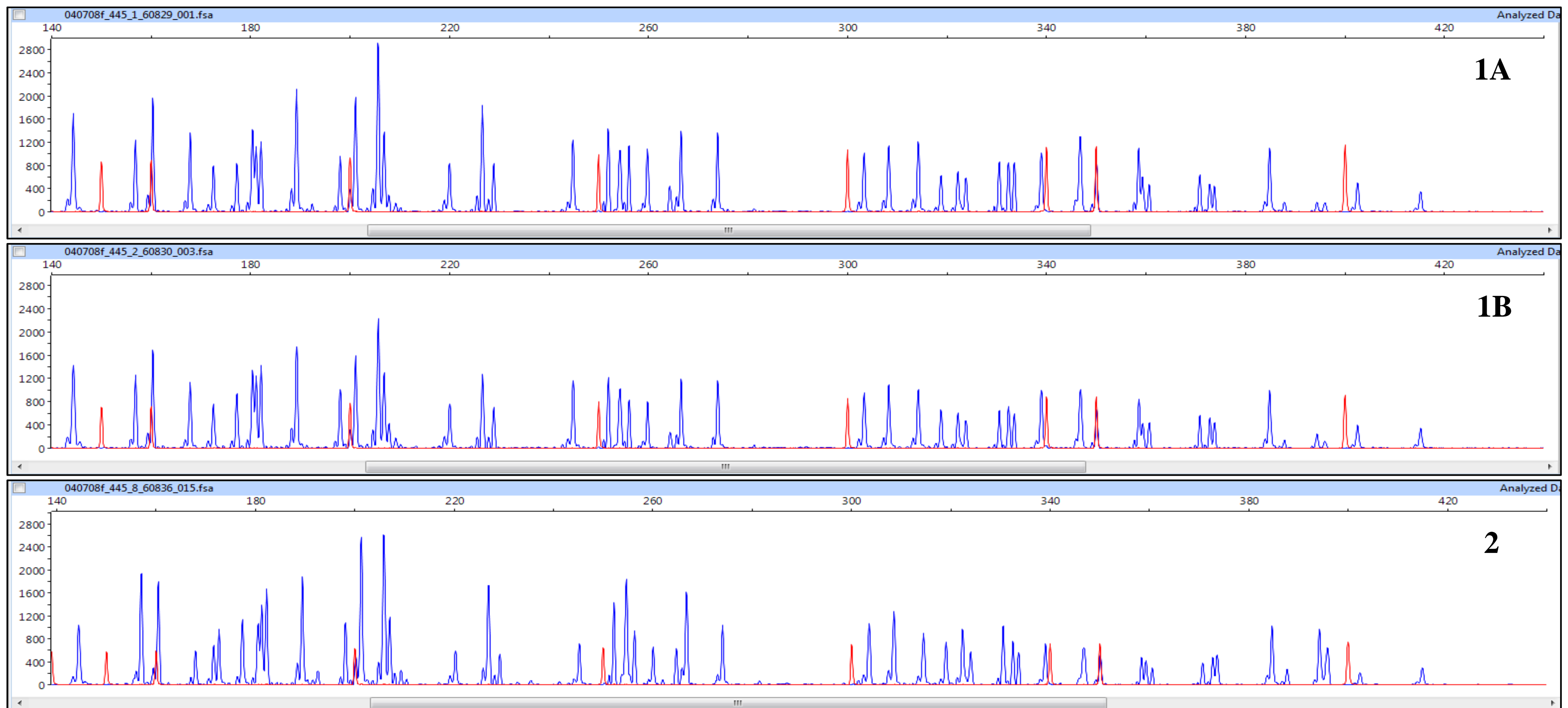

Fig. B.2 Reproducibility test. The figure shows three independent AFLP reactions realised from DNA extractions of the same *S. muticum* sample (CAL) using the selective primer combination *Eco*RI + AG and *Mse*I + CAA. The top and middle profiles (1B and 1B) were generated from the same DNA extract but run on different days. The bottom profile (2) was generated from a replicate DNA extract of the same sample. Horizontal scaling indicates fragment sizing (base pairs); vertical scaling indicates fluorescence signal intensity of amplified fragments (rfu). Blue peaks represent sample fragments; red peaks indicate internal size standard (ROX-500) added to the samples.

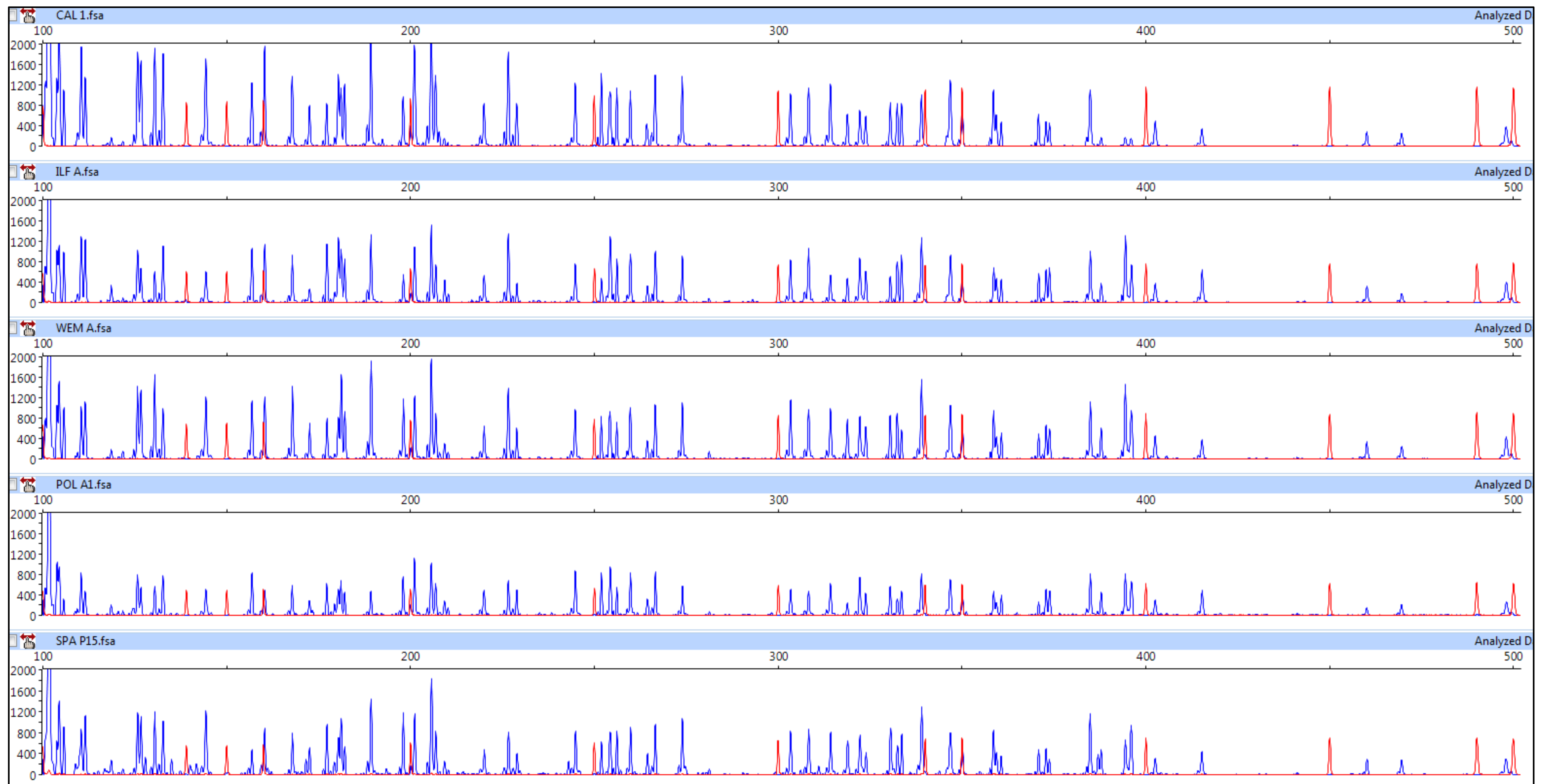

Fig. B.3 AFLP Electropherograms profiles generated from reproducible samples collected from various introduced locations, using the selective primer pair *Eco*RI + AG and *Mse*I + CAA. Horizontal scaling indicates fragment sizing (base pairs); vertical scaling indicates fluorescence signal intensity of amplified fragments (rfu). Blue peaks represent sample fragments; red peaks indicate internal size standard (ROX-500) added to the samples.

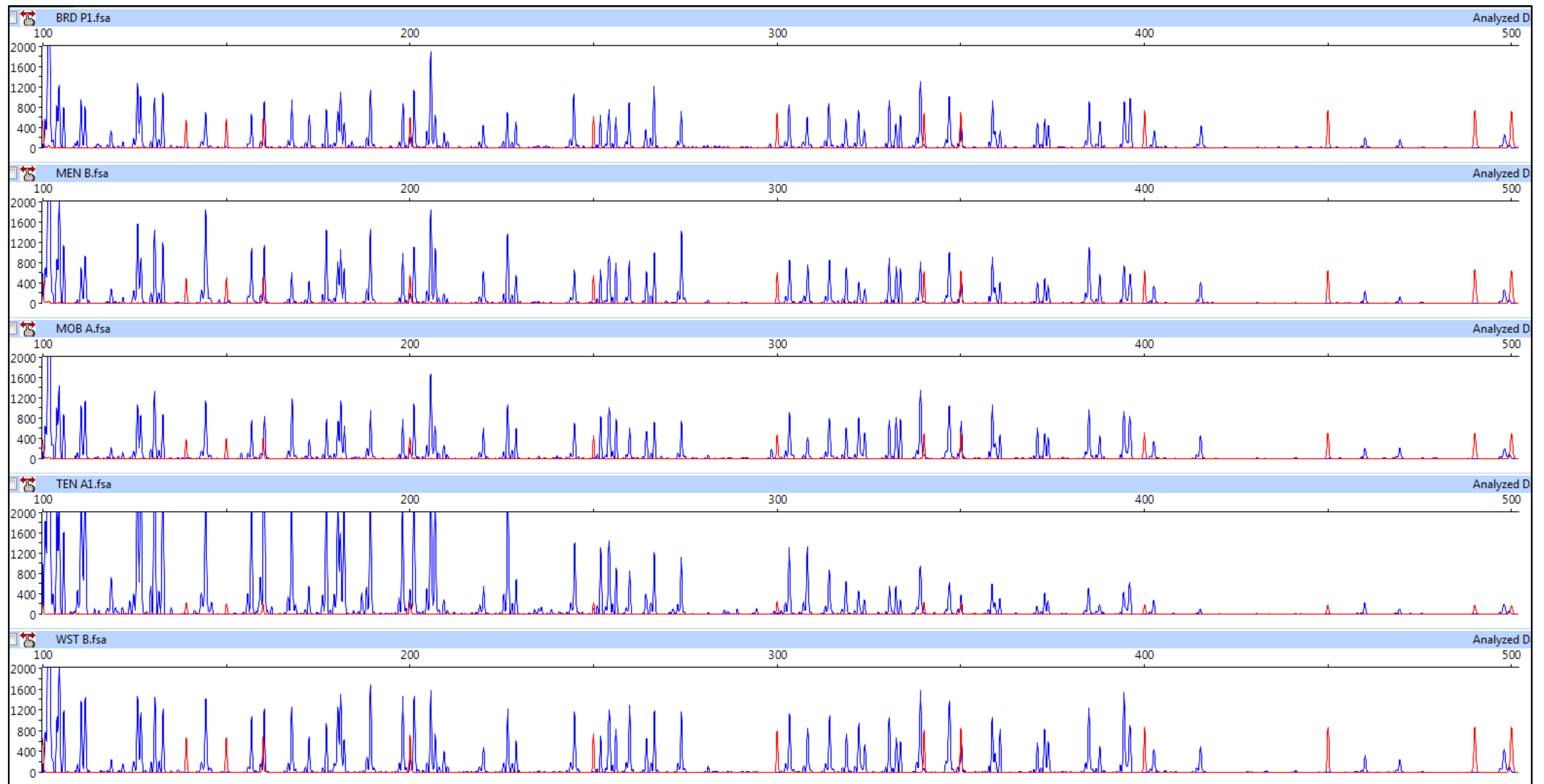

Fig. B.3 continued. AFLP Electropherograms profiles generated from reproducible samples collected from various introduced locations, using the selective primer pair *Eco*RI + AG and *Mse*I + CAA. Horizontal scaling indicates fragment sizing (base pairs); vertical scaling indicates fluorescence signal intensity of amplified fragments (rfu). Blue peaks represent sample fragments; red peaks indicate internal size standard (ROX-500) added to the samples.

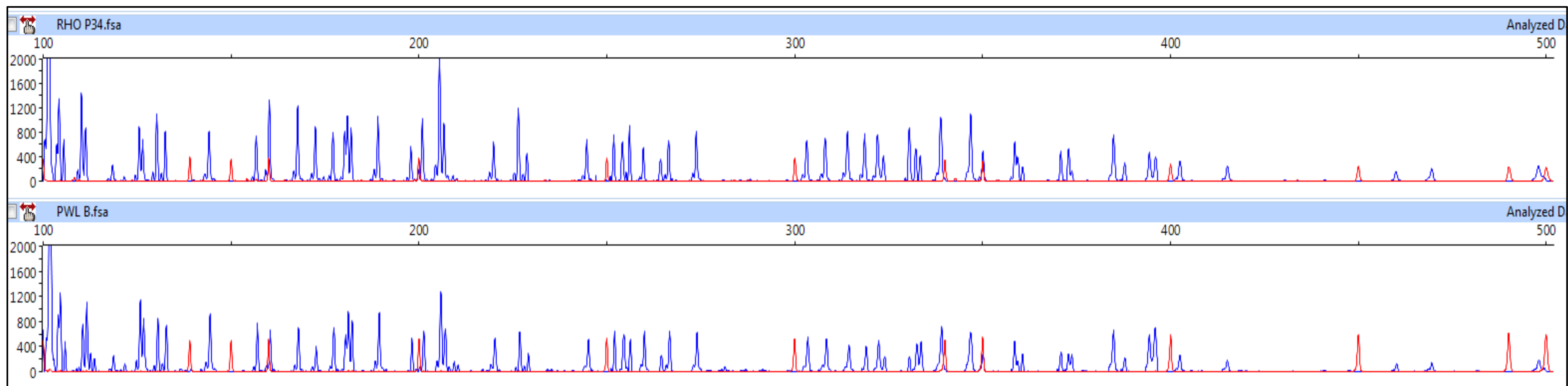

Fig. B.3 continued. AFLP Electropherograms profiles generated from reproducible samples collected from various introduced locations, using the selective primer pair *Eco*RI + AG and *Mse*I + CAA. Horizontal scaling indicates fragment sizing (base pairs); vertical scaling indicates fluorescence signal intensity of amplified fragments (rfu). Blue peaks represent sample fragments; red peaks indicate internal size standard (ROX-500) added to the samples.

Appendix C.1. Raw abundance counts of epifaunal taxa recorded from the different basiphyte species collected from the two contrasting habitats on Anglesey. TS = Tal y Foel, *S. muticum*; TF = Tal y Foel, *F. vesiculosus*.

Epifaunal Taxa	**Samples**															
	TS1	TS2	TS3	TS4	TS5	TS6	TS7	TS8	TF1	TF2	TF3	TF4	TF5	TF6	TF7	TF8
PORIFERA																
Halichondria spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Porifera Unid. spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CNIDARIA																
Dynamena pumila	-	-	-	-	-	-	-	-	-	-	-	25	-	-	-	-
Nemertesia antennina	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Obelia geniculata	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-
Obelia spp.	-	-	-	-	-	-	-	-	7	8	11	-	6	15	-	12
Sertularia cupressina	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-
ANNELIDA																
Nereidae spp. 1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Nereidae spp. 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nereidae spp. 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phyllodocidae spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Spirorbis spp.	-	-	-	-	-	-	-	-	259	48	31	154	52	465	135	293
Polychaete Unid. spp. 1	1	-	-	-	-	-	-	-	2	-	-	2	1	1	-	1
Polychaete Unid. spp. 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polychaete Unid. spp. 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CRUSTACEA																
Copepod Unid. spp.	-	-	14	4	-	-	-	-	-	-	-	-	-	-	-	-
Balanus crenatus	-	-	-	-	-	-	-	-	1	1	1	-	-	-	5	2

Epifaunal Taxa	Samples															
	TS1	TS2	TS3	TS4	TS5	TS6	TS7	TS8	TF1	TF2	TF3	TF4	TF5	TF6	TF7	TF8
Mysidacea Unid. spp.	-	-	-	-	-	3	-	-	-	-	1	-	-	-	-	-
Dynamene bidentata	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Idotea baltica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Idotea granulosa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Idotea pelagica	1	-	-	-	-	-	3	1	-	-	-	-	-	-	-	-
Jaera albifrons	-	-	-	-	2	-	-	-	1	4	3	1	8	13	11	-
Gnathia maxillarus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Isopoda Unid. Spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Caprella acanthifera	1	1	2	-	1	1	1	1	-	-	-	-	-	-	-	-
Caprella equilibra	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ampithoe gammaroides	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ampithoe spp.	2	2	-	-	4	-	-	-	-	-	-	-	-	-	-	-
Aora gracilis	-	-	1	-	2	-	-	-	-	-	-	-	-	-	-	-
Dexamine spinosa	-	1	2	-	15	-	-	4	-	-	-	-	-	-	2	-
Erichtonius difformis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Erichtonius puntatus	2	-	2	2	1	-	-	-	-	-	-	-	-	-	-	-
Gammarus locusta	2	-	3	-	1	-	1	-	62	9	16	11	8	27	4	15
Gammarus spp.	243	192	215	27	121	270	464	73	153	50	137	51	40	13	60	23
Carcinus maenas	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-
Pagurus bernhardus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Palaemon elegans	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Palaemon serratus	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Macropodia spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Crab megalopae larvae	9	1	18	8	5	10	8	5	48	4	18	7	14	1	17	37
PYCNOGONIDA																
Callipallene brevirostris	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-

Epifaunal Taxa	Samples															
	TS1	TS2	TS3	TS4	TS5	TS6	TS7	TS8	TF1	TF2	TF3	TF4	TF5	TF6	TF7	TF8
Nymphon brevirostre	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	1
Nymphon gracile	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pycnogonidae Unid. spp.	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
MOLLUSCA																
Gibbula umbilicalis	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Gibbula cineraria	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Lacuna vincta	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lacuna spp.	1	-	3	2	-	-	-	-	-	-	-	-	-	-	-	-
Lepidochitona cinerea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Littorina littorea	-	-	-	-	-	-	-	-	1	2	1	1	-	1	-	13
Littorina mariae	2	1	2	-	1	-	3	5	6	2	4	7	31	19	5	6
Littorina obtusata	-	-	-	-	-	-	-	-	1	-	-	2	2	-	2	-
Littorina spp.	-	3	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Mytilidae spp.	-	-	3	-	-	-	-	1	-	-	-	-	-	-	-	-
Ostrea edulis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rissoa parva	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Tricolia pullus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BRYOZOA																
Alcyonidium spp.	-	-	-	-	-	-	-	-	15	8	-	8	-	4	6	20
Bowerbankia spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bugula spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Electra pilosa	-	-	-	-	-	-	-	-	4	-	7	-	-	-	2	5
ECHINODERMATA																
Amphipholis squamata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Epifaunal Taxa	Samples															
	TS1	TS2	TS3	TS4	TS5	TS6	TS7	TS8	TF1	TF2	TF3	TF4	TF5	TF6	TF7	TF8
Ophiotrix spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CHORDATA																
Corella eumyota	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Diplosoma spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Entelurus aequoreus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nerophis lumbriciformis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Spinachia spp.	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-

Appendix C.1 continued. Raw abundance counts of epifaunal taxa recorded from the different basiphyte species collected from the two contrasting habitats on Anglesey. RS = Rhosneigr, *S. muticum*; RC = Rhosneigr, *Cystoseira* spp.

Epifaunal Taxa	**Samples**															
	RS1	RS2	RS3	RS4	RS5	RS6	RS7	RS8	RC1	RC2	RC3	RC4	RC5	RC6	RC7	RC8
PORIFERA																
Halichondria spp.	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-
Porifera Unid. spp.	-	-	-	-	1	-	-	-	-	1	2	-	1	-	-	-
CNIDARIA																
Dynamena pumila	-	-	-	-	5	-	-	-	7	-	-	-	-	-	-	-
Nemertesia antennina	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Obelia geniculata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Obelia spp.	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-
Sertularia cupressina	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ANNELIDA																
Nereidae spp. 1	-	-	-	-	-	-	-	-	1	15	3	9	-	1	13	2
Nereidae spp. 2	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-
Nereidae spp. 3	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Phyllodocidae spp.	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Spirorbis spp.	-	-	-	-	-	-	-	-	-	10	-	-	-	1	-	2
Polychaete Unid. spp. 1	1	-	1	-	-	-	1	-	1	1	1	3	-	-	-	1
Polychaete Unid. spp. 2	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Polychaete Unid. spp. 3	-	2	-	-	-	1	-	-	-	-	-	-	-	-	-	-
CRUSTACEA																
Copepod Unid. spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Balanus crenatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Epifaunal Taxa	Samples															
	RS1	RS2	RS3	RS4	RS5	RS6	RS7	RS8	RC1	RC2	RC3	RC4	RC5	RC6	RC7	RC8
Mysidacea Unid. spp.	7	5	3	4	9	11	21	11	-	5	3	3	2	1	2	-
Dynamene bidentata	13	7	2	4	7	5	13	6	1	1	1	-	-	-	-	-
Idotea baltica	-	-	-	-	-	-	1	-	-	2	-	-	-	2	1	-
Idotea granulosa	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Idotea pelagica	1	3	2	-	2	-	1	-	-	1	1	-	-	-	-	-
Jaera albifrons	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Gnathia maxillarus	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Isopoda Unid. Spp.	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Caprella acanthifera	8	14	3	3	4	3	1	3	-	-	-	-	-	-	-	-
Caprella equilibra	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ampithoe gammaroides	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-
Ampithoe spp.	81	76	20	54	48	71	56	64	32	70	194	48	8	67	88	48
Aora gracilis	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-
Dexamine spinosa	151	55	35	90	72	107	237	256	52	86	325	149	17	105	134	22
Erichtonius difformis	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Erichtonius puntatus	-	2	-	2	-	2	1	1	-	-	-	-	-	-	1	1
Gammarus locusta	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gammarus spp.	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-
Carcinus maenas	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pagurus bernhardus	-	-	-	1	-	2	-	-	-	3	-	-	-	-	-	-
Palaemon elegans	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Palaemon serratus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Macropodia spp.	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Crab megalopae larvae	3	7	4	4	12	12	10	23	10	35	35	25	6	17	19	14
PYCNOGONIDA																
Callipallene brevirostris	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Epifaunal Taxa	Samples															
	RS1	RS2	RS3	RS4	RS5	RS6	RS7	RS8	RC1	RC2	RC3	RC4	RC5	RC6	RC7	RC8
Nymphon brevirostre	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nymphon gracile	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Pycnogonidae Unid. spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
MOLLUSCA																
Gibbula umbilicalis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Gibbula cineraria	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lacuna vincta	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-
Lacuna spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Lepidochitona cinerea	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Littorina littorea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Littorina mariae	4	6	8	3	5	3	6	-	-	7	9	2	-	2	1	-
Littorina obtusata	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-
Littorina spp.	3	2	2	-	-	1	1	-	1	4	3	4	1	-	-	5
Mytilidae spp.	3	3	2	-	3	2	-	2	-	10	27	16	1	7	15	1
Ostrea edulis	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Rissoa parva	3	3	-	1	2	-	-	-	2	17	23	13	2	2	1	1
Tricolia pullus	-	-	-	-	-	-	-	-	-	1	3	2	-	-	2	-
BRYOZOA																
Alcyonidium spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bowerbankia spp.	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-
Bugula spp.	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
Electra pilosa	-	-	-	-	-	-	-	-	2	1	-	-	-	-	-	-
ECHINODERMATA																
Amphipholis squamata	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-

Epifaunal Taxa	Samples															
	RS1	RS2	RS3	RS4	RS5	RS6	RS7	RS8	RC1	RC2	RC3	RC4	RC5	RC6	RC7	RC8
Ophiotrix spp.	-	1	-	-	1	-	1	-	-	1	1	-	2	-	4	-
CHORDATA																
Corella eumyota	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diplosoma spp.	2	-	-	-	1	-	4	-	1	-	1	2	1	1	3	-
Entelurus aequoreus	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nerophis lumbriciformis	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
Spinachia spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Appendix C.2. Raw abundance counts of epiphytic algae recorded from the different basiphyte species collected from the two contrasting habitats on Anglesey. Semi-quantitative abundance values: p = present, c = common, a = abundant and d = dominant. TS = Tal y Foel, *S. muticum*; TF = Tal y Foel, *F. vesiculosus*.

Epiphytic Algae	**Samples**															
	TS1	TS2	TS3	TS4	TS5	TS6	TS7	TS8	TF1	TF2	TF3	TF4	TF5	TF6	TF7	TF8
CHLOROPHYTA																
Cladophora rupestris	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cladophora spp.	-	C	-	C	-	P	-	-	C	-	C	-	P	P	P	P
Ulva compressa	P	-	-	-	P	-	P	P	-	-	-	-	-	-	-	-
Ulva intestinalis	P	-	-	-	-	P	-	-	-	-	-	P	-	-	-	-
Ulva lactuca	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-
Ulva prolifera	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ulva spp.	P	-	-	P	-	-	-	-	P	-	P	-	P	-	P	P
Unidentified Chlorophyta spp.	-	-	-	-	-	-	P	-	-	-	-	-	-	-	-	-
PHAEOPHYTA																
Asperococcus fistulosus	-	-	-	-	-	-	-	P	P	-	-	-	P	-	-	-
Cladostephus spongiosus	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-
Colpomenia peregrina	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cystoseira spp.	-	-	-	-	-	-	-	-	-	-	P	-	-	-	-	-
Dictyota dichotoma	-	-	-	-	P	-	-	C	-	-	-	-	-	-	-	P
Ectocarpoid spp.	D	D	D	D	D	D	D	D	D	D	D	D	D	C	D	A
Elachista spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-
Eudesme spp.	-	P	-	-	-	-	-	P	-	-	-	-	-	-	-	-
Halopteris filicina	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Leathesia difformis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Punctaria spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Scytosiphon lomentaria	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Epiphytic Algae	Samples															
	TS1	TS2	TS3	TS4	TS5	TS6	TS7	TS8	TF1	TF2	TF3	TF4	TF5	TF6	TF7	TF8
Sphacelaria spp.	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-
Stilophora spp.	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-
RHODOPHYTA																
Acrosorium venulosum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aglaothamnion spp.	-	-	-	-	-	-	-	-	P	-	-	-	-	-	-	-
Apoglossum ruscifolium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Boergeseniella fruticulosa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ceramium echionotum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ceramium pallidum	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-
Ceramium spp.	P	C	P	-	P	P	P	-	-	-	-	P	-	P	-	-
Chondria dasyphylla	-	-	-	-	-	P	-	-	-	-	-	-	-	-	-	-
Chondrus crispus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cryptopleura ramosa	-	-	-	-	-	-	-	P	-	-	P	-	-	-	-	-
Delessaria sanguinea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Furcellaria lumbricalis	-	-	-	-	-	-	-	P	-	-	-	-	-	-	-	-
Gelidium spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gigartina acicularis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gracilaria gracilis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gracilaria spp.	-	P	-	-	-	-	-	-	P	P	-	-	-	-	P	P
Heterosiphonia plumosa	-	-	-	-	-	-	-	-	P	-	-	-	P	-	P	-
Hypoglossum hypoglossoides	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-
Lithophyllum spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lomentaria articulata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Membranoptera alata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Osmundea pinnatifida	-	-	-	-	P	-	-	-	-	-	-	-	-	-	-	-
Phycodrys rubens	-	-	-	-	-	-	-	-	P	-	-	-	-	-	-	-

Epiphytic Algae	Samples															
	TS1	TS2	TS3	TS4	TS5	TS6	TS7	TS8	TF1	TF2	TF3	TF4	TF5	TF6	TF7	TF8
Plocamium cartilagenum	-	P	P	-	P	-	-	-	P	P	C	P	P	-	-	C
Plumaria plumosa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polyides rotundus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polysiphonia fucoides	-	-	-	-	-	-	-	-	P	C	P	P	P	-	-	-
Polysiphonia lanosa	-	P	-	-	-	P	-	-	-	-	-	-	-	-	-	-
Polysiphonia spp. 1	-	-	P	P	P	C	C	P	-	-	-	-	-	-	-	-
Polysiphonia spp. 2 4 peri	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pterosiphonia parasitica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhodomela confervoides	-	-	-	-	-	-	-	-	-	-	P	-	-	-	-	-
Unidentified Rhodophyta spp. 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Unidentified Rhodophyta spp. 2	-	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-
Unidentified flat red spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Appendix C.2 continued. Raw abundance counts of epiphytic algae recorded from the different basiphyte species collected from the two contrasting habitats on Anglesey. Semi-quantitative abundance values: p = present, c = common, a = abundant and d = dominant. RS = Rhosneigr, *S. muticum*; RC = Rhosneigr, *Cystoseira* spp.

Epiphytic Algae	**Samples**															
	RS1	RS2	RS3	RS4	RS5	RS6	RS7	RS8	RC1	RC2	RC3	RC4	RC5	RC6	RC7	RC8
CHLOROPHYTA																
Cladophora rupestris	-	-	P	C	-	-	-	P	-	-	-	-	P	-	-	-
Cladophora spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	P
Ulva compressa	-	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-
Ulva intestinalis	P	-	-	-	-	-	-	P	-	-	-	P	-	-	-	-
Ulva lactuca	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ulva prolifera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ulva spp.	P	-	-	-	-	-	-	P	P	P	-	-	-	-	-	-
Unidentified Chlorophyta spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PHAEOPHYTA																
Asperococcus fistulosus	-	-	-	P	P	-	-	-	P	P	P	-	-	-	P	P
Cladostephus spongiosus	-	-	-	-	-	-	P	-	-	-	-	-	-	-	-	P
Colpomenia peregrina	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-	-
Cystoseira spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dictyota dichotoma	-	C	-	-	C	A	P	P	P	P	P	-	-	-	-	-
Ectocarpoid spp.	D	A	P	C	D	A	D	D	-	-	-	-	-	-	-	C
Elachista spp.	-	-	-	-	-	-	-	-	P	D	D	D	D	D	D	D
Eudesme spp.	-	-	-	-	P	P	-	-	-	-	-	-	-	-	-	P
Halopteris filicina	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P
Leathesia difformis	-	P	-	P	-	P	-	P	-	P	P	-	P	P	C	P
Punctaria spp.	-	-	-	-	-	-	-	-	-	P	-	-	-	P	P	-
Scytosiphon lomentaria	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Epiphytic Algae	Samples															
	RS1	RS2	RS3	RS4	RS5	RS6	RS7	RS8	RC1	RC2	RC3	RC4	RC5	RC6	RC7	RC8
Sphacelaria spp.	-	P	P	P	-	-	-	-	P	P	-	-	-	P	-	-
Stilophora spp.	-	-	-	-	-	-	-	-	P	P	P	P	P	-	-	C
RHODOPHYTA																
Acrosorium venulosum	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-
Aglaothamnion spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-
Apoglossum ruscifolium	P	-	-	-	-	-	-	-	-	P	-	-	-	-	-	-
Boergeseniella fruticulosa	-	-	-	-	P	P	-	P	C	P	P	-	P	P	P	P
Ceramium echionotum	-	-	-	-	-	-	-	-	P	-	-	P	-	-	-	-
Ceramium pallidum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ceramium spp.	A	-	A	C	A	P	P	P	P	P	P	P	P	-	P	P
Chondria dasyphylla	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P
Chondrus crispus	-	-	P	-	-	-	P	-	P	-	-	-	-	-	-	-
Cryptopleura ramosa	P	P	-	-	-	P	P	-	P	-	P	-	-	P	P	P
Delessaria sanguinea	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-
Furcellaria lumbricalis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gelidium spp.	P	-	-	-	P	-	P	-	C	A	P	P	P	-	A	-
Gigartina acicularis	-	-	-	-	-	-	-	-	-	-	P	-	-	-	C	-
Gracilaria gracilis	-	-	-	-	-	P	-	-	-	P	-	-	-	-	-	-
Gracilaria spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Heterosiphonia plumosa	P	-	-	P	-	-	-	P	-	P	-	P	-	-	P	P
Hypoglossum hypoglossoides	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lithophyllum spp.	-	-	-	-	-	-	-	-	-	P	-	-	-	-	-	-
Lomentaria articulata	-	P	-	-	P	P	P	P	-	P	-	-	P	-	P	C
Membranoptera alata	-	-	-	-	-	P	-	-	-	-	-	-	-	-	-	-
Osmundea pinnatifida	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phycodrys rubens	P	-	-	-	P	-	P	-	-	-	-	-	-	-	-	-

Epiphytic Algae	Samples															
	RS1	RS2	RS3	RS4	RS5	RS6	RS7	RS8	RC1	RC2	RC3	RC4	RC5	RC6	RC7	RC8
Plocamium cartilagenum	C	P	-	-	-	P	P	-	-	P	-	P	P	P	P	-
Plumaria plumosa	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	P
Polyides rotundus	-	-	-	-	-	P	P	-	-	P	-	-	-	-	P	P
Polysiphonia fucoides	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P
Polysiphonia lanosa	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-
Polysiphonia spp. 1	-	P	P	-	A	P	-	-	-	-	-	P	P	P	-	P
Polysiphonia spp. 2 4 peri	-	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-
Pterosiphonia parasitica	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-	-
Rhodomela confervoides	-	-	-	-	-	-	-	-	-	-	-	-	-	P	-	-
Unidentified Rhodophyta spp. 1	-	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Unidentified Rhodophyta spp. 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Unidentified flat red spp.	-	-	-	-	-	-	-	-	-	-	-	P	-	-	-	-

APPENDIX C.3 FEEDING HABITS OF THE MOBILE EPIFAUNAL SPECIES FOUND IN THIS STUDY INCLUDING REFERENCES

Herbivores

Dynamene bidentata	(Arrontes, 1990)
Idotea baltica	(Salemaa, 1987)
Idotea granulosa	(Salemaa, 1987)
Idotea pelagica	(Salemaa, 1987)
Jaera albifrons	(Pavia et al., 1999)
Ampithoe gammaroides	(Duffy, 1990)
Ampithoe spp.	(Duffy, 1990)
Dexamine spinosa	(Zimmerman et al., 1979, Viejo, 1999)
Gibbula umbilicalis	Withers et al. 1975
Gibbula cineraria	Withers et al. 1975
Lacuna vincta	(Johnson and Mann, 1986)
Lacuna spp.	(Johnson and Mann, 1986)
Lepidochitona cinerea	(Hayward and Ryland, 1995)
Littorina littorea	(Withers et al., 1975)
Littorina mariae	(Hawkins and Hartnoll, 1983, Watson and Norton, 1987)
Littorina obtusata	(Hawkins and Hartnoll, 1983)
Littorina spp.	(Norton et al., 1990)
Rissoa parva	(Steneck and Watling, 1982, Viejo, 1999)

Predators

Phyllodocidae spp.	(Rouse and Pleijel, 2001)
Carcinus maenas	(Cohen et al., 1995)
Crab megalopae larvae	(Barnes, 1984)
Nymphon brevirostre	(Barnes, 1984)
Nymphon gracile	(Barnes, 1984)
Entelurus aequoreus	(Oliveira et al., 2007)
Nerophis lumbriciformis	(Lyons and Dunne, 2004)
Spinachia spp.	(Bobsien, 2006)

Omnivores

Caprella acanthifera	(Duffy, 1990, Brawley, 1992)
Caprella equilibra	(Duffy, 1990, Brawley, 1992)
Erichtonius difformis	(Duffy, 1990)
Erichtonius puntatus	(Duffy, 1990)
Gammarus locusta	(Greze, 1968, Zimmerman et al., 1979)
Gammarus spp.	(Greze, 1968, Zimmerman et al., 1979)
Pagurus bernhardus	(Gerlach et al., 1976)
Palaemon elegans	(Smaldon, 1979)
Palaemon serratus	(Smaldon, 1979)
Macropodia spp.	(Barnes, 1984)
Callipallene brevirostris	(Barnes, 1984)
Nereidae spp.	(Rouse and Pleijel, 2001)

Detritivores

Aora gracilis	(Dixon and Moore, 1997)
Tricolia pullus	(Fretter and Manly, 1977)
Amphipholis squamata	(Martin, 1968, Emson and Whitfield, 1989)
Ophiotrix spp.	(Hayward and Ryland, 1995)