DOCTOR OF PHILOSOPHY

The invasion potential of the non-native Chilean oyster (Ostrea chilensis Philippi 1845) in the Menai Strait (North Wales, UK) : present observations and future predictions

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Award date:
2012

Awarding institution:
Bangor University

The invasion potential of the non-native Chilean oyster (*Ostrea chilensis* Philippi 1845) in the Menai Strait (North Wales, UK): present observations and future predictions

A thesis presented to Bangor University for the degree of Doctor of Philosophy by

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September 2012

Cyflwynaf y cyfanwaith hwn i'm teulu oll, ac er cof annwyl am Taid a Nain Corris (Mr a Mrs Luther a Gwyneth Morgan) a Taid Rhuthun (Mr Emlyn Morris) - diolch am eich holl gefnogaeth, caredigrwydd a chyngor ar hyd y blynyddoedd, er gwaetha'r ffaith i bethau gymryd ychydig yn hirach na'r disgwyl i gydio ynof ar brydiau.

"Gwyn eu byd, daw dydd a'u clyw,
Dangnefeddwyr, plant i Dduw" - Waldo (1941)

Datganiad a Chaniatâd

Manylion y Gwaith

Rwyf trwy hyn yn cytuno i osod yr eitem ganlynol yn y gadwrfa ddigidol a gynhelir gan Brifysgol Bangor ac/neu mewn unrhyw gadwrfa arall yr awdurdodir ei defnyddio gan Brifysgol Bangor.

Enw'r Awdur:	Eilir Hedd Morgan
Teitl:	'The invasion potential of the non-native Chilean oyster (*Ostrea chilensis* Philippi 1845) within the Menai Strait (North Wales, UK): present observations and future predictions'
Goruchwyliwr/Adran:	Yr Athro Christopher Alan Richardson (Ysgol Gwyddorau'r Eigion)
Corff cyllido (os oes):	Coleg Cymraeg Cenedlaethol
Gradd a enillwyd:	PhD

Mae'r eitem hon yn ffrwyth fy ymdrechion ymchwil fy hun ac mae'n dod o dan y cytundeb isod lle cyfeirir at yr eitem fel "y Gwaith". Mae'n union yr un fath o ran cynnwys â'r eitem a osodwyd yn y Llyfrgell, yn amodol ar bwynt 4 isod:

Hawliau Anghyfyngol

Mae'r hawliau a roddir i'r gadwrfa ddigidol trwy'r cytundeb hwn yn gwbl anghyfyngol. Rydw i'n rhydd i gyhoeddi'r Gwaith yn ei fersiwn presennol neu mewn fersiynau i ddod mewn man arall.

Cytunaf y gall Prifysgol Bangor gadw ar ffurf electronig, copïo neu drosi'r Gwaith i unrhyw gyfrwng neu fformat cymeradwy at bwrpas ei gadw a mynd ato yn y dyfodol. Nid yw Prifysgol Bangor o dan unrhyw rwymedigaeth i atgynhyrchu neu arddangos y Gwaith yn yr un fformatau neu ddyraniadau y cadwyd ef ynddynt yn wreiddiol.

Cadwrfa Ddigidol Prifysgol Bangor

Deallaf y bydd y gwaith a osodir yn y gadwrfa ddigidol ar gael i amrywiaeth eang o bobl a sefydliadau, yn cynnwys asiantau a pheiriannau chwilio awtomataidd trwy'r We Fyd Eang.

Deallaf unwaith y gosodir y Gwaith, y gellir ymgorffori'r eitem a'i metadata yn y catalogau neu'r gwasanaethau mynediad cyhoeddus, cronfeydd data cenedlaethol theses a thraethodau hir electronig megis EthOS y Llyfrgell Brydeinig neu unrhyw wasanaeth a ddarperir gan Lyfrgell Genedlaethol Cymru.

Deallaf y bydd y Gwaith ar gael trwy Wasanaeth Theses Electronig Ar-Lein Llyfrgell Genedlaethol Cymru o dan y telerau a'r amodau defnydd a ddatganwyd (http://www.llgc.org.uk/index.php?id=4676). Cytunaf fel rhan o'r gwasanaeth hwn y gall Llyfrgell Genedlaethol Cymru gadw ar ffurf electronig, copïo neu drosi'r

Gwaith i unrhyw gyfrwng neu fformat cymeradwy at bwrpas ei gadw a mynd ato yn y dyfodol. Nid yw Llyfrgell Genedlaethol Cymru o dan unrhyw rwymedigaeth i atgynhyrchu neu arddangos y Gwaith yn yr un fformatau neu ddyraniadau y cadwyd ef ynddynt yn wreiddiol.

Datganiad 1:

Nid yw sylwedd y gwaith hwn wedi cael ei dderbyn o'r blaen ar gyfer unrhyw radd, ac nid yw'n cael ei gyflwyno ar yr un pryd mewn ymgeisiaeth am unrhyw radd onid fel y cytunwyd gan y Brifysgol ar gyfer cymwysterau deuol cymeradwy.

Llofnod: .... (ymgeisydd)
Dyddiad: ....

Datganiad 2:

Canlyniad fy ymchwil fy hun yw'r thesis hwn, ac eithrio lle nodir yn wahanol. Lle defnyddiwyd gwasanaethau cywiro, mae maint a natur y cywiriad wedi'i nodi'n glir mewn troednodyn/troednodiadau. Caiff ffynonellau eraill eu cydnabod gan droednodiadau yn rhoi cyfeiriadau eglur. Mae llyfryddiaeth ynghlwm.

Llofnod: .... (ymgeisydd)
Dyddiad: ....

Datganiad 3:

Rhoddaf ganiatâd drwy hyn i'm thesis, os caiff ei dderbyn, fod ar gael ar gyfer llungopïo, ar gyfer benthyciad rhyng-lyfrgellol ac i'w gadw'n electronig (yn amodol ar unrhyw gyfyngiadau fel y nodir yn natganiad 4), ac i'r teitl a'r crynodeb fod ar gael i sefydliadau allanol.

Llofnod: .... (ymgeisydd)
Dyddiad: ....

Datganiad 4:

Cytunaf i osod copi electronig o'm thesis (y Gwaith) yng Nghadwrfa Ddigidol Sefydliadol Prifysgol Bangor, system ETHOS y Llyfrgell Brydeinig, ac/neu mewn unrhyw gadwrfa arall y rhoddwyd awdurdod i Brifysgol Bangor ei defnyddio, a lle bo angen wedi cael y caniatâd angenrheidiol i ddefnyddio deunydd trydydd parti.

Llofnod: .... (ymgeisydd)
Dyddiad: ....

Yn ogystal â’r uchod rydw i hefyd yn cytuno â’r canlynol:

1. Mai myfi yw’r awdur neu wedi cael awdurdod yr awdur(on) i ddod i’r cytundeb hwn a fy mod i felly’n rhoi’r hawl i Brifysgol Bangor i sicrhau bod y Gwaith ar gael yn y ffordd a ddisgrifiwyd uchod.

2. Bod y copi electronig o’r Gwaith a gadwyd yn y gadwrfa ddigidol ac sydd o dan y cytundeb hwn, yn union yr un fath o ran ei gynnwys â’r copi papur o’r Gwaith a osodwyd yn Llyfrgell Prifysgol Bangor, yn amodol ar bwynt 4 isod.

3. Fy mod i wedi cymryd gofal rhesymol i sicrhau bod y Gwaith yn wreiddiol a, hyd eithaf fy ngwybodaeth, nad yw’n torri unrhyw gyfreithiau – yn cynnwys y rhai hynny sy’n ymwneud â difenwi, enllib a hawlfraint.

4. Fy mod i mewn achosion lle mae eiddo deallusol awduron eraill neu ddeiliaid hawlfraint wedi ei gynnwys yn y Gwaith, a lle bo’n briodol, wedi cael caniatâd eglur i gynnwys y deunydd hwnnw yn y Gwaith, ac yn ffurf electronig y Gwaith fel y ceir mynediad ato trwy’r gadwrfa ddigidol mynediad agored, *neu* fy mod i wedi canfod ac wedi dileu’r deunydd hwn na roddwyd caniatâd digonol a phriodol ar ei gyfer ac na fydd modd mynd ato trwy’r gadwrfa ddigidol.

5. Nad oes unrhyw ymrwymiad gan Brifysgol Bangor i gymryd camau cyfreithiol ar ran y sawl sy’n cyflwyno’r gwaith, neu ddeiliaid hawliau eraill, os digwydd bod hawliau eiddo deallusol yn cael eu torri, neu unrhyw hawl arall yn y deunydd a gedwir.

6. Y byddaf yn indemnio ac yn cadw wedi ei indemnio Prifysgol Bangor a Llyfrgell Genedlaethol Cymru rhag ac yn erbyn unrhyw golled, atebolrwydd, hawl neu ddifrod, gan gynnwys yn ddigyfyngiad unrhyw ffioedd cyfreithiol a chostau llys cysylltiedig (ar sail indemniad llawn), sy’n gysylltiedig ar unrhyw achos lle’r ydw i wedi torri unrhyw amod yn y cytundeb hwn.

Llofnod: .... (ymgeisydd)
Dyddiad: ....

Acknowledgements

As anybody who has completed a PhD thesis will testify, the 'end product' is never the result of the sole efforts of the author named on the front cover. Attempting to produce a comprehensive list of all those to whom I am indebted would undoubtedly prove futile, such is the sheer number of people that have kindly provided advice and support to me during the last four years. I therefore endeavour to thank as many as my mind serves to remember, with my heartfelt apologies and equal gratitude to all whose names I have failed to include herein.

Firstly, I would like to thank my supervisor, Prof. Chris Richardson, for his continued enthusiasm and support throughout the duration of this study. Even since his appointment as Head of Department at the School of Ocean Sciences, Chris continued to find time for informal discussions regarding all aspects of the project and always acted as a positive and supportive counterpoint to my many doubts and concerns.

Several people provided expert advice regarding the finer details of all data chapters, either informally or during my supervisory committee meetings. In this respect, the help of the following is kindly acknowledged: Dr Andy Beaumont, Mrs Alison Bell, Dr Jim Bennell, Mr Paul Brazier, Dr Noel Craine, Dr Andrew Davies, Dr Louise Firth, Dr Luis Giménez, Dr Enrique González-Ortegón, Prof. Steve Hawkins, Dr Jan Hiddink, Dr Stuart Jenkins, Mr Paul Kennedy, Dr Ian McCarthy, Prof. Ray Seed, Dr Martin Skov and Mrs Gabe Wyn. I am also indebted to several anonymous referees and journal editors who provided detailed comments on abridged versions of two of the data chapters, now published in peer-reviewed journals. Special thanks goes to Dr Stuart Jenkins and Dr Dai Roberts (The Queen's University, Belfast) for providing extremely helpful feedback during the *viva voce* examination.

Funding for this study was offered through a 4-year post-graduate scholarship, kindly provided by the Coleg Cymraeg Cenedlaethol (CCC). Under this incentive, I was extremely fortunate to further my pedagogic development and receive the opportunity to complete a 'Post-Graduate Certificate in Higher Education' (PGCertHE). Staff members at the CCC, as well as Canolfan Bedwyr (Bangor University), continue to be instrumental figures in the development of Welsh-medium provision within Higher Education institutes. Specifically, Dr Lowri Ahronson, Dr Dei Huws, Dr Llion Jones, Dr Dylan Phillips and Mr Wyn Thomas were all highly influential in igniting my passion for developing and delivering new academic provision in the field of marine science through the medium of Welsh.

Often the 'unsung heroes' of many scientific endeavours, the administrative and technical staff at the School of Ocean Sciences provided exceptional support throughout the duration of my studies, often taking time from their own busy schedules to help out. In this instance, my many thanks go to Dr David Assinder, Mr Vallen Astley, Miss Judy Davies, Mr John East, Mrs Anwen Griffiths, Mrs Joan Griffiths, Mr

Gwyn Hughes, Mr Peter Hughes, Mrs Marilyn Lorrison, Mr Ian Nichols, Mr Gwynne Parry-Jones, Mr Ben Powell, Mr Berwyn Roberts, Mr David Roberts, Mr Len Roberts, Mrs Lorna Roberts and Mrs Lynne Roberts.

A billion thanks also go to all of my colleagues and friends, whose encouragement and friendship never diminished, even when things looked pretty grim. Gareth (O' Johnson), Iain (Ridgo), Nick (The Steam), Osh (Bach), Tim (Whitto), Benny, Cara and Rich (+ Ioan!), Glen (No. 10), Gruff, Jim, Jon and Lou (Aidial, Gei!), Lloyd, Paula, Prysor, Rich and Carrie (+ the chickens!), as well as everyone in the 'Nautilus' Suite – thanks for an incredible four years!

The beautiful 'pen and ink' artwork that accompany the title pages of each data chapter are courtesy of my girlfriend, Miranda. I was especially keen to include something of her work in this thesis as its formation has always been a large and often dictatorial part of our lives together. Thanks for all your patience and for always believing in me, Mir; I could never have done it without you.xx

Lastly, I would like to take this opportunity to thank my family for being there for me throughout the course of my studies. Too often during the last four years have I neglected friendships and the support of my family at the expense of many a late evening in the office. I Dad a Mam, Aled, Rhydian, Nain Rhuthun, Bethan a Dave, Eirian a Glyn, Eirian a Ray, Sioned a Llŷr (+ Martha!), Alaw, Betsan a Gerwyn, Elain, Jim a Sarah, Miranda, Becks a Jari – diolch o waelod calon am bopeth!

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Cydnabyddiaeth

Fel y gall unrhyw un sydd wedi cwblhau traethawd PhD ei ardystio, nid ymdrechion un person yn unig mo'r cyfanwaith hwn, er gwaethaf beth sydd wedi'i ysgrifennu ar y dudalen flaen. Gyda chynifer wedi bod o gymorth i mi trwy gydol y pedair blynedd ddiwethaf, mai'n anochel mai ofer fyddai unrhyw ymdrech i lunio rhestr gyflawn o'r rhai hynny a fuodd mor garedig â'm helpu yn ystod y cyfnod hwn. Ymdrechaf felly i ddiolch i gynifer o bobl ag sy'n bosibl, gydag ymddiheuriadau a'm diolch diffuant i'r rhai hynny rwyf wedi methu a'u cofio.

Yn gyntaf, hoffwn ddiolch i'm goruchwyliwr, yr Athro Chris Richardson, am ei frwdfrydedd di-ben-draw a'i gymorth trwy gydol y prosiect. Hyd yn oed ar ôl ei benodiad fel Pennaeth Adran yn Ysgol Gwyddorau'r Eigion, parhaodd Chris i neilltuo amser ar gyfer nifer o drafodaethau anffurfiol ar holl agweddau o'r gwaith, gyda'i agwedd bositif a chefnogol bob amser yn falm i'm holl bryderon.

Cafwyd trafodaethau cyffrous gyda nifer o bobl ynglŷn â nifer o agweddau gwahanol o'r penodau data, unai ar lefel anffurfiol neu trwy gyfrwng cyfarfodydd gyda'm panel goruchwyliol. Hoffwn felly ddiolch i'r canlynol: Dr Andy Beaumont, Mrs Alison Bell, Dr Jim Bennell, Mr Paul Brazier, Dr Noel Craine, Dr Andrew Davies, Dr Louise Firth, Dr Luis Giménez, Dr Enrique González-Ortegón, yr Athro Steve Hawkins, Dr Jan Hiddink, Dr Stuart Jenkins, Mr Paul Kennedy, Dr Ian McCarthy, yr Athro Ray Seed, Dr Martin Skov a Mrs Gabe Wyn. Rwyf hefyd yn hynod werthfawrogol i nifer o ganolwyr anhysbys a golygyddion cyfnodolion am eu sylwadau manwl ar fersiynau talfyredig o ddau o'r penodau data sydd bellach wedi'u cyhoeddi mewn cyfnodolion rhyngwladol. Hoffwn ddiolch yn arbennig i Dr Stuart Jenkins a Dr Dai Roberts (Prifysgol y Frenhines, Belfast) am yr holl adborth defnyddiol a gefais ganddynt yn ystod yr arholiad *viva voce*.

Cyllidwyd y prosiect hwn trwy garedigrwydd y Coleg Cymraeg Cenedlaethol (CCC). Fel deiliad un o'u hysgoloriaethau ôl-radd 4-mlynedd, bues yn ffodus iawn o dderbyn y cyfle i gwblhau'r 'Tystysgrif Uwchraddedig Addysgu mewn Addysg Uwch' (TUAAU), gan ddatblygu fy sgiliau ymchwil pedagogaidd a fydd o gymorth i'm gyrfa fel addysgwr. Mae staff y CCC, yn ogystal â Chanolfan Bedwyr (Prifysgol Bangor), yn parhau i chwarae rôl flaenllaw yn natblygiad darpariaeth cyfrwng Cymraeg ar draws holl sefydliadau Addysg Uwch Cymru a thu hwnt. Hoffwn ddiolch yn benodol i Dr Lowri Ahronson, Dr Dei Huws, Dr Llion Jones, Dr Dylan Phillips a Mr Wyn Thomas am eu holl ysbrydoliaeth ynghylch datblygu a darparu addysg cyfrwng Cymraeg ym maes gwyddorau'r eigion.

Cefais gefnogaeth eithriadol gan holl staff gweinyddol a thechnegol Ysgol Gwyddorau'r Eigion trwy gydol fy nghyfnod yma, a rhoesant o'u hamser prin i'm helpu bob amser. Hoffwn felly estyn fy niolch diffuant i Dr David Assinder, Mr Vallen Astley, Miss Judy Davies, Mr John East, Mrs Anwen Griffiths, Mrs Joan Griffiths, Mr Gwyn Hughes, Mr Peter Hughes, Mrs Marilyn Lorrison, Mr Ian Nichols, Mr Gwynne Parry-

Jones, Mr Ben Powell, Mr Berwyn Roberts, Mr David Roberts, Mr Len Roberts, Mrs Lorna Roberts a Mrs Lynne Roberts.

Hoffwn hefyd ddiolch o galon i'm holl gyfoedion a'm ffrindiau am eu brwdfrydedd a'u cyfeillgarwch diflino, hyd yn oed pan oedd pethau'n edrych yn o ddu arnaf ar brydiau! I Gareth (O'Johnson), Iain (Ridgo), Johnson (PTS-AI!), Nick (The Steam), Osh (Bach), Paula, Prysor, Tim (Whitto), Benny, Cara a Rich (+ Ioan!), Glen (No. 10), Gruff, Jim, Jon a Lou (Aidial, Gei!), Lloyd, Rich a Carrie (+ yr ieir!), yn ogystal â phawb yn y 'Nautilus' - diolch am bedair blynedd anhygoel!

Daw'r darluniau 'pin ac inc' hyfryd ar ddechrau pob pennod data o waith fy nghariad, Miranda. Roeddwn yn awyddus iawn i gynnwys rhywbeth o'i gwaith hi yma gan i'r traethawd fod yn feistr corn ar draul amryw o nosweithiau a phenwythnosau yng nghwmni'n gilydd. Diolch am dy amynedd ac am ddal i gadw dy ffydd ynof, Mir; ni fuaswn byth wedi gallu cwblhau'r gwaith yma hebddo ti.xx

Yn olaf, hoffwn gymryd y cyfle i ddiolch i'm teulu oll am ddal i gredu ynof trwy gydol y daith. Yn rhy fynych o lawer yn ystod y pedair blynedd ddiwethaf yr esgeulusais gyfeillgarwch a chymorth gan fy nheulu ar draul nosweithiau hir a hwyr yn y swyddfa. I Dad a Mam, Aled, Rhydian, Nain Rhuthun, Bethan a Dave, Eirian a Glyn, Eirian a Ray, Sioned a Llŷr (+ Martha!), Alaw, Betsan a Gerwyn, Elain, Jim a Sarah, Miranda, Becks a Jari – diolch o waelod calon am bopeth!

Swyddfa PhD
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Contents

Abstract

'Biological invasions' are currently recognised as one of the most prevalent drivers of global change. The proliferation of species in areas beyond their natural geographic range can have significant implications for the invaded community, endangering native biodiversity and ecosystem function, as well as jeopardising the provision of several ecosystem services. Critically, non-native ecosystem engineers such as oysters are far more likely to have profound impacts upon their new environment than those which do not exhibit habitat modification abilities. Using the recent spread of the Chilean oyster (*Ostrea chilensis* Philippi 1845) within the Menai Strait and Conwy Bay Special Area of Conservation (SAC) (North Wales, UK), this thesis demonstrates what can happen to a seemingly innocuous non-native species under the currently uncoordinated UK legislation framework. Despite minimal dispersal away from the invasion foci during the first 30 years following its introduction, this chapter shows that *O. chilensis* has expanded its distribution by at least 30 km of shoreline during the last 20 years. Mean densities within several oyster beds are high (>20 m^{-2}), with a maximum density of 232 oysters m^{-2} observed over 1 km to the north-east of the invasion foci. Whilst restricted to a relatively narrow breeding season during the summer months (June-September), the Chilean oyster population consistently recruits a high number of offspring each year (maximum mean monthly spat settlement = 2,570 m^{-2} y^{-1}), particularly following periods of high food concentrations (up to 14.2 µg L^{-1}) during early gametogenesis. Preliminary data suggests that *O. chilensis* may be exempt from heavy predation pressure within the SAC due to the absence of natural predators, thus increasing its persistence and eventual dominance along the low shores of the Menai Strait. The observed strong stock-recruitment relationship and highly reduced natural dispersal capacity (generally <100 m) of this species is in stark contrast to its relatively substantial range expansion, suggesting that other, potentially anthropogenically-mediated transport vectors are in operation. The commercial collection of oyster-fouled periwinkles (*Littorina littorea* L. 1758) is identified as one example of a currently unregulated anthropogenic activity which may facilitate its dispersal. Up to 10.5% of all periwinkles at several localities within the Menai Strait were fouled by oysters, with all but the largest oysters accidentally collected during the collection process. The process of 'winkle farming', whereby under-sized or overly-fouled periwinkles are returned to the Menai Strait by the central wholesaler (although, critically, not necessarily back to the site from which they were initially collected) to help maintain healthy stocks, may facilitate the local spread of *O. chilensis*. Additionally, the survival of several oyster epibionts throughout a laboratory simulation of the harvesting process highlights the potential of transfer to continental Europe, where live periwinkles are brought as grazers which help to reduce algal fouling on commercial Pacific oyster (*Crassostrea gigas* Thunberg 1793) trestles. The negative associations observed between the presence of oyster epibionts and proxies of general periwinkle marketability (namely dry flesh weight and orientation ability) could be utilised as a tool to dissuade collectors from harvesting oyster-fouled periwinkles. Finally, evidence is presented which suggests that further warming of the Earth's atmosphere is likely to further extend its breeding season. However, whilst unlikely to halt the poleward migration of non-native species indefinitely, future cold snaps (also predicted to increase in both their frequency and intensity with climate change in north-western Europe) are hypothesised to act as a critical ‘reset’ mechanism which may impede the rate of biological invasions. Laboratory experiments, mimicking present conditions and future projections of acute periods of extreme freezing air temperatures, were conducted to estimate how the proliferation of the intertidal *O. chilensis* population may become compromised due to cold winter temperature aberrations. Non-native oysters did not perform as well as their native co-inhabitants under simulated future cold snap conditions. 16% and 63% of all oysters had died within 4 weeks following a 2h exposure period to -6°C and 10°C respectively, increasing to 63% and 100% mortality when cold snap frequency was effectively doubled. Small oysters (likely to be experiencing their first winter) cooled and thawed as much as three and nine times quicker than their larger counterparts respectively. Small oysters were also subjected to significantly greater periods of extracellular ice formation. However, no significant difference was observed between oyster survival rates across size classes within each temperature treatment. A case of 'strength in numbers' is presented, whereby small oysters, in the presence of several other conspecifics, are buffered against the effects of freezing air temperatures compared with those exposed to freezing temperatures in isolation. This has critical implications for the future invasion dynamics of this non-native oyster population within a designated SAC. In light of the findings presented within this thesis, future management options regarding this non-native oyster population are proposed, aimed at stimulating discussion among all those with environmental and economical interests within the SAC.

Crynodeb

Cydnabyddir fod 'goresgyniadau biolegol' yn chwarae rôl flaenllaw mewn newid byd-eang. Gall cynyddiad rhywogaethau mewn ardaloedd y tu hwnt i'w ffiniau daearyddol naturiol gyflwyno goblygiadau arwyddocaol i'r gymuned sy'n cael ei bygwth. Peryglir bioamrywiaeth swyddogaeth ecosystem cynhenid, yn ogystal â darpariaeth mewn nifer o wasanaethau ecosystem. Mae ffyniant peirianyddion ecosystem anfrodorol megis wystrys yn llawer mwy tebygol o gael sgîl-effeithiau negyddol ar y gymuned dan fygythiad o'u cymharu â'r rhywogaethau hynny sydd ddim yn meddu ar rinweddau o'r fath, Gan ddefnyddio ffyniant diweddar y wystrysen Tsileaidd (*Ostrea chilensis* Philippi 1845) yn Ardal Cadwraeth Arbennig (ACA) y Fenai a Bae Conwy (Gogledd Cymru, DU), dengys y traethawd ymchwil hwn beth sy'n gallu digwydd i rywogaeth anfrodorol digon diniwed yr olwg o dan fframwaith deddfwriaethol dryslyd presennol y DU. Er nad iddi ledaenu ymhell oddi wrth ffocws yr ymlediad yn ystod y 30 mlynedd gyntaf yn dilyn ei chyflwyniad i'r ardal, dangosir yr astudiaeth bresennol fod *O. chilensis* wedi cynyddu ei amrediad yn ystod yr 20 mlynedd ddiwethaf, gan bontio o leiaf 30 km o arfordir. Mae'r dwysedd cymedrol oddi mewn i nifer o wlâu wystrys yn uchel (> 20 m^{-2}), gydag uchafswm dwysedd o 232 wystrys m^{-2} wedi'i ardystio tua 1 km i'r gogledd-ddwyrain o ffocws yr ymlediad. Er bod y tymor atgenhedlu wedi'i gyfyngu i dymor yr haf yn bennaf (Mehefin-Medi), mae poblogaeth y wystrysen Tsileaidd yn parhau i recriwtio nifer fawr o epil bob blwyddyn (uchafswm cymedrol anheddiad misol = 2,570 m^{-2} y^{-1}), yn enwedig yn dilyn cyfnodau ble mae crynodiad bwyd yn uchel (hyd at 14.2 µg L^{-1}) yn ystod cyfnodau cynnar gametogenesis. Mae data rhagarweiniol yn awgrymu yn eithriedig o gyfnodau o bwysau ysglyfaethol trwm oddi mewn i'r ACA o ganlyniad i absenoldeb ei hysglyfaethwyr naturiol, gan gynyddu ei dyfalwch a'i goruchafiaeth ar hyd rhannau isel o arfordir rhynglanw'r Fenai. Mae'r berthynas gref rhwng 'stoc' a 'recriwtiaid' ac analluogrwydd y rhywogaeth yma i ledaenu dros bellteroedd arwyddocaol trwy ddull naturiol yn unig (<100 m) yn gwrthgyferbynnu'n llwyr gyda'r lledaeniad cymharol eang a nodwyd, ac felly'n awgrymu fod fectorau cludiant dynol hefyd ar waith. Adnabyddir y broses fasnachol o gasglu gwichiaid (*Littorina littorea* L. 1758) wedi'i llychwino gan wystrys Tsileaidd fel un enhgraifft o weithgaredd anreoledig, ddynol ei natur sydd o bosibl yn gyfrifol am y lledaeniad yma yn nosraniad *O. chilensis*. Nodwyd fod hyd at 10.5% o bob gwichiaid mewn nifer o ardaloedd o fewn yr ACA wedi'i llychwino gan wystrys, gyda phob un namyn y rhai hynny sy'n cario'r wystrys mwyaf eu maint yn cael eu casglu yn ystod y broses o gynaeafu. Adnabyddir hefyd fod 'ffermio gwichiaid', sef y broses o ddychwelyd gwichiaid sydd un ai'n rhy fach neu wedi'u llychwino’n ormodol yn ôl i'r Fenai (ond nid o'r rheidrwydd i'r man lle casglwyd hwy yn wreiddiol) er mwyn hybu'r boblogaeth, yn debygol o ychwanegu tuag at y lledaeniad yma. Yn ychwanegol i hyn, defnyddir efelychiadau o'r broses gynaeafu yn y labordy i ddangos fel y gallai'r wystrys oresgyn a chael eu cludo i gyfandir Ewrop, lle mae rhai gwichiad yn cael eu gwerthu'n fyw i ffermwyr pysgod cregyn sy'n defnyddio'r malwod fel porwyr i leihau llychwiniad algâu ar gregyn a chyfarpar ffermio wystrysen y Môr Tawel (*Crassostrea gigas* Thunberg 1793). Hyderir y gellir defnyddio'r perthnasau negyddol hynny rhwng presenoldeb llychwinol y wystrys Tsileaidd a^ iechyd cyffredinol y gwichiaid (trwy fesur biomas sych y cnawd a gallu cyfeiriadedd) i anghymell y casglwyr i beidio â chasglu gwichiaid wedi'i llychwino â'r wystrys,

Yn olaf, cyflwynir tystiolaeth sy'n awgrymu y bydd cynyddiad yn nhymheredd y môr yn sgîl cynhesu byd-eang yn ehangu’r tymor atgenhedlu yn y rhywogaeth hon. Er nad yn debygol o roi terfyn ar ymfudiad tua'r pegynau yn gyfan gwbl, dangosir, ar y llaw arall, fel y gallai cynyddiad yn amlder ac arddwysedd cyfnodau llym o rewi caled (a ragdybir ar gyfer gogledd-orllewin Ewrop yn y dyfodol agos) weithredu fel sustem 'ailosod' gritigol i arafu'r gyfradd lledaeniad mewn rhywogaethau anfrodorol. Defnyddwyr efelychiadau o gyfnodau llym o rewi caled arfaethedig a'r rhai hynny a ragdybir ar gyfer y dyfodol agos yn y labordy i ddangos sgîl-effeithiau posibl y fath gyfnodau ar gyfradd lledaeniad *O. chilensis* yn y Fenai o'i gymharu â rhywogaethau brodorol eraill. Ni ffynnodd wystrys anfrodorol cystal â'u cyfoedion brodorol o dan gyfnodau llym o rewi caled. Bu farw 16% a 63% o'r wystrys Tsileaidd o fewn 4 wythnos o'u cyflwyno i gyfnod o 2 awr yn unig ar dymereddau aer o -6 a -10°C yn ôl eu trefn. Trwy ddyblu'r cyfnod rhewi (2 awr, unwaith bob dydd am gyfnod o ddeuddydd), bu cynnydd arwyddocaol yn eu marwolaeth ar yr un tymereddau (-6°C = 63%, -10°C = 100%). Roedd cyfradd rhewi a meirioli wystrys bychan (h.y. y rhai hynny a oedd yn agored i dymereddau gaeafol am y tro cyntaf) gymaint â thair a naw gwaith yn fwy na'r hynny a recordiwyd mewn wystrys mwy yn ôl eu trefn. Dangoswyd hefyd fod wystrys bychan yn dioddef cyfnodau hirach lle'r oedd rhew allgellog yn ffurfio. Serch hynny, ni welwyd gwahaniaeth arwyddocaol yng ngoresgyniant *O. chilensis* ar draws yr holl feintiau o fewn pob triniaeth tymheredd. Cynigir tystiolaeth sy'n awgrymu mai 'mewn undod y mae nerth', lle mae wystrys bychan, ym mhresenoldeb nifer o wystrys eraill, yn cael eu clustogi rhag effeithiau'r rhewi caled o'u cymharu â phan breswyliant ar eu pen eu hunain. Mae gan y canlyniad yma arwyddocâd arbennig i ddeinameg ymlediad y wystrysen yma yn yr ACA yn y dyfodol agos. Yn sgîl yr holl ganlyniadau a gyflwynir oddi mewn i'r traethawd hwn, trafodir nifer o opsiynau rheolaeth addas ar gyfer y boblogaeth o wystrys Tsileaidd anfrodorol, gyda'r bwriad o ennyn trafodaethau brwd rhwng yr holl hapddalwyr sydd â diddordeb yn lles ac iechyd yr ACA yma.

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Glossary

Term	**Synonyms**	**Definition**
Biological invasions	n/a	Comprises of the anthropogenically-mediated movement of a *non-native species* across a biogeographic barrier and into an area beyond its *native geographic range*, as well as its subsequent proliferation, ecological interactions and impacts within its novel environment.
Biotic resistance	n/a	The resistance of *native* species to either the *establishment* of or *invasion* by *non-native species*.
Establishment or *Established*	Naturalisation or Naturalised	*Non-native* population which are capable of producing viable offspring that are, in turn, recruited either into the originally-introduced population or into a new geographic location to form inter-connecting or self-sustaining populations.
Establishment-Invasion continuum	Naturalisation-Invasion continuum	A conceptualisation of the progression of *non-native* propagules from forming an *established* population to becoming *invasive* (see Figure 1.1).
Introduction	Transfer	The act of transferring, either deliberately or accidentally via human-mediated activities, a *non-native species* into an area beyond its *native geographic range*, although not always leading to the *establishment* of an *invasive species.*
Invasibility	n/a	A measure of the resistance of habitats to biological invasions. Habitats with a high degree of *invasibility* are more likely to be impacted by the introductions of *non-native species*.
Invasion foci	Site of original introduction	The area to which *non-native* propagules were initially introduced prior to the commencement of *range expansion.*
Invasiveness	n/a	A measure of the overall capacity of a *non-native species* to become *invasive*, usually based upon specific life-history characteristics and reproductive dynamics.

Invasive species	n/a	A *non-native species* which has managed to establish a self-sustaining population within its *novel environment*, producing several generations of viable propagules which have subsequently spread over significant distances away from the site of original introduction in large numbers. It may become dominant in places and often capable of exerting economic and ecological changes within its new environment.
Native	Indigenous	Any species which has evolved within a given geographical area over geological time scales or has arrived there more recently solely by natural dispersal mechanisms as opposed to anthropogenically-mediated transfers (see *range expansion*).
Non-native species	Alien Non-indigenous species	Any species that, via anthropogenically-mediated activities, has overcome a biogeographic barrier and thus been transferred into an area beyond its natural geographic range.
Novel environment	New geographic region	An area beyond the *native geographic range* of a particular species (i.e. where all propagules from their native range are unable to colonise due to a biogeographic barrier or a lack of adequate natural dispersal capacity).
Propagule pressure	n/a	A composite measure of the number of propagules of a *non-native species* entering a *new geographic region*. It is widely-regarded as one of the only consistent predictors of invasion success across numerous taxa and geographic locations. Propagule pressure may be calculated by multiplying the number of introduction events with the number of non-native propagules within each event. As either one of these factors increases, propagule pressure also increases (see *propagule rain* or *secondary spread* below for comparison).

Propagule rain	n/a	Refers to the probability of non-native propagules extending their distribution further away from the invasion foci following an *introduction* event, rather than *propagule pressure* originating from their *native* region *per se*.
Range expansion	Range extension (also see *'secondary spread'* - right)	A concept relevant to both *native* and *non-native species* concerning their spread into *new regions* either by natural or anthropogenically-mediated dispersal, although not across biogeographic boundaries. A *'secondary spread'* is a form of range expansion whereby propagules spread away from an *invasion foci.*
Transient	Casual Innocuous	*A non-native species* which, despite its own ability to survive within its *novel geographic region*, is not yet capable of producing viable offspring.
Transport vector	n/a	A broad term to define the causation, mode, speed and duration of the transfer of *non-native* propagules across a biogeographic barrier and into their *novel environment.*

Chapter 1

General Introduction

1.1 Biological invasions: what, where, when and why?

For a species to occupy a wide geographical range, it must rely upon some degree of dispersal. Some species disperse by active movements such as walking, swimming or flying, whilst others rely on exogenous transport mechanisms such as oceanic currents or wind dispersal. Some disperse over several hundreds of kilometres, whilst others stay relatively close to their parents. Despite this plethora of dispersal strategies, no species has a fully cosmopolitan distribution. In fact, most taxa are confined to relatively small geographical areas, whilst relatively few are geographically widespread (Darwin 1859; MacArthur 1972; Gaston 1996). This relationship also holds true within evolutionary clades across the majority of taxa and ecosystems investigated (Calosi *et al.* 2009). Theoretical propositions as to *how* geographic ranges are limited far outweigh empirical-based evidence, although an intricate combination of several biological, physical and evolutionary mechanisms are likely to be operational towards the frontiers of any organism's distribution (Brown *et al.* 1996; Lester *et al.* 2007; Gaston 2009). Some species may be confined to a particular region due to an impermeable physical barrier, ranging from waterfalls and localised areas of unsuitable habitat or terrain to entire mountain ranges and oceanic basins (Cox and Moore 1980). Others may be constrained by their lack of physiological tolerance and acclimation abilities to environmental stressors experienced towards the perimeters of their respective geographic distributions, including temperature, light availability, salinity and hypoxic conditions (Somero 2011). Temperature plays a critical role in the functioning of physiological mechanisms and is of particular relevance to ectothermic organisms, who must endure wide fluctuations in body temperature over both short- and long-term time scales. Dispersal in itself may also be a major determinant of geographic range extent in a rapidly-changing climate, when the rate of change may exceed that of the species' dispersal capacity (Burrows *et al.* 2011). Occasionally, however, anthropogenically-mediated activities allow species to breach these otherwise impassable barriers and thrive in areas *beyond* their natural geographic ranges. 'Biological invasions' encompass all aspects of such transfers, as well as the subsequent proliferation of these species (hereafter termed 'non-native species') beyond their native ranges, including their survival, dispersal, ecological interactions with other co-inhabitants and impacts upon their novel ecosystems.

Humans may directly or indirectly facilitate the transfer of non-native species across biogeographic barriers and into new regions beyond their natural dispersal capacity. Direct transfers concern instances where the invasion barrier has been overcome due to the implicit actions of man and can be further sub-divided into 'accidental' and 'deliberate' introductions. Deliberately-transferred organisms is a term usually reserved for species that have been intentionally introduced into new regions for the purpose of aquaculture, recreational use (e.g. sport fishing, shooting) or biological pest control. Accidental introductions, on the other hand, encompass a wide-range of anthropogenic activities where the surmounting of the invasion barrier was unintentional. Examples include the transfer of species attached to the hulls of shipping vessels (Gollasch 2002), within ship ballast water (Carlton 1985) or as accessory species of those which have been deliberately introduced for the purpose of aquaculture (Minchin 1996). Indirect transfers of non-native species are rather less conspicuous. In this instance, the invasion barrier is, in effect, removed by an anthropogenic activity, which then allows for the introduction of species to new geographic regions by natural dispersal mechanisms (i.e. a form of range extension). Biological invasions, in this instance, thus occur as an *indirect* result of human activities. A classic example would be the completion of the construction of the Suez Canal in 1869, which subsequently enabled connectivity between species inhabiting the Mediterranean and Red Seas (i.e. Lessepsian migrations; see Galil 2008). A further caveat which somewhat clouds the definition of a biological invasion involves the anthropogenically-mediated warming of the Earth's atmosphere as a result of fossil fuel burning and land use changes. Ocean warming is known to facilitate the poleward migration of many species across several taxa and geographic regions, both native and non-native (Southward *et al.* 1995, 2005; Hawkins *et al.* 2009). Some elements of global climate change may thus *move* but not *remove* invasion barriers *per se*. Poleward migrations of non-native species may therefore be considered to be an expansion of their geographic range following their introduction (i.e. part of the 'secondary spread'), or alternatively, as part of the invasion process outright (see Colautti and MacIsaac 2004; Hodges 2008; Richardson *et al.* 2011).

1.2 The invasion process

The field of biological invasions has gained widespread attention in recent years, partly due to the extensive use of emotive ‘buzzwords’ such as ‘alien’, ‘exotic’, ‘noxious’, ‘nuisance’ and ’pest’ to describe those species that have become established within areas beyond their natural geographic range. However, respective definitions are often incongruent, leading to misinterpretation of important ecological concepts and thus undermining policy formation and management efforts (Colautti and MacIsaac 2004; Riccardi and Cohen 2007). For clarity, a glossary section is included herein which serves as a compendium of definitions of all relevant invasion-based terms that will be used throughout the remainder of this thesis.

Several key papers have attempted to describe the biological invasion process (e.g. Carlton 1985; Williamson 1996; Marchetti *et al.* 2004; Freckleton *et al.* 2006; Reise *et al.* 2006) and it is beyond the scope of this thesis to form a comprehensive critique of all proposed concepts. Suffice to say that significant advances in our understanding of the invasion process have come from stage-based approaches, which depict the invasion process as the passage of non-native species through a series of distinct stages between their native and novel environments. It may be argued that stage-based approaches unwittingly suggest that the invasion process is strictly discrete, with the attainment of each subsequent stage dependent on the termination of the previous stage. In reality, of course, it is known that *"activities in prior stages do not stop with the inauguration of a subsequent stage"* (Davis 2009), thus invasion may often undergo simultaneous periods of establishment, recruitment failure and dispersal. However, the practicality of stage-based approaches has undoubtedly facilitated better connectivity and understanding between the scientific community and those involved in the formation of management strategies concerning biological invasions, and are thus to be commended.

A favoured model in modern-day invasion ecology is that of Colautti and MacIsaac (2004) and is based on the paradigm of ‘propagule pressure’; a *"composite measure of the number of individuals released into a region to which they are non-native"* (Lockwood *et al.* 2005). The concept states that as the number of discrete release events and/or the number of individuals released increases, propagule pressure also increases. The prospective invaders begin as propagules within a potential ‘donor region’ (stage 0), and their passage into subsequent stages of the invasion process is controlled by a series of filters (see Figure 1.1). Some propagules are transferred into the transport vector (stage I) by an anthropogenically-mediated activity. Survival within the transport medium leads to introduction to a novel area (stage II), with the possibility of becoming established (stage III), providing that the species is able to survive and reproduce within its new geographic region. As seen in Figure 1.1, propagule pressure (determinant A) is heavily associated with all stages of the invasion process, which can also be facilitated (positive B and/or C determinants) or inhibited (negative B and/or C determinants) by the physicochemical requirements of the invader (B) and also by community interactions (C). In a similar vein, 'propagule rain' (*sensu* Lockwood *et al.* 2009, defined as a function of the number of release events and the density of propagules within each release event, dispersed from the invasion foci following establishment) is linked with the post-establishment success of an invasive species. Both the dispersal of an established species away from its invasion foci and its dominance within its new environment are highly dependent on

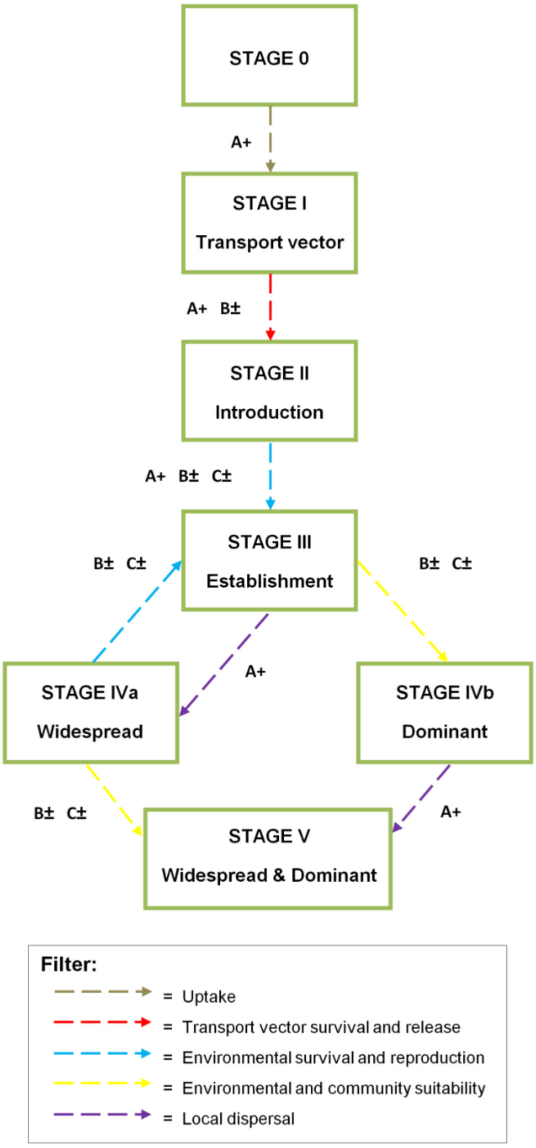


Figure 1.1 Suggested framework for defining operationally important terms in invasion studies (redrawn from Colautti and MacIsaac 2004). Potential invaders begin as propagules residing in a donor region (stage 0), and pass through a series of filters that may preclude transition to subsequent stages. A non-native species may be localised and numerically rare (stage III), widespread but rare (stage IVa), localised but dominant (stage IVb) or widespread and dominant (stage V). Adjectives are intended only to aid in conceptualising each stage, but should not be used to refer to the stage of interest. Three classes of determinants affect the probability that a potential invader will pass through each filter: (A) propagule pressure; (B) physicochemical requirements of the potential invader; (C) community interactions. Determinants may positively (+) or negatively (–) affect the number of propagules that successfully pass through each filter (Colautti and MacIsaac 2004).

propagule rain and how physiologically well-suited a species is to its new environment. Whilst abundant within its area of initial introduction, a species can be highly restricted in terms of its geographic range extent by low propagule pressure (e.g. low fecundity, reduced breeding season and duration of larvae in the plankton, often linked with an increased period of maternal brooding) (stage IVb). The ability of a species to overcome such barriers restricting geographic spread and local dominance within the community is not temporally or intra-specifically 'fixed' and can change naturally and/or anthropogenically by several possible processes. These include the anthropogenic removal of a physical barrier previously restricting further dispersal (Rilov *et al.* 2004), the removal of a predator previously controlling invader density (Paine 1974), as well as the accelerated warming of the oceans due to global warming, with the resulting increase in spawning events and extension of the breeding season (see Reise *et al.* 2005) culminating in a widespread and abundant non-native, invasive population (stage V). As well as aiding in the clarification of one's perceived definition of specific 'invasive' terminology, this supplementary terminology proposed identifies the factors that influence the relative 'success' of the potential invader at each stage of the invasion process (determinants A, B and C; Figure 1.1). Conflicting and often biased views due to subconscious associations with preconceived terms are also eliminated by the supplementation of with 'operational' terms (i.e. 'stages') with no *a priori* meaning (Colautti and MacIsaac 2004).

1.3 Why should we care?

To become invasive, an introduced species must often withstand extremely stressful conditions both within the transport vector and following its transfer into a new geographic region. Temperatures within ballast water tanks, for example, can increase as much as 16°C between points of uptake and release, whilst hypoxic conditions are also a regular occurrence (Seiden *et al.* 2011). Likewise, non-native epibionts of deliberately-introduced oysters may spend several days in transit and may thus be exposed to numerous stresses, including temperature, desiccation and hypoxia (Minchin 1996). Following their arrival into a new geographic region, introduced species must also be capable of producing viable propagules that are then capable of some degree of dispersal away from the adult population. Species which show a high degree of behavioural or phenotypic plasticity are thus generally considered to possess a higher degree of invasiveness (Davidson *et al.* 2011). It is therefore unsurprising that the majority of biological invasions are rendered unsuccessful.

The 'Tens Rule' (*sensu* Williamson and Fitter 1996) describes the distribution of the probability of the successful progression of a non-native species through each stage of invasion along the establishment-invasive continuum. Initially based on European plant data, it is suggested that approximately one in ten of introduced species survive the transport vector to become transient species within their novel environment. In turn, approximately one in ten of all transient species are capable of proliferation within their new geographical region, with only one in ten of all established species capable of becoming invasive. It should be noted that the point estimate of 0.1 is simply a measure of central tendency, and is not intended to represent a definitive proportion of propagules which progress into each subsequent invasion stage (see Caley *et al.* 2008). These predicted proportions will also vary depending on how one defines each stage along the established-invasive continuum and are likely to increase over time as the residence time of non-natives within new geographic regions increases. Nevertheless, the Tens Rule serves as a useful working estimate and concept which highlights that biological invasions are consistently rare events which are heavily reliant upon propagule pressure, life history characteristics and geographic location. Despite a relatively low number of successful invasions, the significance of this small portion of invaders becomes noteworthy when one considers the colossal socio-economic and ecological impacts that several previously-documented invasive species have bestowed upon their respective novel environments.

1.3.1 Socio-economic impacts

Whilst relatively rare, introductions of some non-native species beyond their native geographic range have been socio-economically catastrophic. For example the European shore crab, *Carcinus maenas* (L. 1758), is an invader to the Atlantic shores of both Canada and the United States of America, and costs relating to its dominant predatory impacts upon commercial shellfisheries are estimated to exceed US$44 million annually (Pimentel *et al.* 2005). Likewise, the manual removal of high densities of freshwater zebra mussels, *Dreissena polymorpha* Pallas 1771, from the inlet pipes of several water treatment and power plants in North America is estimated to cost the US government over US$161-467 million a year (Connelly *et al.* 2007). Growing concerns are developing regarding the European invasion of the Chinese mitten crab, *Eriocheir sinensis* Milne-Edwards 1853, and its potential impact on several aspects of human health and biosecurity. Its extensive burrowing activity (see Panning 1938) have led to the accelerated erosion and collapse of riverbanks and levees, posing serious threats to flood defence systems and the management of water supplies. The crab is also a secondary host for the Oriental lung fluke, *Paragonimus westermani* Kerbert 1878, which can lead to severe pulmonary discomfort, paralysis and even death among humans (see Clark *et al.* 1998).

1.3.2 Invasion lags

Whilst the advent of biological invasions far precedes that of any scientific records, a continual intensification in globalisation and transport, particularly since the industrial revolution, has directly led to an exponential rise in the rate of biological invasions (Hulme 2009). Regrettably, financial constraints and the occasional lack of effective collaboration between scientists and policy makers have meant that management efforts have generally focused on a few of the more pressing invaders, whilst the majority of transient and less invasive non-natives have received little or no attention. However, population growth and secondary spread following the establishment of a non-native species may vary dramatically over both time and space. Some transient species can persist for many years within their novel environment until conditions may later become favourable for reproduction (see Crooks and Soulé 1999). Although the biological factors operating following establishment are not well understood, three factors have been postulated as potential explanatory factors of time lags (see Table 1.1). The case of the Red Sea mussel, *Brachidontes pharaonis* (P. Fischer 1870), (introduced to the Mediterranean following the opening of the Suez Canal in 1869), its spread along the coast of Israel and its subsequent dominance over the native mussel, *Mytiliaster minimus* (Poli 1795), provides a rare documented example of a lag phase of approximately 120 years (Rilov *et al.* 2004). It is proposed that the onset of invasion was permitted due to recent shift in habitat conditions towards lower sedimentation rates and improved water exchange in areas previously devoid of mussels, as well as the ability of *B. pharaonis* to subsequently outcompete its indigenous counterpart following settlement within their novel environment (termed an 'environmental lag').

Table 1.1 Description of different types of lag phases (*sensu* Crooks and Soulé 1999).

Type of Lag	**Description**
Inherent lag	Caused by the nature of population growth and range expansions, and heavily influenced by the larval dispersal capabilities of the invading species.
Environmental lag	Caused by shifts in ecological conditions towards a more favourable environment for the invading species.
Genetic lag	Caused by the relative lack of genetic fitness of the invading species within its new environment.

1.3.3 Changes in the rate of invasion

Anthropogenically-mediated transfers of species across the globe are expected to continue to bridge the gaps between several biogeographically-distinct regions. This unprecedented exchange of species is rapidly leading to the replacement of native species with non-native invaders, reducing spatial diversity and promoting biotic homogenisation (McKinney and Lockwood, 1999). The duration of environmental lags may become reduced in the near future due to our rapidly-changing climate. Biological invasions and climate change are currently recognised as two of the most prevalent modifiers of environmental change on a global scale. Whilst the independent impacts of both environmental drivers continue to receive ample attention in the scientific literature, empirical studies regarding the intricate interactions between both processes are required (Dukes and Mooney 1999; Stachowicz *et al.* 2002; Ward and Masters 2007). The Earth's atmosphere has warmed by 0.74±0.18°C since the early 1900s and a further warming of 1-3°C is predicted by the end of the 21st century (IPCC 2007).

Worryingly, native and non-native species are responding disproportionately to a warming climate (Southward *et al.* 1995; Hawkins *et al.* 2003; Mieszkowska *et al.* 2005; Hiddink and ter Hofstede 2008). For native species, which have evolved within their unique environment for several thousands of years, a rapidly-warming climate may be disastrous. Many species are already pushed to their physiological limits within their current native range and further physiological stresses may hamper their competitive resistance (Somero 2011). The invasiveness of newly-introduced non-natives may, on the other hand, be facilitated by climate change, with the generally broader thermal tolerance and greater dispersal capacity of several established non-natives favouring their proliferation at the expense of several native competitors (Sorte *et al.* 2010). Studies investigating phenological adaptations (i.e. changes in the timing of key events, including reproductive maturity) to climate change have also revealed interesting differences between native, transient non-natives and invasive species (Willis *et al.* 2010). Moore *et al.* (2011) showed that, in a single geographic region, the spawning season of warm water limpets is becoming increasingly prolonged, whilst more cold-acclimated species are expressing increasing reproductive failure. As well as an increase in mean global surface temperature, the Earth's climate is also expected to become increasingly variable. Extreme climatic events, including hurricanes, flooding, droughts, heat waves and cold snaps, are likely to become more common. However, the responses of both native and non-native species to such changes require urgent attention (Smith 2011). Whilst climate change-related ocean warming is likely to extend the duration of the brooding season of several non-native species, it remains to be seen whether or not future plankton dynamics will match or mismatch with the nutritional requirements of adult conspecifics (see Cushing 1990) and have positive or negative effects on the future proliferation and invasiveness of non-native species.

1.4 Transfers of non-native oysters around the world

Several oyster fisheries around the world have undergone 'boom and bust' phases, including the Eastern oyster, *Crassostrea virginica* (Gmelin 1791), in Chesapeake Bay (USA) (Mann *et al.* 1991), the Chilean oyster, *Ostrea chilensis* Philippi 1845, in Foveaux Strait (New Zealand) (Doonan *et al.* 1994) and the native European oyster, *Ostrea edulis* L. 1758, in the UK (see Yonge 1960). The United Kingdom, in particular, has a long tradition of oyster fishing, with landings peaking during the early 1900s. Although mainly considered as the foodstuff of the 'working class' during this time, a rise in the demand from increasingly populated cities resulted in overfishing, severely depleting the natural oyster populations (Yonge 1960). Pathogenic infections (e.g. a haplosporidian parasite from the genus, *Bonamia*; see Sprague 1971), spatfall failure and mortalities due to natural disasters (including the abnormally cold winter of 1962-1963; see Crisp 1964) have also contributed to the decline of *O. edulis* landings in the UK. Between 1920 and 1972, oyster landings in England and Wales declined rapidly from 40 million to 3 million individuals per annum (Davidson 1976). Even by 2010, only 206 T (equating to approximately 4 million oysters) were commercially fished from UK waters (FAO 2005). Such a drastic loss has prompted the need for oyster cultivation and, more specifically, research into the culture of more suitable, alternative species with which to replenish the native oyster stocks; a field of study led initially in the UK by the Ministry of Agriculture, Fisheries and Food (MAFF) (see Walne 1974). Considering that oysters have been introduced to 73 countries worldwide (Ruesink *et al.* 2005), such a lack of understanding of the ecological implications of non-native oyster introductions is cause for concern.

1.5 The ecological importance of oysters

Initial studies into transfers of non-native oysters across biogeographical boundaries were conducted due to an increased focus on novel methods of fishery stock enhancement and water quality management issues (e.g. Mann *et al.* 1991; Coen *et al.* 2000). However, recent emphasis on biodiversity preservation and other conservation matters has ignited much interest into the potential impacts of oyster culture, (re)introductions of native oysters and transfers of non-native oysters upon the environment (see Table 1.2). Historically, most ecological publications concerning interactions between non-natives and their associated native co-inhabitants focus solely on negative ecological interactions pertaining from competition, parasitism and predation. However, more recent work on facilitation (i.e. a term

Table 1.2 A non-exhaustive list of studies demonstrating the ecosystem engineering abilities of oysters and their subsequent effects on other associated habitats.

Oyster Species	Location	Impacted Habitat	Mode of Engineering	Source
Crassostrea virginica	Swansboro Region North Carolina USA	*Spartina alterniflora* Salt marsh	Oyster cultch laid seaward to the salt marsh buffered the erosive effects of wave action and storm events. Oyster reef also reduced current flow, leading to increased sedimentation and stabilisation within the marsh. Oyster cultch provided a complex physical structure that was inhabited by numerous species of economic importance.	Meyer *et al.* (1997)
Crassostrea virginica	Neuse River Estuary North Carolina USA	*Crassostrea virginica* Oyster reef	Positive correlation between reef height and flow rate resulted in an increase in the delivery rate of suspended particulate material, leading to improvements in oyster growth, condition and survival.	Lenihan (1999)
Crassostrea virginica	Chesapeake Bay Virginia USA	Estuarine community	Along with increasing anthropogenic inputs of nitrogenous compounds (mainly fertiliser), the loss of oysters and their filter-feeding activity are thought to have led to shifts from primarily benthic to pelagic primary production, as well as an increase in harmful algal blooms. Such changes may have caused a shift in community dominance from macroalgae and nekton to microbial organisms and jellyfish.	Jackson *et al.* (2001)
Crassostrea virginica	Chesapeake Bay Virginia USA	Estuarine community	Loss of oysters and their filter-feeding ability was linked to an increase in turbidity, leading to negative implications for ecologically-important habitats, such as seagrass beds and other primary producers.	Newell and Koch (2004)

Crassostrea gigas	Bay of Mont Saint-Michel France	*Sabellaria alveolata* Biogenic reef	Larvae from nearby oyster culture facilities settled on cultch, increasing species richness. Oyster reefs altered local hydrodynamics, leading to nearby areas of increased sedimentation and the creation of a novel environment for several infaunal species. Oysters may also be outcompeting *Sabellaria alveolata* for food due to a higher filtration rate. Sporadic discoveries of new predators within the reef habitat in the presence of oysters suggested the possibility of the creation of new multi-trophic level species associations.	Dubois *et al.* (2006)
Crassostrea hatcheri (ancient population)	Patagonia Argentina	Shallow-shelf benthos	Oysters provided a hard substrate which was colonised by a wide range of epibionts from a wide range of taxa. High biodiversity was also facilitated by its wide geographic distribution, high abundance and longevity.	Parras and Casadío (2006)
Crassostrea gigas	Bay of Veys France	Macrobenthic assemblage associated with the tubeworm, *Lanice conchilega*	Oysters induced a top-down effect by modifying water quality and food input and quality, leading to a trophic shift in the underlying infaunal community from suspension-feeders to predators. High oyster densities increased secondary production, causing a shift from pelagic to benthic consumers, thus modifying benthic-pelagic coupling and trophic dynamics within the community.	Dubois *et al.* (2007)
Saccostrea glomerata	Sydney Harbour Sydney Australia	Artificial seawall	Facilitation of whelk (*Morula marginalba*) densities due to presence of oysters led to a trophic shift in the dominant species within the community.	Jackson *et al.* (2008)
Crassostrea gigas	Wadden Sea Germany	Intertidal native mussel (*Mytilus edulis*) Mussel reef	Shift in dominance from mussels to non-native oysters altered habitat structure, leading to a change in the associated benthic community. Community structure was changed due to the differences in ecosystem engineering functioning between mussels and oysters.	Kochmann *et al.* (2008)

integrating all types of intra- and inter-specific positive interactions whereby at least one species benefits and none are harmed in any way) has stimulated scientific endeavours that have significantly improved our understanding of the key drivers which help structure ecological communities (see Rodriguez 2006). Habitat modification has been identified as the most commonly reported mechanism by which invasive species facilitate native species and, in some cases, habitat modification can have as significant an effect on community dynamics as other biotic driving forces such as competition and predation (Bertness *et al.* 1999). By instigating physical state changes in biotic and abiotic materials, thus altering the availability of resources to other species, oysters have the ability to create, maintain and modify their habitat in such a way as to significantly affect the associated biological community. Formal terminology was devised by Jones *et al*. (1994), who termed such organisms ‘ecosystem engineers', and their habitat modifying activities ‘ecosystem engineering’. Ecosystem engineers may also be further divided into two subclasses; ‘autogenic’ and ‘allogenic’ engineers. Autogenic engineers modify the environment via their own physical structures, whilst allogenic engineers modify their environment by causing physical state changes in biotic or abiotic materials. Oysters can be incorporated into both classes of ‘engineering’, thus highlighting their undeniable significance as environmental modifiers. Ecosystem engineers are far more likely to have profound impacts within their new environment compared to those non-natives that do not exhibit habitat modification abilities. Whilst both engineering and non-engineering non-native species may present biological stresses in the form of competition and predation, the native biota must also contend with changes to their physical environment as a result of invasions by ecosystem engineers (Vitousek 1986).

1.5.1 Oysters as habitat modifiers

Oyster shells provide a hard substratum upon which fouling organisms may settle, often in areas otherwise consisting of soft sediments. Due to their gregarious nature, oysters (particularly reef-forming species such as *C. virginica*) are capable of forming complex, three-dimensional assemblages on the sea bed, creating crevices that offer spatial refuge for both juvenile conspecifics (Bartol and Mann 1999) and a range of other organisms (Coen *et al.* 2000) from both predators and physical stresses. Lehnert and Allen (2002) also demonstrated the essential role of oyster shell aggregations as nursery grounds for several phyla, including juveniles of key members of the trophic web. Importantly, oyster shell is highly resistant to degradation and therefore persists on the seabed long after the death of its former occupant. The significance of oysters as autogenic ecosystem engineers thus extends far beyond their lifespan. Artificially-created oyster reefs (formed from cultch i.e. a collection of single oyster valves) have been shown to facilitate macrofaunal diversity and recruitment of oyster larvae at a comparable rate to that of naturally-formed reefs (Meyer and Townsend 2000). It has been suggested that such ‘persistent’ habitat modifiers are likely to have a delaying effect on ecological change (Jones *et al.* 1994), thus enhancing ecosystem ‘stability’ (Pimm 1984). However, the allogenic habitat modification capabilities of oysters are lost *post-mortem*, and a comparison of the effects of the ecosystem engineering abilities of live and dead oysters/cultch on the associated benthic community is lacking.

Oyster reefs are also known to indirectly affect local community dynamics due to physical-biological coupling. Their physical structure results in the modification of physical variables, leading to changes in biodiversity and ecosystem function. The construction of artificially-formed American oyster cultch reefs at the seaward periphery of a *Spartina alterniflora* salt marsh has been shown to buffer the erosive effects of wave action. Such reefs also instigated sediment accretion within the marsh, leading to improved structural stabilisation (Meyer *et al.* 1997). Biologically, the creation of habitat-fringing cultch beds provides a suitable habitat for several species, including juvenile and economically-important organisms (Meyer and Townsend 2000). Similarly, manipulations of mussel bed density within a laboratory flume have shown that flow speeds within the mussel assemblage decreases with increasing density. This leads to an increase in sediment loading and a reduction in erosion potential of the underlying sediment, thus a transition from destabilisation to consolidation of the substratum (Friedrichs 2004). Earlier flume experiments by Weissburg and Zimmer-Faust (1993) also demonstrated a positive relationship between turbulence at the benthic boundary layer with both current speed (analogous to the findings of Lenihan (1999) at the upper regions of oyster reefs) and sediment particle size, which subsequently led to a reduction in the chemosensory abilities of the predatory blue crab, *Callinectes sapidus* (Rathbun 1896), when exposed to odour plumes emanating from actively filtering hard clams, *Mercenaria mercenaria* (L. 1758). A similar effect due to the physical structure of oyster reefs may well be instigated, although no published evidence was found in support of this deduction.

The effect of oyster reef structures upon local flow patterns has been identified as the most influential factor controlling physical-biological coupling. Lenihan (1999) observed that the reef structure not only controlled local physical variables, but also had subsequent implications for the resident oyster community. Filter-feeding bivalves require sufficient water movement to ensure adequate provision of suspended organic material and removal of waste material. However, water flow should not be too high so as to inhibit larval settlement (Butman 1987) growing near the upper crest of the reef were subjected to quicker flow than those near the basal fringes of the reef, resulting in improved food supply and reduced sedimentation rates, and thus a decrease in hypoxia stress. It is therefore clear that habitats such as oyster reefs have the ability to indirectly control local population production through physical-biological coupling, the understanding of which is fundamental to improve our conservation, restoration and management of such habitats and their natural resources (Lenihan 1999).

1.5.2 Oysters as translocators of energy from the water column to the benthos

Oysters are proficient filter-feeders and are considered to be significant contributors to the translocation and transformation of large quantities of energy between the overlying water column and the benthos (Dame *et al.* 1980). A conceptual diagrammatic representation of the multiple roles played by dense aggregations of filter-feeding organisms is given in Figure 1.2. By filtering large quantities of organic matter from the water column and directly incorporating such material as tissue biomass, oysters function as important trophic links that provide a previously inaccessible source of energy to a range of carnivorous predators and detritivors. *Callinectes sapidus*, for example, is recognised as a highly-voracious predator of juvenile American oysters in Chesapeake Bay (Eggleston 1990). Mature oysters are known to lose a large percentage of their body mass during spawning (Brown and Russell-Hunter 1978) and as the spring phytoplankton bloom declines, oyster gametes may also become an important source of nutrition for bentho-pelagic carnivores. Oysters of the genus *Crassostrea*, in particular, are highly fecund, with larvae remaining in the plankton for approximately three weeks following external fertilisation (Galtsoff 1964). In the same species, Bernard (1974) estimated a release of 500 kcal m^{-2} of energy as gametes in a population with mean oyster density of 190 g m^{-2}. By reducing the deposition rate of organic carbon into deeper waters during spring phytoplankton bloom events, the role of oysters in reducing the extent of summer hypoxia within stratified embayments (thus initiating a top-down grazing control on phytoplankton) has been intensively argued within the scientific community (see Newell 1988; Pomeroy *et al.* 2006; Newell *et al.* 2007).

Not all energy acquired from plankton consumption is accumulated as oyster tissue (see Figure 1.2). All epibenthic filter feeders have the ability to actively remove suspended particulate matter from the water column and deposit it as faeces or pseudofaeces, which either sinks to the bottom as a result of gravity or is carried away from the area by water movements. The process of particle filtration, digestion and subsequent release as faecal material is termed ‘biodeposition’ and the voided products termed ‘biodeposits’. It has been shown that suspended particulate matter of 1-12 µm diameter is routinely filtered by the American oyster, *C. virginica*, with optimal efficiency at 3 µm (Haven and Morales-Alamo 1970). Such material is subsequently released as larger faecal pellets of 500-3,000 µm diameter. It has been estimated that the Pacific oyster, *Crassostrea gigas*, voids 8.9 g g

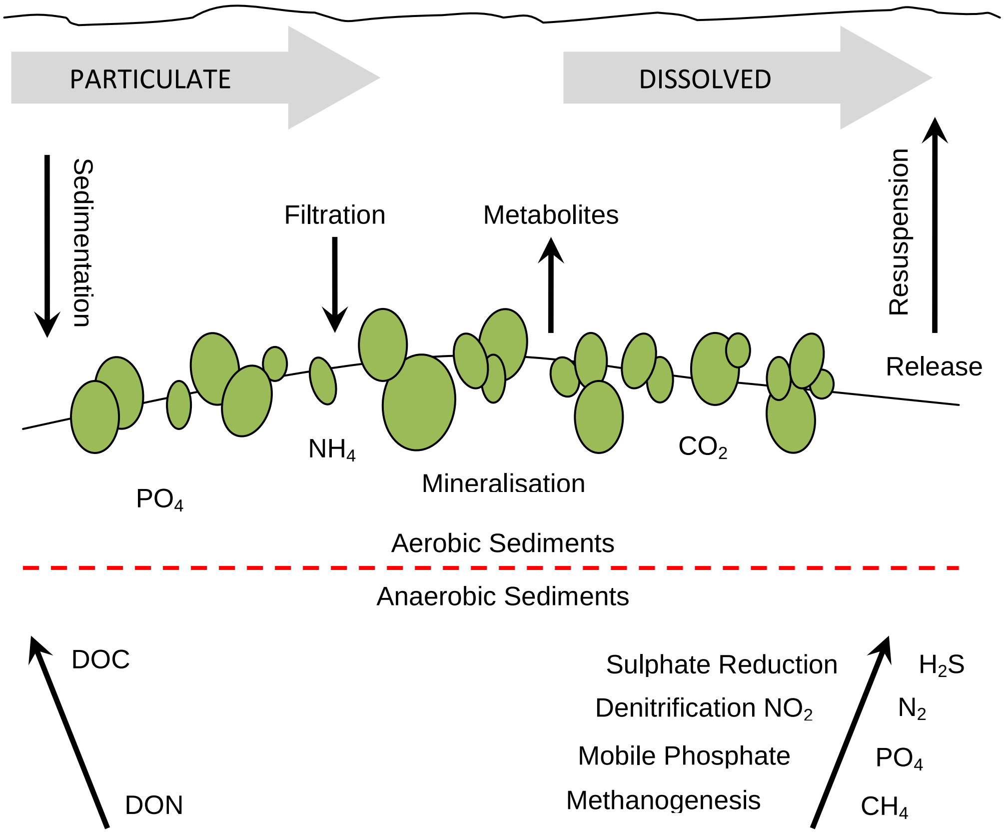


Figure 1.2 A conceptual summary of processes occurring in and around dense systems of filter feeding bivalves such as mussels and oysters (redrawn from Dame 1993).

oyster^{-1} y^{-1} as biodeposits, giving an estimated calorific value of 1,545 kcal m^{-2} (Bernard 1974). Biodeposits provide a highly suitable substrate for microbial colonisation. When re-suspended in the water column by water movements or other means, biodeposits can be reutilised by oysters or carried away from the oyster bed, thus further increasing the productivity of the oyster reef and adjacent areas. Bernard (1974) suggested that sedimentation of a large quantity of biodeposits can modify the physical and chemical properties of the underlying sediment, allowing for the establishment of a diverse group of organisms, although no evidence was given to support his theory.

Due to an exponential increase in marine aquaculture, as well as an increasing awareness of the importance of conserving biodiversity for maintaining ecosystem services, more recent studies concerning the impacts of oysters as ecosystem engineers have focused on evaluating the impacts of oyster aquaculture on the underlying sediment and its associated fauna, often with conflicting conclusions. Oyster trestles have been shown to decrease current flow, thus increasing the local deposition of sediment and organic material (Nugues *et al.* 1996). Biodeposition by the cultured species also contributes to the organic enrichment of the underlying sediment, particularly in 'low energy' areas where there is insufficient flow to inhibit sedimentation. Unlike finfish aquaculture, which requires the addition of processed feed, bivalve aquaculture relies on natural sources of suspended organic material for food. Although no net input of organic material is added into the environment, the packaging of seston into larger, heavier faecal material can cause a localised accumulation of organic material in the underlying sediment (Grant *et al.* 1995). A subsequent increase in the biological oxygen demand of aerobic microbial communities can lead to hypoxia/anoxia in the top layers of the sediment and overlying water (Lenihan and Micheli 2001), with mass mortalities of the least tolerant organisms. Conceptual patterns in species abundance, biomass and richness with increasing organic enrichment within a soft-sediment benthic community were shown by Pearson and Rosenberg (1978) (Figure 1.3). Recovery of a soft-sediment community is often characterised by a succession of community members, beginning with opportunistic, r-strategists such as worms from the genus, *Capitella*. These species are usually surface and/or shallow sub-surface deposit feeders. Their bioturbation activities irrigate and oxygenate the top few millimetres of sediment. Alterations to the sediment community allows for further colonisation by a range of species that are less tolerant to toxic conditions or unstable and unstructured sediment habitats (Lenihan and Micheli 2001), progressing to the re-establishment of a similar community to that observed in unaffected, neighbouring regions. Minor organic enrichment, on the other hand, can give rise to an increase in species abundance, biomass and richness (Figures 1.3

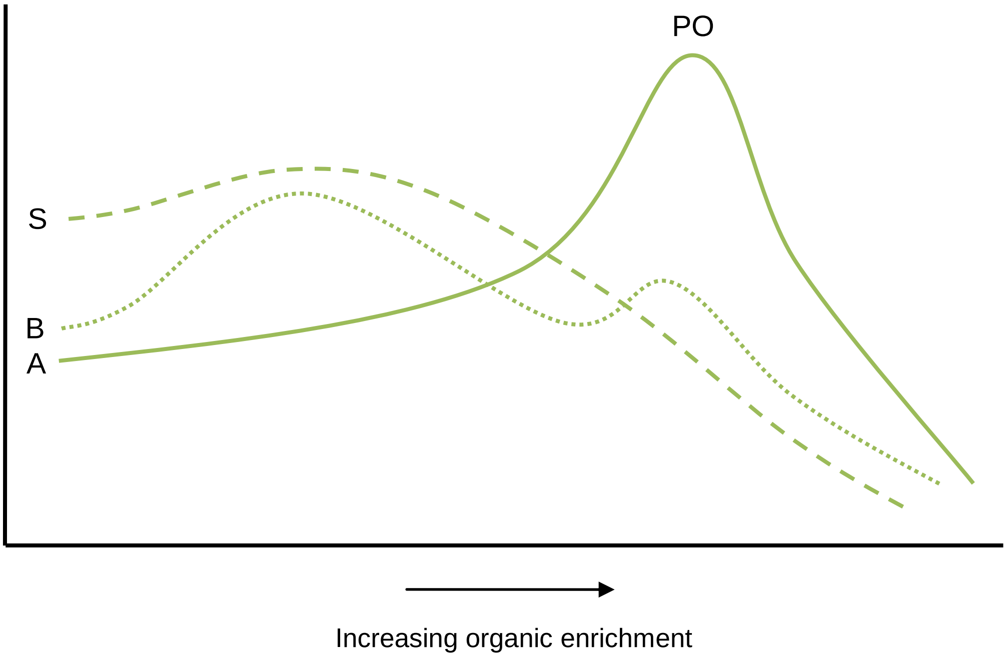
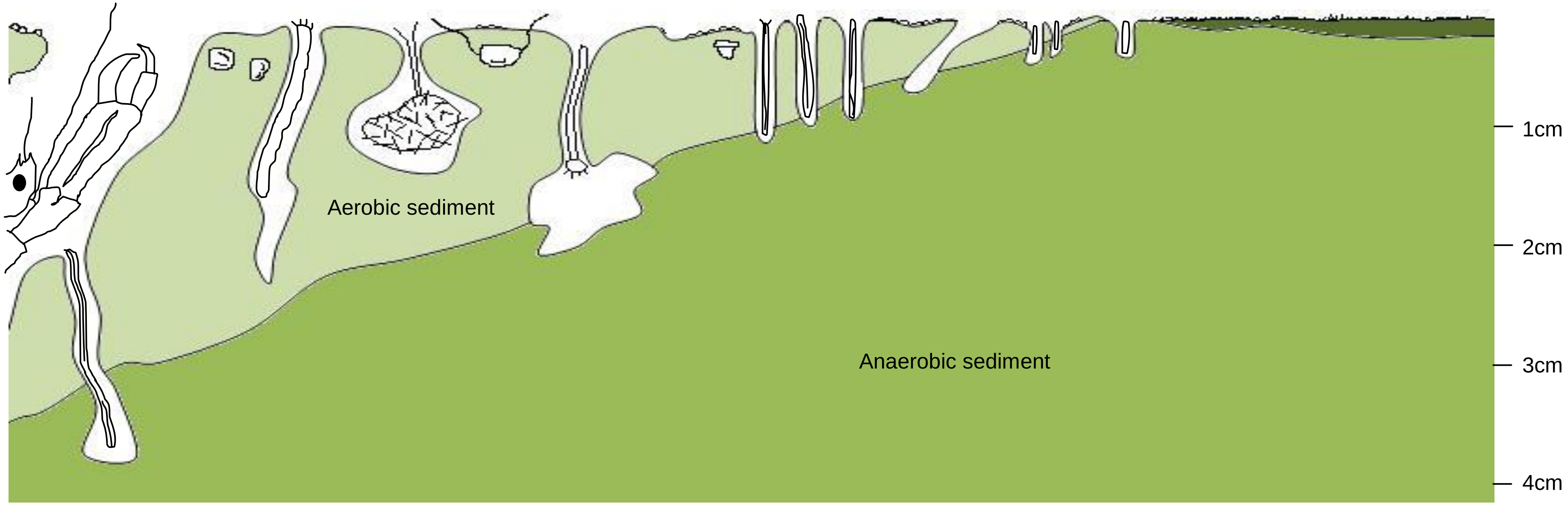


Figure 1.3 Changes in abundance (A), biomass (B) and species richness (S) within an infaunal benthic community along an organic enrichment gradient (redrawn from Pearson and Rosenberg, 1978). PO = peak in abundance of opportunistic species.



ZONE	NORMAL	TRANSITORY	POLLUTED	GROSSLY POLLUTED
TYPICAL DOMINANT MACROFAUNA	*Nucula* *Amphiura* *Terebellides* *Rhodine* *Echinocardium* *Nephrops*	*Lobidoplax* *Corbula* *Goniada* *Thyasira* *Pholoe* *Chaetozpne* *Anaitides* *Pectinaria* *Myriochele* *Ophiodormus*	*Capitella* *Scoleptis*	No macrofauna Surface covered by fibrous 'blanket'

Figure 1.4 Temporal or spatial changes in soft-sediment community located along a temporal or spatial gradient in organic enrichment (redrawn from Pearson and Rosenberg 1978).

and 1.4).

1.5.3 Oysters as prey items for keystone predators

Due to their sessile, epibenthic lifestyle, oysters are susceptible to predation by a wide range of mobile organisms, including crabs, fish, gastropods, lobsters, seabirds and starfish. A detailed assessment of the significance of each individual predator species and the importance of oysters as part of their respective diet is well beyond the scope of this introductory thesis chapter. However, some oyster predators have been identified as keystone species, having a disproportionately large influence upon their environment relative to their abundance. Keystone species, such as the predatory ochre starfish, *Pisaster ochraceous* (Brandt 1835), are capable of controlling the density and distribution of influential benthic organisms, thus manipulating the structure of the biological community (see Paine 1974). Studies concerning diet preference of keystone predators provide useful insights into foraging behaviour, leading to improvement in our understanding of community dynamics. Crabs, in particular, are voracious predators of several bivalve species, and are considered to be significant contributors to the structuring of marine benthic habitats (Leber 1985; Raffaelli *et al.* 1989; Mascaró and Seed 2001b). The known preferential behaviour of crabs, in terms of both prey size and species selection, has direct implications for the abundance and distribution of prey species, which are themselves modifiers of the benthic community. 'Optimal foraging theory' (see Hughes 1980), where a predator selects the most energetically profitable prey item per unit handling time, often forms the premise by which size-selective predation is explained (Elner and Hughes 1978; Dare *et al.* 1983). However, although prey handling times (and thus net energetic profitability) vary with both prey and predator species (Hughes and Seed 1981; Mascaró and Seed 2001a, 2001b), leading to variation in foraging tactics when presented with different prey items (Juanes 1992).

Unlike those concerning size-selective predation, investigations into species preference are not so well documented. However, it has been established that shore crabs, when presented with a range of bivalve species (including oysters) of pre-determined 'optimal' sizes, show preference towards mussels (e.g. Dare *et al.* 1983; Mascaró and Seed 2001a, 2001b). Although known to feed indifferently on both flat (*Ostrea edulis*) and cupped (*Crassostrea gigas*) oysters (Mascaró and Seed 2001a), the reluctance of both *Carcinus maenas*, and the edible crab, *Cancer pagurus* L. 1758, to feed on the Chilean oyster, *Ostrea chilensis* Philippi 1845, even when presented in the absence of any other prey species, was attributed to mechanical difficulties during handling (Richardson *et al.* 1993b). Bishop and Peterson (2006) established the tendency of the blue crab, when presented with equal numbers of the native Eastern oyster, *Crasostrea* *viginica*, and the non-native Suminoe oyster, *Crassostrea ariakensis* (Fujita 1913), to select the non-native prey; the latter once a candidate species to replenish stocks of the decimated native *C. virginica* stocks in Chesapeake Bay (North America). Selection was thought to be based upon the contrasting shell strength, with significantly less energy required to crush open the shell of *C. ariakensis*. The implications of such findings have undoubted importance to the success of management of natural resources (Mascaró and Seed 2001b) and demonstrate the value of relating life-history theory with results from contained mesocosm experiments that compare ecological responses of native and non-native oysters in response to dominant features within the recipient environment. However, one must avoid the formulation of over-generalistic conclusions regarding the influence of keystone predators on community dynamics based solely upon prey preference trials involving adult predators. Differences in the spatial distribution, feeding habits and prey preference of juvenile crabs compared to their adult conspecifics have been established. Compared to their adult conspecifics, patterns in size-selective predation patterns are rather more inconsistent in juvenile crabs, possibly due to the physical constraints imposed on smaller individuals that have limited access to larger prey (Mascaró and Seed 2001b).

1.6 Case Study – the non-native Chilean oyster (*Ostrea chilensis* Philippi 1845) population in the Menai Strait (North Wales, UK)

Despite the ever-increasing volume of scientific publications regarding the potential economic and ecological impacts pertaining from biological invasions, financial constraints and the occasional lack of coordination between the scientific community and policy makers mean that monitoring and management strategies must be prioritised to focus on those species. Despite strong evidence, information regarding several seemingly transient non-native species is often lacking.

Native to both Chile and New Zealand (see O'Foighil *et al.* 1999), the Chilean oyster (*Ostrea chilensis*) has supported a highly profitable fishery in New Zealand since the mid-nineteenth century (NZMF 2008), although epizootics of the haplosporidian parasite, *Bonamia exitiosa* (Hine *et al.* 2001) have impeded commercial output during the last 20 years or so (see Dinamani *et al.* 1987). Based solely on its life history characteristics, it may be hypothesised that the offspring of *O. chilensis* is highly unlikely to disperse great distances away from adult conspecifics (Millar and Hollis 1963; Cranfield 1968; Westerskov 1980); a desirable feature of any species in terms of both fisheries and aquaculture management (Walne 1974). The Chilean oyster is a protandric hermaphrodite. Unlike most other oyster species, it has a low fecundity and a highly extended brooding period. An individual female oyster (50-85mm shell length) will typically brood ~50,000 larvae within the mantle cavity (Cranfield and Allen 1977) for up to eight weeks (Chaparro 1990; Chaparro *et al.* 1993). The proportion of brooding females within a population can be as low as 6% (Cranfield and Allen 1977), although this is highly variable between populations (see Buroker *et al.* 1983). It is thought that the larvae are predominantly released as pediveligers, thus explaining their imminent settlement in close proximity to adult conspecifics, providing that a suitable substratum is available (Hollis 1962; Cranfield 1968; Westerskov 1980). Evidence of the premature release of small numbers of larvae has also been documented (Cranfield and Michael 1989), although the ability of such larvae to undergo metamorphosis and settlement, as well as their survival rate and fitness, has not been investigated.

The Chilean oyster was experimentally introduced into the UK during the early 1960s (Walne 1974) by the Ministry of Agriculture, Fisheries and Food (MAFF) (now part of the Centre of Environment, Fisheries and Aquaculture Sciences). Laboratory-reared juvenile *O. chilensis*, cultured under strict quarantine conditions by the MAFF from broodstock imported from both Chile and New Zealand, were transplanted onto the low intertidal shore at Tal y Foel, Menai Strait (North Wales, UK), in an attempt to establish its potential as a replacement species with which to supplement the dwindling native European oyster stocks (Walne 1974). However, the subsequent growth trials soon demonstrated that *O. chilensis* suffered high spat mortalities during the winter months, was relatively slow growing and was also susceptible to infection by haplosporidian parasites of the genus *Bonamia*; traits which quickly ruled out the species as a suitable oyster species with which to supplement the dwindling native *O. edulis* stocks. Despite evidence of spat recruitment in 1970 (Walne 1974), the focus of the MAFF was subsequently turned to other avenues of research, and the remaining surviving oysters at Tal y Foel were abandoned and left to their own devices. Interest in the status of this non-native oyster population within what is now part of a designated Special Area of Conservation (SAC) is restricted to a single survey, conducted in 1992 by Richardson *et al.* (1993b), who note that *O. chilensis* generally remained confined to a 0.4 km stretch of the shoreline at Tal y Foel. However, more recent anecdotal observations and unpublished data suggest that the local geographic range of this population has recently expanded (see Morgan 2007a).

1.7 Conclusions and questions addressed

This introductory chapter highlights the multiple ecosystem engineering properties of non-native oysters and how their future invasion potential may become further augmented in the face of global climate change. Preliminary data have repeatedly shown that *O. chilensis* promotes species richness within the Menai Strait and Conwy Bay SAC, primarily due to its provision of a hard substratum in an area otherwise predominating of soft sediments. In high densities, several mobile species also take refuge within its intricate shell matrix (Appendix I). However, no analysis has yet been conducted to investigate how this increase may endanger the qualifying habitats of the SAC and their ecosystem function. Regrettably, a worryingly low amount of robust scientific endeavour has been dedicated to elucidate the past, present and future invasion dynamics of the Chilean oyster population within the Menai Strait and Conwy Bay SAC. Following a 30-year lag phase confined to the site of original introduction, anecdotal observations of occurrences of *O. chilensis* as far as 30 km away from the invasion foci during the last 8 years signifies an urgent need to update the distribution records of this non-native oyster species. Moreover, no information exists regarding its reproductive dynamics or its future as a significant invader within its introduced region.

The primary aim of this thesis is to investigate past records, present observations and future predictions relating to the biological invasion of the non-native Chilean oyster within the Menai Strait and Conwy Bay SAC. Chapter 2 presents the finding of a quantitative survey of the current distribution of the oyster population within the area. The data are compared with the findings of Richardson *et al.* (1993b) which, prior to scientific studies herein, served as the only comprehensive survey of the Chilean oyster population to date. The chapter also outlines how the current UK legislation framework does not offer adequate mitigation measures for those species that are currently innocuous beyond their native geographic range. Chapter 3 provides a comprehensive account of the reproductive dynamics of the Chilean oyster population, investigating both the spatial and temporal variability over three years of study. Despite its high settlement rates, the highly-reduced planktonic larval phase and highly-gregarious nature of this species suggests that the dispersal of this species away from the site of original introduction is heavily-reliant upon secondary dispersal mechanisms related to anthropogenically-mediated activities. Following anecdotal observations of oyster-fouled common periwinkles (*Littorina littorea* L. 1758) within the area, Chapter 4 investigates the potential role of a previously unidentified anthropogenic activity, namely the commercial collection of periwinkles, as a transport vector responsible for both the small- and large-scale dispersal of this non-native oyster species. Using both field observations and laboratory experiments, Chapter 5 investigates the potential impact of forecasted increases in both the frequency and intensity of cold winter climatic extremities on the future proliferation of this non-native oyster population. Finally, Chapter 6 provides a synthesis of all experimental chapters and discusses possible future regulation and management advice regarding the proliferation of a non-native oyster species in areas beyond its native geographic range. It is my intention for each data chapter to function equally as stand-alone chapters when read in isolation and a comprehensive synthesis when read as a full document, thus explaining some overlap within the introduction and discussion sections of all data chapters. Additional information relevant to the main body of text is also included in the form of appendices at the end of each chapter and all references cited throughout the entire thesis are compiled in Chapter 7.

Appendix I: Assessing oyster reef complexity and its relationship with biodiversity

Preliminary studies have been carried out on the changes in community composition associated with an increasing density of *O. chilensis*. The following figures are just some of the results from two MSc projects which I co-supervised with Drs Jan Hiddink and Gwladys Lambert (Stäbler 2011) and Prof. Chris Richardson (Vearey-Roberts 2011) respectively. Reef complexity was estimated from a digital image of a standard profile gauge, whose 'needles' followed the outline of the underlying oyster bed (see Figure I). This outline could then be converted to several indices of complexity using the formulas presented in Figure II. Several measures of oyster reef complexity were shown to be highly correlated with oyster density (see Figure III for example).

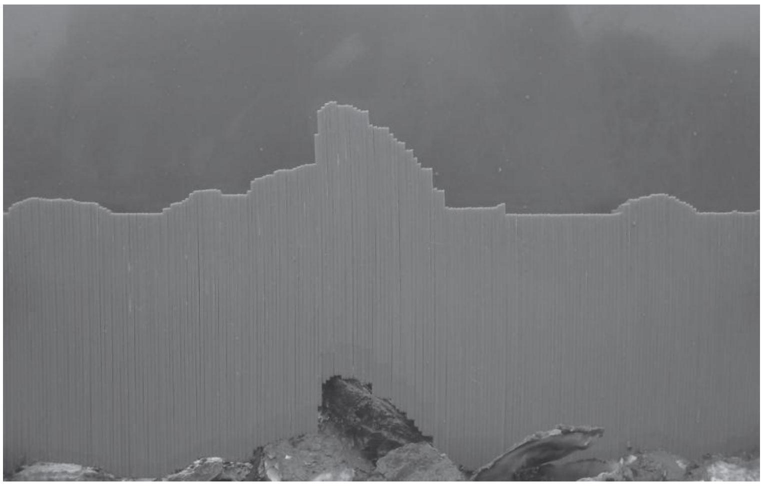


Figure I Digital image (taken parallel to the seabed) showing the relative positions of numerous 'needles' of a profile gauge, held tightly to both the oyster reef (bottom of image) and the camera by a modified copy stand (from Stäbler 2011).

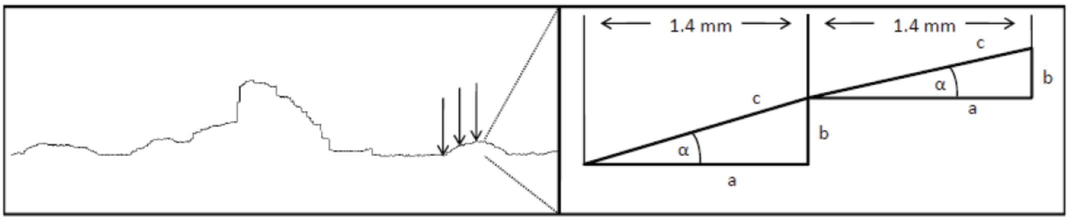


Figure II Schematic representation of the calculations of three indices of complexity, namely 'chain and tape' = ∑(c) / ∑(t), 'vector dispersion' = var(α) and 'height difference' = $\sum(b^2)$ (from Stäbler 2011).

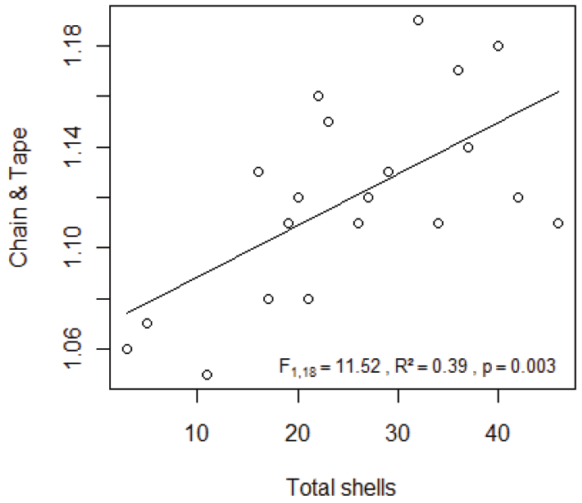


Figure III Relationship between and the 'chain and tape' index of oyster reef complexity total oyster shell density, observed at Plas Trefarthen (North Wales, UK) (from Stäbler 2011).

Figures IV and V show how total biomass, number of individuals and species richness all increase with increasing oyster density. Both epifaunal (Stäbler 2011) and mobile (Vearey-Roberts 2011) species showed significant increases in richness with increasing oyster densities, although no such difference was observed within the infaunal community.

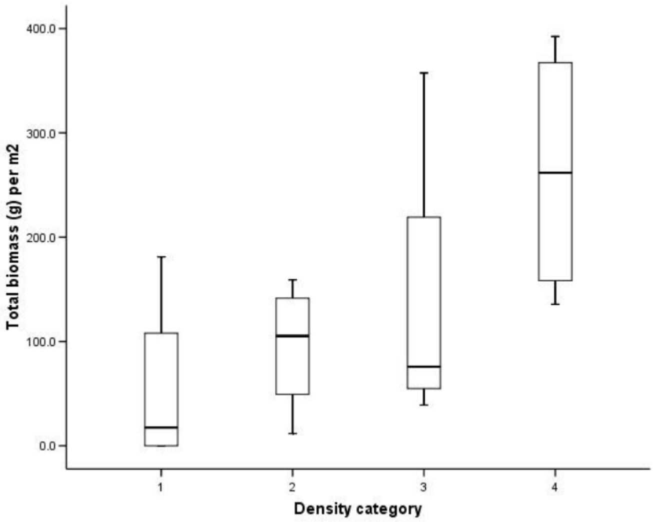


Figure IV Boxplot of total biomass (g) of all organisms found within 1 m^2 plots at Plas Trefarthen (North Wales, UK). Density category: 1 = no oysters, 2 = low oyster density (<10 m^{-2}), 3 = medium oyster density (~50 m^{-2}), 4 = high oyster density (>100 m^{-2}) (from Vearey-Roberts 2011).

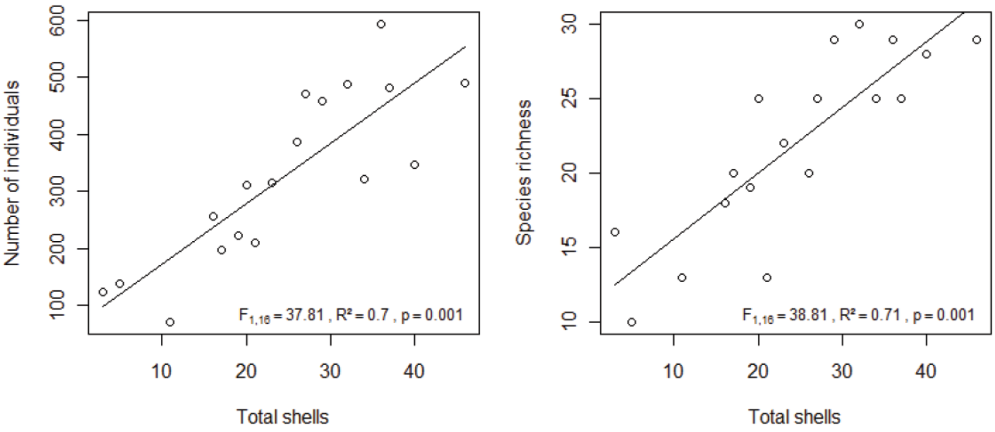


Figure V Relationships between both total number of individuals (left) and species richness (right) with oyster shell density at Plas Trefarthen (North Wales, UK) (from Stäbler 2011).

This suggests that allogenic ecosystem engineering (*sensu* Jones *et al.* 1994; Chapter 1) is currently of relatively low importance to the non-native oyster population in the Menai Strait. Given the dynamicity of the tidal currents and the relatively recent formation of the Plas Trefarthen oyster reef (<20 years old), it is likely that rates of sedimentation is low within the region, thus explaining the difficulties experienced in standardising core volumes between replicates. However, the ever-increasing build-up of oyster shells is likely to aid in the trapping of sediment (see Chapter 1). The allogenic engineering properties of non-native oysters and their potential impacts upon the native biodiversity and ecosystem function may not be stable in time and space, and should thus not be disregarded.

Chapter 2

Capricious bioinvasions versus uncoordinated management strategies: how the most unlikely invaders can prosper under the current UK legislation framework

2.1 Abstract

Biological invasions are known to be highly unpredictable and context-dependent, varying both spatially and temporally, particularly in areas of intense anthropogenic activity and disturbance. Even the most unlikely invader can rapidly become problematic in the absence of frequent, coherent and flexible management strategies. Using the recent spread of the Chilean oyster (*Ostrea chilensis* Philippi 1845) within a designated Special Area of Conservation (SAC), this chapter describes what can happen to seemingly innocuous non-native species under the often complicated and uncoordinated current UK legislation framework. Following >30 years of containment at Tal y Foel (North Wales, UK), *O. chilensis*, a species with a highly-reduced natural dispersal capacity, has now spread over a range of >30 km of shoreline. Alternative transport vectors, including rafting and several anthropogenic activities, are likely to have facilitated the dispersal of *O. chilensis* away from Tal y Foel. Areas of high oyster densities (maximum = 232 oysters m^{-2}) have become established both close to and distant from the site of original introduction. The presence of all year classes throughout the observed age range (≤7 and ≤9 years old in the intertidal and subtidal populations, respectively) confirms regular annual recruitment within the SAC. Information is now urgently required regarding the factors that promote the persistence and spread of *O. chilensis* within its new environment, as well as the impacts of its increasing localized dominance on the native biodiversity and ecosystem function. As well as providing valuable, up-to-date information on the recent spread of this non-native species, this chapter highlights discrepancies in the current UK legislation framework that allow for the successful establishment and spread of even the most unlikely invaders. The formation of a comprehensive and dedicated EU legal framework for managing invasives is advocated that also promotes coherence and continuity with impending legislative instruments concerning other relevant sectors.

The following chapter has been published in the journal 'Aquatic Conservation: Marine and Freshwater Ecosystems' (2011 5-year impact factor = 2.217) and is thus subject to copyright by the publisher John Wiley and Sons Ltd. Please consult the original journal article and cite as follows:

Morgan EH and Richardson CA. 2012. Capricious bioinvasions versus uncoordinated management strategies: how the most likely invaders can prosper under the current UK legislation framework. *Aquatic Conservation: Marine and Freshwater Ecosystems.* **22:** 87-103.

2.2 Introduction

Biological invasions have long been recognised as a key component of anthropogenically-mediated changes to the environment on a global scale (Vitousek *et al.* 1997). Whilst only a small fraction of introduced non-native species (NNS) are thought to proliferate and become ecologically and/or economically damaging within their new environment (see Williamson 1996), the costs associated with some of the most severe biological invasions can often be catastrophic (Pimentel *et al.* 2005). Means of predicting which NNS are most likely to become invasive, as well as the spatial and temporal dynamics of their respective invasions, have thus become major focal points of both management and research efforts in recent years. Of particular prevalence is the identification of biological traits that are shared amongst the most effective invaders (e.g. Ehrlich 1989; Williamson and Fitter 1996; Pattison *et al.* 1998). Whilst reviews of the biology and invasive history of NNS (e.g. Eno 1996; Eno *et al.* 1997; Hill *et al.* 2005) provide useful insights into their potential invasiveness, evidence in support of consistent biological traits (including high fecundity and high natural dispersal capabilities) across multiple invasive taxa is often lacking (Lodge 1993; Kolar and Lodge 2001; Hayes and Barry 2008). Furthermore, both species invasiveness and habitat invasibility can be spatially and temporally variable, especially in areas of intense anthropogenic activity and disturbance (Colautti *et al.* 2006), meaning that even the most unlikely invader can rapidly become problematic in the absence of regular risk assessment and monitoring protocols. The present study documents the recent spread of one such species, namely the non-native Chilean oyster (*Ostrea chilensis* Philippi 1845) within the Menai Strait and Conwy Bay Special Area of Conservation (SAC).

Native to both Chile and New Zealand (see O'Foighil *et al.* 1999), *O. chilensis* has supported a highly profitable fishery in New Zealand since the mid-nineteenth century (NZMF 2008), although commercial yields have varied in the last two decades due to epizootics of the haplosporidian parasite, *Bonamia exitiosa* (Hine *et al.* 2001) (see Dinamani *et al.* 1987). Based solely on its life history characteristics (see Millar and Hollis 1963; Cranfield 1968; Westerskov 1980), it is thought that the offspring of this oyster species is highly unlikely to spread far from adult conspecifics; a desirable implication for both fisheries and aquaculture management (Walne 1974). It is a protandric hermaphrodite and, unlike most other oyster species, it has a low fecundity and a highly extended brooding period. An individual female oyster (50-85 mm shell length) will typically brood ~50,000 larvae within the mantle cavity (Cranfield and Allen 1977) for up to eight weeks (Chaparro 1990). The proportion of brooding females within a population can be as low as 6% (Cranfield and Allen 1977), although this is highly variable between populations (see Buroker *et al.* 1983). The larvae are predominantly released as pediveligers, rapidly settling in the vicinity of their adult conspecifics providing that a suitable substratum is available (Hollis 1962; Cranfield 1968; Westerskov 1980). Evidence of the premature release of small numbers of larvae has also been documented (Cranfield and Michael 1989), although the ability of such larvae to undergo metamorphosis and settlement, as well as their survival rate and fitness, is unclear.

O. chilensis was experimentally introduced into the UK by a branch of the Ministry of Agriculture, Fisheries and Food (MAFF) during the early 1960s. Following a strict quarantine regime, releases of laboratory-reared juvenile *O. chilensis*, cultured by the MAFF from broodstock imported from both Chile and New Zealand, were transplanted onto the low intertidal shore at Tal y Foel (now part of the Menai Strait and Conwy Bay SAC - see Figure 2.1), in an attempt to establish the potential of this oyster as an aquaculture species (see Walne 1974). Subsequent growth trials soon demonstrated that *O. chilensis* suffered high spat mortalities during the winter months. The species was also deemed to be relatively slow-growing and susceptible to the disease, *Bonamiasis*; traits that quickly ruled out the species as a possible replacement oyster for the native oyster, *Ostrea edulis* L. 1758*.* Despite evidence of recruitment in 1970 (see Walne 1974), with the focus of the MAFF was subsequently turned to other avenues of research, and the remaining surviving oysters at Tal y Foel were abandoned and left to their own devices.

A census of the *O. chilensis* population in 1992 reported that a small, discrete population had become established at Tal y Foel (see Figure 2.2), restricted to a 0.4 km stretch of the intertidal (mean density = 2.3±0.9 oysters m^{-2}, maximum oyster density = 12 oysters m^{-2} in very close proximity to the invasion foci) (see Richardson *et al.* 1993b). A lack of suitable settlement substrata surrounding the area of original introduction was believed to have impeded the further spread of *O. chilensis*, although a few isolated examples were also found attached to commercial oyster trestle frames, located 0.5 km northward from Tal y Foel. More recently, several anecdotal sightings of *O. chilensis* within other areas of the SAC have been reported, although no specific, formal monitoring of this non-native oyster population has been carried out.

As well as providing valuable, up-to-date information on the recent spread of this NNS within and around a designated marine SAC, this chapter suggests likely vectors responsible for the successful propagation of *O. chilensis*. Current discrepancies in UK legislation and management strategies concerning the effective regulation of NNS, allowing for the successful establishment and spread of even the most unlikely invaders are also discussed.

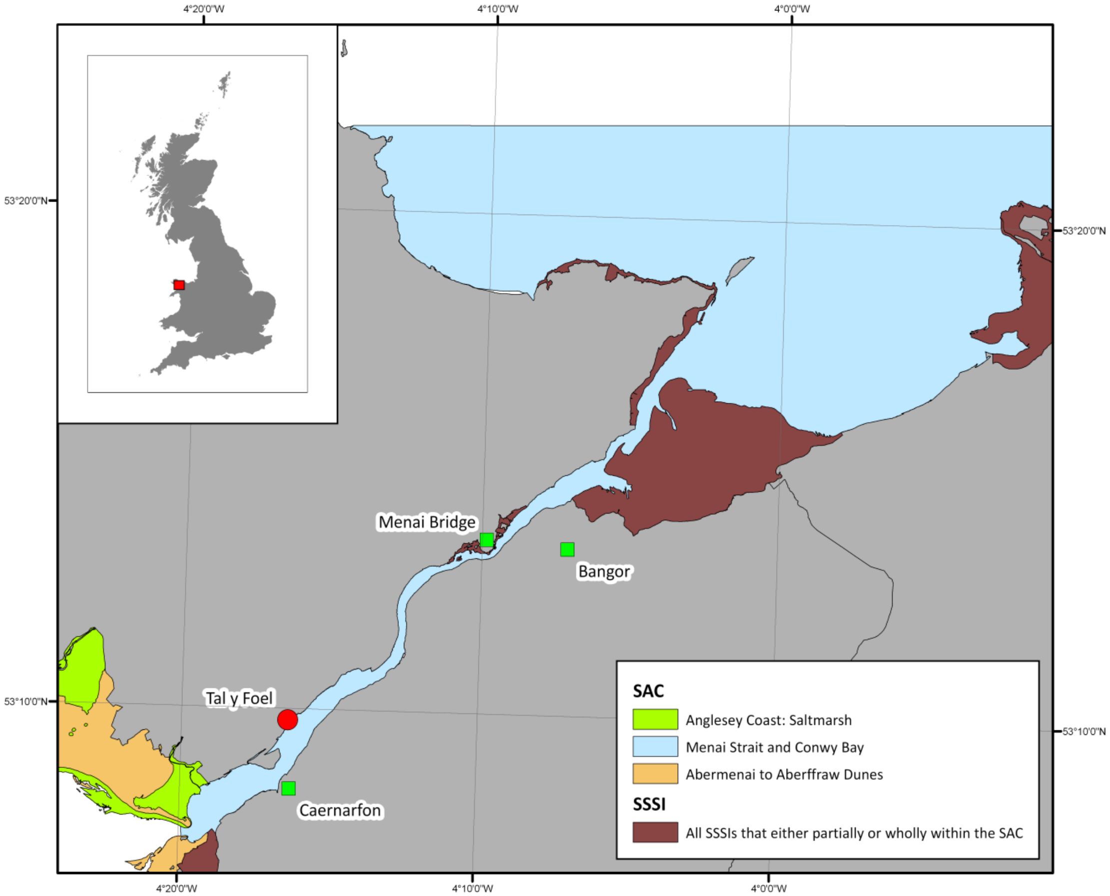


Figure 2.1 Map showing the location of the Menai Strait and Conwy Bay Special Area of Conservation (SAC) (North Wales, UK; see inset map), as well as the site of original introduction of the Chilean oyster (*Ostrea chilensis*) at Tal y Foel. Two other SACs (bordering the Menai Strait and Conwy Bay SAC) and all Sites of Special Scientific Interest (SSSIs) (occurring either partially or wholly within the Menai Strait and Conwy Bay SAC) are also displayed, showing areas where provision under the Habitats Directive 1992 is therefore extended to mean high water. Data used to generate SAC and SSSI boundaries is subject to Crown Copyright (reserved). Countryside Council for Wales, Licence No. 100018813.

2.3 Methods

2.3.1 Study site

The Menai Strait is a narrow tidal channel (mean width = 0.8 km) that separates the Isle of Anglesey from mainland Wales (Figure 2.1). Due to a large anomaly between tidal ranges at opposing ends of the Menai Strait, the area is subjected to strong quadri-diurnal tidal currents of up to 2.5 m s^{-1}. A residual flow from Liverpool Bay in the north-east to Caernarfon Bay in the south-west and a relatively short seawater residence time of 2-3 days (Rippeth *et al.* 2002) results in a continuous supply of relatively nutrient-rich sea water; a key feature to the success of the large-scale commercial mussel (*Mytilus edulis* L. 1758) farming industry in the north-eastern end of the Menai Strait (Simpson *et al.* 2007). Small-scale cultivation of the Pacific oyster (*Crassostrea gigas* (Thunberg 1793)) also occurs in the southern part of the Menai Strait at Tal y Foel and Plas Menai (see Figure 2.2).

Despite the strong tidal flow in the Menai Strait, the area is sheltered from wave action, thus creating a unique environmental setting with an associated high biodiversity. The area forms part of the Menai Strait and Conwy Bay SAC (see CCW 2009), primarily selected due to the presence of four qualifying marine habitat types ('Mudflats and sandflats not covered by sea water at low tide', 'Reefs', 'Sandbanks slightly covered by sea water all the time' and 'Large shallow inlets and bays'), listed under Annex 1 of the EC Habitats Directive 1992, along with their associated biota. The SAC also contains, either partially or wholly, a number of Sites of Special Scientific Interest (SSSIs), as well as two Special Protection Areas (SPAs), classified under the EC Birds Directive 1979 and its subsequent amendments. The majority of the SAC is subtidal, with its landward boundary following the mean low water mark (approximately 2.0 m above chart datum). Some areas of the intertidal are also protected when seaward boundaries of SSSIs or SPAs adjoin or overlap the landward fringe of the SAC (see Figure 2.1). The region is considered to be of major ecological and economic interest, and has been the focus of several scientific studies since the early 1960s (Young 1994; Morris and Goudge 2006).

2.3.2 Intertidal population survey

Surveys of the distribution of the intertidal *O. chilensis* population were conducted in October, 2009. Twenty-four sites were chosen, based on the following criteria: a) the presence of a suitable habitat/substratum type for oyster settlement, b) close proximity to the site of the original introduction of *O. chilensis* by the MAFF, c) evidence of natural spat settlement of other bivalve mollusc species, such as mussels (*Mytilus edulis* L. 1758) and cockles (*Cerastoderma edule* (L. 1758)), d) anecdotal evidence of possible oyster presence, and e) high anthropogenic activity (e.g. aquaculture, bait collecting, periwinkle collection, yachting).

Each site was surveyed during a 5-day period of extreme low water spring tides (tides less than 0.5 m above chart datum). Three replicate 80m transect lines were laid parallel to the low water mark at two tidal levels (0.5 m and 1.0 m above chart datum) at each site. Four replicate 0.25 m^2 quadrats were randomly placed either side of each transect line at 20 m intervals, giving a total coverage of 10 m^2 per transect (60 m^2 per site, 1080 m^2 in total). Pre-survey observations showed that employing this sampling strategy accounted for the 'clumped' distribution of *O. chilensis* and the high small-scale variability in density. The numbers of live and dead oysters were counted within all quadrats. A digital image was acquired of the first of each set of four quadrats, and used to estimate oyster shell percentage cover and biotope type of each site.

All live oysters within each photographed quadrat were measured along the dorso-ventral axis of the flat (right) shell valve (hereafter 'shell length') to the nearest 0.1 mm using Vernier callipers. A 30-minute 'timed search' was conducted at any site where no oysters occurred within any of the quadrats. This gave an indication of whether or not oysters were present in the area, but at densities too low to be detected by the sampling strategy.

2.3.3 Subtidal population survey

Observations of the subtidal oyster population were conducted adjacent to 17 of the 24 intertidal sites during November, 2009. Digital images of the shallow subtidal at each site were obtained using a purpose-built camera sled, fitted with a Canon EOS 400d Digital SLR camera housed inside a water-proof casing and towed using a small boat (90 bhp outboard motor) at ~2 knots along single transect lines (810.0±94.9 m) during periods of extreme high water spring tides (6.0 m above chart datum). The camera settings were pre-calibrated in a tank of sea water in the laboratory, ensuring a 0.15 m^2 field of vision. Still images were captured every 12 seconds, ensuring an average coverage of 11.81±1.43 m^2 at each site. Sampling depth was estimated by subtracting tidal range away from observed depth, giving depths of approximately 3-8 m below chart datum. For comparative purposes, images were also obtained from deeper parts (>20 m below chart datum) of the Menai Strait where possible. The images were later analysed for the presence/absence of *O. chilensis* and to give an indication of the habitat type at each site.

Samples of subtidal *O. chilensis* were also obtained for size-frequency analysis using a mussel dredge (750 x 200 mm steel frame, mesh size = 5mm at cod end), trawled along each transect line in order to obtain relative densities of adult *O. chilensis* at each site. Geographic coordinates (decimal degrees) were obtained at the beginning and end of each trawl, giving an estimation of the total area sampled during each trawl. The shell length of each live-caught oyster was measured to the nearest 0.1 mm using Vernier callipers. A comparison of the estimates of oyster density obtained by dredging and from images of the sea bed showed that the fishing efficiency of the dredge was in the region of 20%.

2.3.4 Age determination

The age of various sizes of *O. chilensis* (approximately 20-80 mm shell length), collected from intertidal and subtidal sites, was determined from the presence of annual growth lines in acetate peel replicas of resin embedded and etched shell sections (see Richardson *et al.* 1993a). Acetate peel replicas were viewed using a transmitted light microscope fitted with a Ricoh Caplio R7 digital camera. Photomontages of the sectioned umbo region were produced using Omnimet® image analysis software and the number of annual growth lines was counted. The distance between each growth band was also calculated (see Richardson *et al.* 1993a).

2.4 Results

O. chilensis occupies a narrow tidal range along the shores of the Menai Strait, extending from mean low water into the shallow subtidal (2.0 m above to 8.0 m below chart datum), meaning that the entire oyster population resides within the SAC boundary. No oysters were found at depths >20 m below chart datum, where fast currents and a lack of suitable substrata most likely inhibit larval settlement. Both the mean intertidal density and the range of the population have increased markedly since 1992 (Table 2.1). Oysters are now found intertidally from the southernmost tip of the Menai Strait (Abermenai Point) to Glyn Garth, covering a distance of >30 km of shoreline (Figure 2.2). This distribution pattern was generally closely mirrored in the shallow subtidal, with the highest subtidal oyster densities observed at Abermenai Point, Tal y Foel, Plas Trefarthen and Llanidan (Figure 2.3). No oysters were found subtidally at sites where *O. chilensis* was absent or rarely found intertidally. Furthermore, mean oyster density was highly correlated with the habitat type, with significantly higher densities present in areas where hard substrata was predominant. *O. chilensis* has now become established on the mainland side of the Menai Strait, near Caernarfon.

Using intertidal observations made in 1992 by Richardson *et al.* (1993b), a linear decrease in log-transformed oyster densities is generally evident in both north-easterly and south-westerly directions away from the invasion foci (Figure 2.4). This exponential decline is consistent with the highly-reduced natural dispersal capacity of this species. Areas of soft sediment which flank either side of the oyster bed may have also restricted further dispersal (Figure 2.4). Repeating the analysis with the data obtained during the current study period (Figure 2.5) highlights several interesting points. Although the current mean oyster density observed at Tal y Foel (12.8±1.8 m^{-2}) is comparable to those recorded in 1992 (see Table 2.1), the

Table 2.1 Comparative table of distribution parameters for the Chilean oyster (*Ostrea chilensis*) population in the Menai Strait and Conwy Bay Special Area of Conservation (North Wales, UK) between 1992 and 2009. 1992 data obtained from Richardson *et al.* (1993b).

	1992	**2009**
Site of overall maximum oyster density	Tal y Foel	Plas Trefarthen
Mean (±SE) Intertidal Oyster Density at site of maximum density	2.3±0.9 oysters m^{-2}	59.2±6.9 oysters m^{-2}
Mean (±SE) Subtidal Oyster Density at site of maximum density	n/a	35.2± 4.5 oysters m^{-2}
Maximum observed density (intertidal) and location	12 oysters m^{-2} (Tal y Foel)	232 oysters m^{-2} (Plas Trefarthen)
Maximum observed density (subtidal) and location	n/a	112 oysters m^{-2} (Plas Trefarthen)
Intertidal Size Range (shell length)	10-100 mm	Spat-90 mm
Subtidal Size Range (shell length)	20-95 mm	Spat-100 mm
Age classes present (intertidal)	Spat – 5 years old	Spat – 7 years old
Age classes present (subtidal)	Spat – 7 years old	Spat – 9 years old
Total range covered	<1 km	>30 km

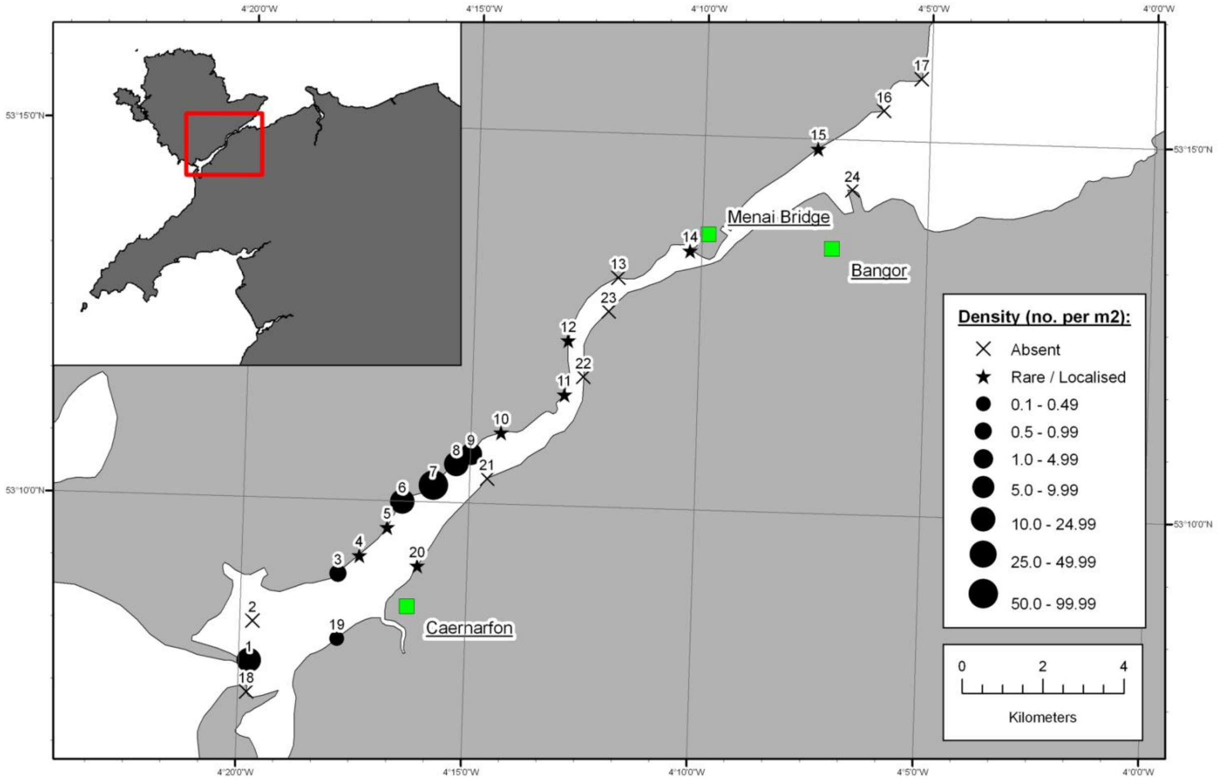


Figure 2.2 Map showing intertidal sampling sites in the Menai Strait (North Wales, UK; see inset map), along with respective mean Chilean oyster (*Ostrea chilensis*) densities (number of oysters m^{-2}, pooled from 0.5 m and 1.0 m above chart datum for each site). Rare / localised densities refer to areas where no oysters were recorded within the transects, but at least one individual found during a 30-minute timed search of the lower intertidal. Site names = 1: Abermenai Point, 2: Traeth Melynog, 3: Stud Farm, 4: Cae Aur, 5: Mermaid, 6: Tal y Foel (MAFF), 7: Plas Trefarthen, 8: Llanidan, 9: Mussels, 10: Castell Gwylan, 11: Moel y Don, 12: Plas Newydd, 13: Pwll Fanogl, 14: Church Island, 15: Glyn Garth, 16: Gallows Point, 17: Beaumaris, 18: Fort Belan, 19: Tŷ Calch, 20: Waterloo Port, 21: Plas Menai, 22: Y Felinheli, 23: Y Faenol, 24: Porth Penrhyn.

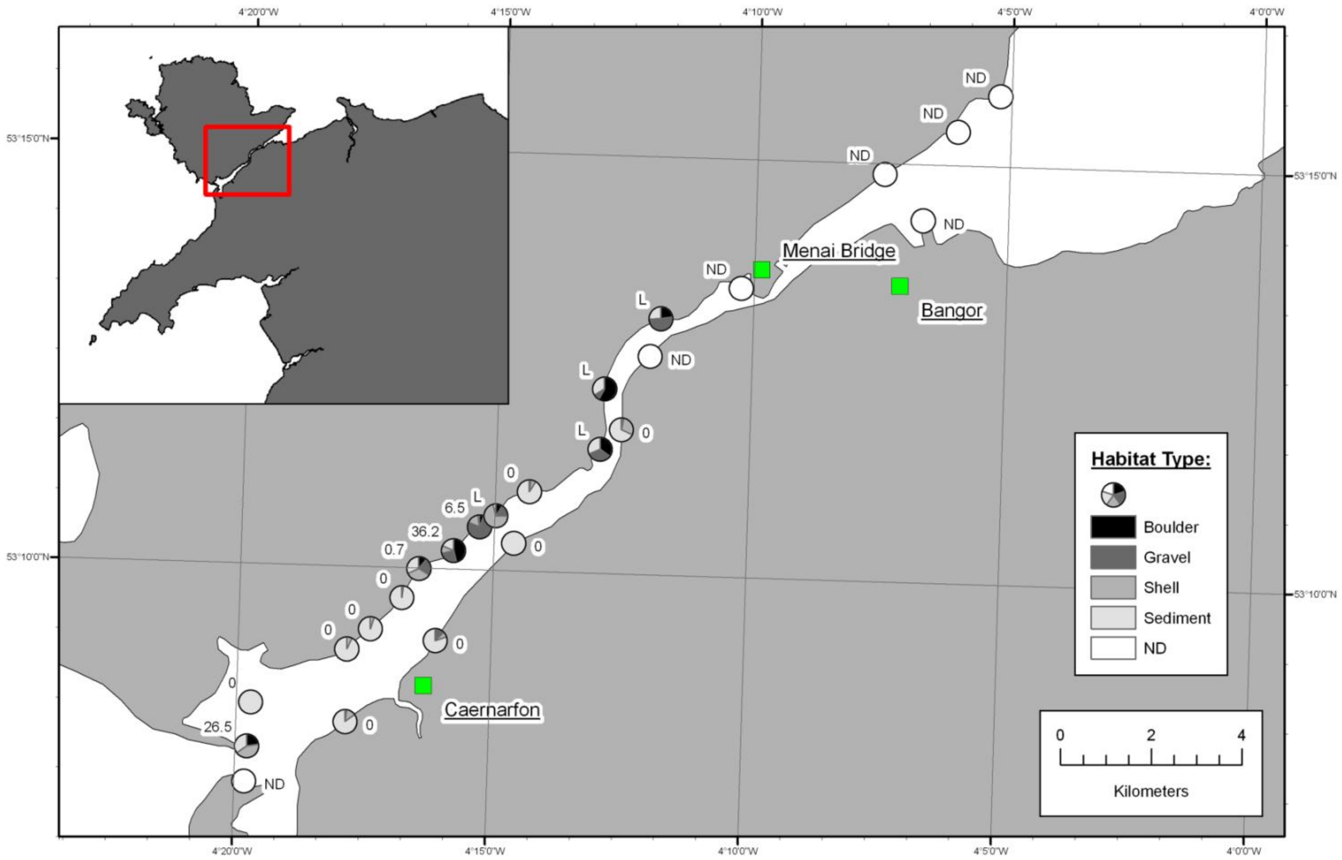


Figure 2.3 Map showing subtidal sampling areas (3-8 m below chart datum), adjacent to each intertidal sampling sites in the Menai Strait (North Wales, UK; see inset map), along with respective mean Chilean oyster (*Ostrea chilensis*) densities m^{-2}. L = areas where no oysters were found in any digital image quadrats, but at least one individual was collected by trawling a mussel dredge along the respective transect line. ND = no data. Pie charts indicate mean relative proportions of various substrata at each site. See Figure 2.2 for site names.

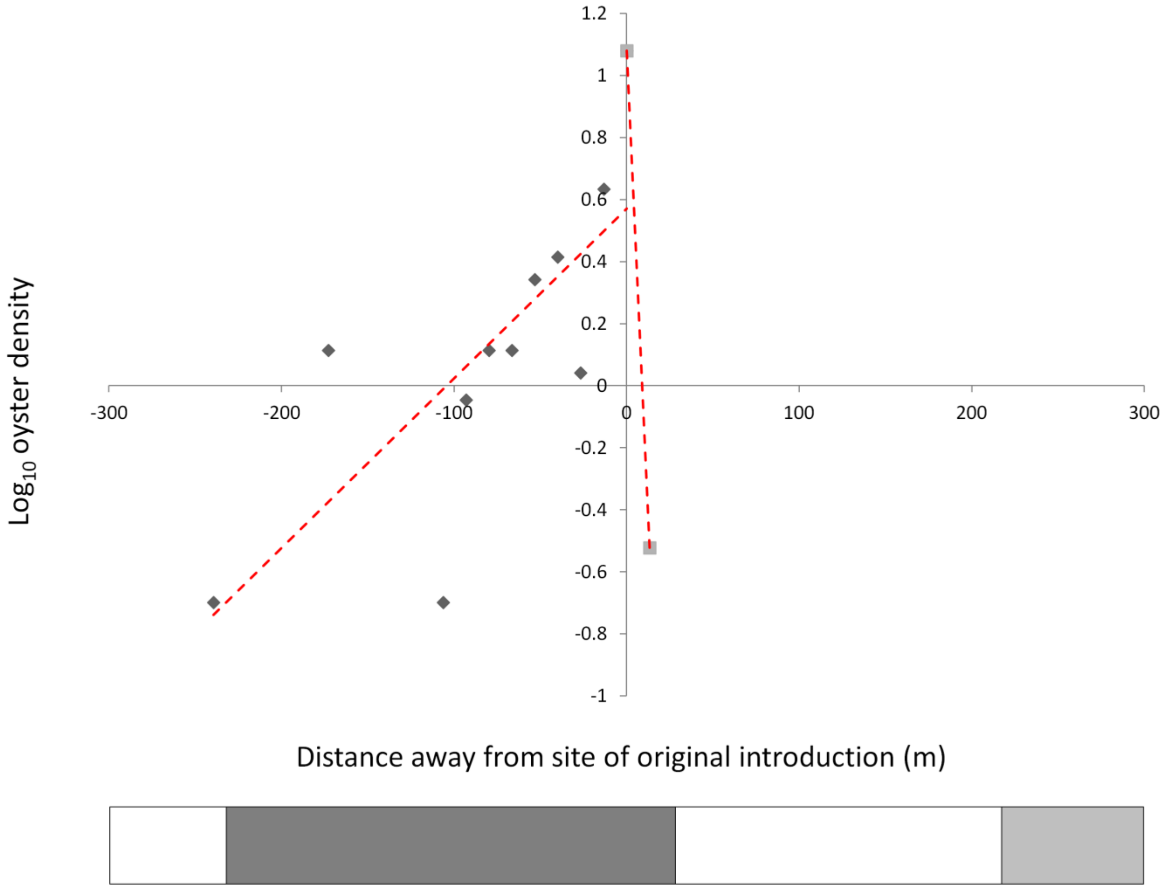


Figure 2.4 Change in Chilean oyster (*Ostrea chilensis*) densities (log-transformed) with distance (in metres) away from the invasion foci (Tal y Foel = 0 m) within the Menai Strait and Conwy Bay Special Area of Conservation (North Wales, UK) as of 1992. Positive and negative values of x indicate movements to the north-west and south-east respectively. Patterned bar below graph shows the change in predominant substrate type with distance away from the invasion foci. Dark grey = hard substrate, Light grey = soft sediment overlaid with patches of boulders, pebbles and other debris, Open = sand / mud. Raw data obtained from Richardson *et al.* (1993b).

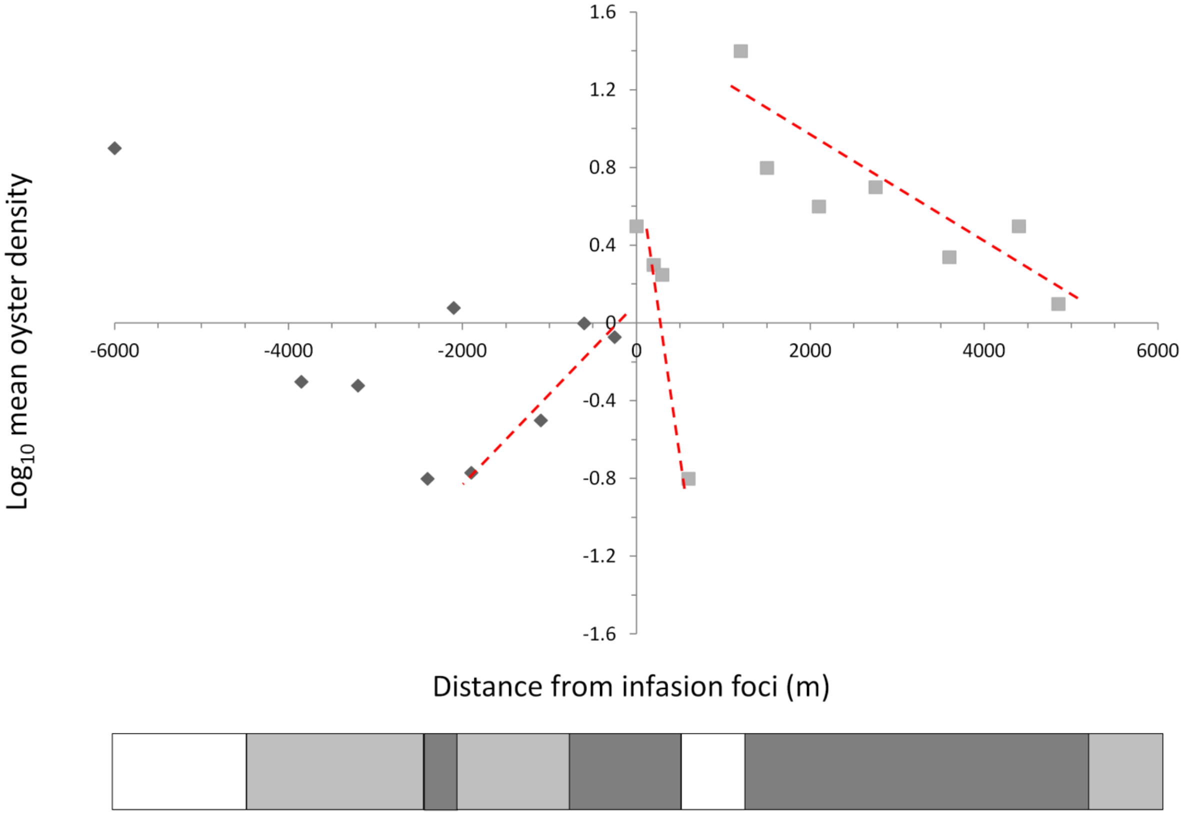


Figure 2.5 Change in Chilean oyster (*Ostrea chilensis*) densities (log-transformed) with distance (in metres) away from the invasion foci (Tal y Foel = 0 m) within the Menai Strait and Conwy Bay Special Area of Conservation (North Wales, UK) as of 2009. Positive and negative values of x indicate movements to the north-west and south-east respectively. Patterned bar below graph shows the change in predominant substrate type with distance away from the invasion foci. Dark grey = hard substrate, Light grey = soft sediment overlaid with patches of boulders, pebbles and other debris, Open = sand / mud.

Figure 2.6 Exceptionally high densities of the Chilean oyster (*O. chilensis*) observed at Plas Trefarthen, part of the Menai Strait and Conwy Bay Special Area of Conservation (North Wales, UK).

predominant habitat type of this locality has changed markedly such that mussels lays now dominate the majority of an area once covered by cobble stones and mixed hard substrata (Figures 2.4 and 2.5). The soft sediment barrier to the north-east of Tal y Foel has now been breached, giving rise to a second exponential decline in oyster density originating at Plas Trefarthen (Figure 2.5) with mean intertidal oyster density of 59.9±6.9 oysters m^{-2} and a maximum of 232 oysters m^{-2}. A steady linear decline in logarithmic densities persists north-east of Plas Trefarthen, with the lowest densities correlating with a significant decrease in hard substrata towards Moel y Don. Although a somewhat erratic decline in oyster densities was observed south-west of Tal y Foel, unusually high densities (21.1±6.0 oysters m^{-2}) were also found at Abermenai Point. Whilst only low densities (<0.2 oysters m^{-2}) have become established on the soft-sediment substratum separating the site of original introduction and Plas Trefarthen, a large number of oysters were found attached to trestle frames at this location (approximately 66 oysters per frame).

Size-frequency distributions of both intertidal and subtidal populations of *O. chilensis* along the Menai Strait displayed clear modal size-class peaks that corresponded to the population year classes determined from the number of growth lines in the sectioned shells. However, this relationship breaks down after 4-5 years as the size classes of the oysters merge together and overlap so that there is no longer a distinction between subsequent modal (year) classes (Figure 2.7). The oldest oysters collected from the intertidal and subtidal were seven and nine years old respectively (both >80mm shell length). The presence of all year classes from newly settled spat (<1 year) to oysters up to 7 and 9 years old, in the intertidal and subtidal respectively, indicates that there has been regular annual recruitment into the populations over the last 10 years (Figure 2.7).

2.5 Discussion

The present investigation documents a significant increase in both the density and range of the non-native Chilean oyster in the Menai Strait and Conwy Bay SAC over the last 20 years. Following at least 30 years of containment at Tal y Foel (see Richardson *et al.* 1993b), it has now spread over a range of more than 30 km along the Menai Strait. It is also likely to have spread outside the southern boundary of the SAC. Areas of very high densities have become established both near and distant from the site of original introduction. The Chilean oyster is the dominant benthic organism within such patches.

Regular annual recruitment is likely to have contributed to the localised dominance of *O. chilensis* within areas of the SAC. Within its native range, *O. chilensis* is known to produce larvae at seawater temperatures as low as 9-10°C (Stead 1971; Westerskov 1980), with peak larval

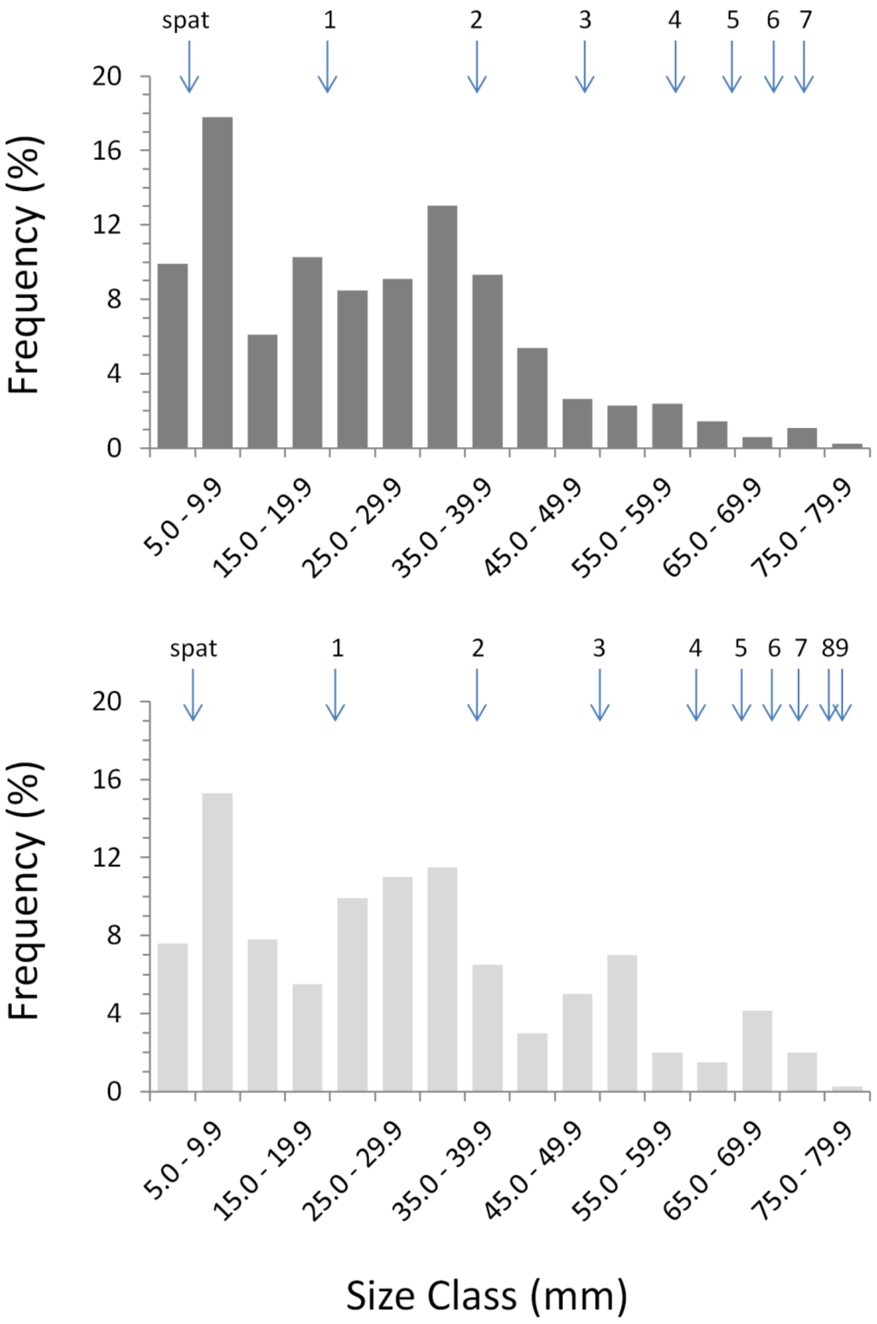


Figure 2.7 Relative size-frequency distribution of the Chilean oyster (*Ostrea chilensis*), collected intertidally (dark grey) and subtidally (light grey) at Plas Trefarthen, Menai Strait (North Wales, UK) during October-November, 2009. Arrows denote mean size-at-age, obtained from analysis of acetate peel replicas of the hinge region of the shell. Star denotes mean shell length of oysters born during the 2009 spawning season.

production coinciding with water temperatures of approximately 13-18°C (Hollis, 1962; Stead, 1971; Cranfield and Allen 1977; Westerskov 1980; Jeffs *et al.* 1996). Hayes and Barry (2008) suggest climate similarity between the native and new environments to be one of the only consistent predictors of NNS establishment success over several biological groups. The Menai Strait shares many similar environmental conditions to those found in several areas harbouring commercial densities of *O. chilensis* within its native range (Table 2.2). Whilst the UK is positioned several latitudinal degrees higher than both Chile and New Zealand, interactions between atmospheric circulation and seasonal patterns in oceanic heat exchange augments its relatively mild winters (Seager *et al.* 2002) and temperate oceanic climate, giving a climatic match of 70% similarity between the native and non-native range of *O. chilensis* ('CLIMATCH'; Bureau of Rural Sciences 2009). Information regarding potential regulators of recruitment success (e.g. predation, intra- and inter-specific competition) within its novel environment is currently lacking (although see Appendix III).

2.5.1 Possible avenues of spread during the last 20 years

Considering its highly-reduced planktonic larval phase, its inability to spread along the Menai Strait during the first 30 years following its establishment, and the largely unfavourable conclusions to the assessment of its suitability as a potential aquaculture species in the UK, the recent and relatively substantial spread of *O. chilensis* along the Menai Strait may seem somewhat surprising. However, prolonged delays in population expansions have been commonly observed in nature amongst even the most notorious alien invaders (termed 'lag phases' *sensu* Crooks and Soulé 1999). Due to the nature of population growth, particularly in relation to sedentary and slow-moving species, a NNS might need to reproduce and reach a critical effective population level before it can expand its distribution from the site of original introduction (i.e. an 'inherent lag'). Lag phases can also be surpassed either from a direct or indirect alteration to environmental conditions or geographical features which hitherto restricted the successful proliferation of a particular NNS. Warmer sea temperatures, arising from global climate change, may lead to earlier spawning events and an extended breeding period (Stachowicz *et al.* 2002), potentially increasing the invasibility of an NNS. The construction of the Suez Canal, linking the waters of the Mediterranean and Red Seas, has led to several instances of Lessepsian migrations (see Galil 2008). Alternatively, lag phases may be overcome by a product of a change in genetic fitness which previously inhibited the ability of a NNS to compete and proliferate within its novel environment. Whilst the hybrid product of the UK native (*Spartina maritima* (Curtis) Fernald) and North American non-native (*Spartina alterniflora* Loisel) cord grasses is infertile (*Spartina townsendii* H. and J. Groves), its later allotetraploid derivative (*Spartina anglica* C.E. Hubb) is

Table 2.2 Comparative table of environmental parameters, likely to affect the reproductive capabilities of the Chilean oyster (*Ostrea chilensis*) in both its native range (New Zealand and Chile) and in the Menai Strait and Conwy Bay Special Area of Conservation (North Wales, UK).

Location	Peak minimum seawater temperature	Peak maximum seawater temperature	Salinity	Water flow	Sources of data
Foveaux Strait (New Zealand)	8.0°C	16.0°C	34.5-35.5	<2-3 knots	Westerskov (1980)
Otago Harbour (New Zealand)	6.5°C	16.0°C	31.7-35.6	0.6-2.75 knots	Westerskov (1980)
Quempillén Estuary (Chile)	9.5°C	18.8°C	28-33	n/a	Toro *et al.* (1995)
Menai Strait (North Wales, UK)	5.0°C	18.0°C	32-35	1-4 knots	Evans *et al.* (2003)

fertile and is now a dominant of the intertidal fringe of saltmarshes through most of north-west Europe (see Williamson 1996).

Assuming an exponential decline in densities away from a central point of release, a logarithmic transformation of oyster densities should display a linear decrease with distance away from the parental stock, so long as the natural dispersal ability of the larvae remains consistent and is the only operating factor. This pattern has been observed in species with low reproductive dispersal capabilities over very short distances, such as the samphire, *Sargassum spinuligerum* Sonder (Kendrick and Walker 1991). However, considerable range expansions in populations of introduced species are not exclusive to those species possessing a significant planktonic larval dispersal phase (e.g. Jackson 1986; Johannesson 1988; O'Foighil 1989; Thiel 2003). NNS of limited natural dispersal abilities must rely on alternative sources of transport away from the adult population, often related to a wide range of anthropogenic and anthropogenically-mediated activities (e.g. Johnson *et al.* 2001; Hewitt *et al.* 2007; Mineur *et al.* 2007). Such alternative vectors of NNS spread often stem from sporadic and unregulated activities (see below), or are simply unknown, providing further challenges to management strategies. Since 1992, it appears likely that alternative vectors (see below, with additional references in Appendix II) may have facilitated the relatively long-distance dispersal of *O. chilensis* to areas away from Tal y Foel.

Increase in Anthropogenic Activities

The breaching of physical or environmental barriers previously inhibiting the geographic spread of NNS (e.g. waterfalls, mountain ranges, unsuitable habitat) can often be facilitated by anthropogenic activities. For example, facilitation of upstream anadromous fish migrations, through the removal of natural physical barriers such as waterfalls, has been called into question due to the possibility of promoting the spread of invasive species (Kerby *et al.* 2005). A lack of suitable settlement substrata flanking the Chilean oyster bed at Tal y Foel was previously identified by Richardson *et al.* (1993b) as a likely factor that had constrained the spread of *O. chilensis* away from Tal y Foel. Anecdotal evidence indicates that breaching of this physical environmental barrier has occurred due to several anthropogenic activities, the intensity of which has significantly increased in the area during recent years. Activities include bivalve culture, fishing and associated bait collecting (including ‘peeler crabs’ (pre-ecdytic shore crabs, *Carcinus maenas* L. 1758) and periwinkle (*Littorina littorea* L. 1758) collection (see Chapter 4).

During the late 1990s, more than 300 oyster trestles were moved from Tal y Foel towards Plas Trefarthen (now the current site of maximum mean oyster density in the Menai Strait), following a change of ownership of the Tal y Foel commercial oyster beds and a significant increase in the intensity of bivalve culture in the area. Man-made coastal structures (e.g. boats, breakwaters, moorings, off-shore wind turbines) can be colonized by NNS, and are thought to occasionally facilitate their spread by providing 'hitch-hiking' or 'stepping stone' opportunities (e.g. Petersen and Malm 2006; Hewitt *et al.* 2007; Locke *et al.* 2007). This chapter shows commercial oyster trestles to be a suitable settlement substratum for *O. chilensis* in areas otherwise lacking hard substrata.

Several large *O. chilensis* of various sizes (up to 90 mm shell length) were found on a patch of ground near Porth Penrhyn, along with some large Pacific oyster specimens. It is believed that they were accidentally transferred from the southwestern end of the Menai Strait by a trawler carrying mussels from Tal y Foel (E.I.S. Rees, pers. comm.). During our surveys, juvenile oysters were quite often found attached to mussels at both Abermenai Point and Llanidan. Oysters ranging between 5 and 45 mm shell length were also found attached to the shells of common periwinkles at several sites, particularly those containing high oyster densities. Periwinkle collection is an unregulated activity within the area and novel information regarding the periwinkle fishery within the SAC is presented in Chapter 4. Approximately 50 adult *O. chilensis* are also known to have been deliberately transferred (a legal activity under the current legal framework) from Tal y Foel to the mainland shore at Tŷ Calch during 2006/7, but were never retrieved. A small population of *O. chilensis* has subsequently become established on the mainland side of the Menai Strait.

Rafting

The passive transport of sessile organisms upon floating debris (termed 'rafting') is a known significant route for the geographic expansion of some marine organisms, often over large distances (e.g. Jackson 1986; O'Foighil 1989). Rafting upon floating pumice stones has been identified as the most likely method of trans-Pacific range expansion of *O. chilensis* (O'Foighil *et al.* 1999). Canopy-forming macroalgae are well-known rafting vectors (e.g. Hobday 2000; Thiel 2003). Both the serrated wrack (*Fucus serratus* L.) and the kelp (*Laminaria digitata* (Hudson) Lamouroux) are commonly found along the Menai Strait, particularly in areas of high oyster densities (pers. obs.). Several independent records of *O. chilensis* attached to rafts (particularly macroalgae) have been reported. A single oyster (52 mm shell length) was found attached to the holdfast of *Laminaria digitata* at Traeth Melynog (T.A. Whitton, pers. comm.); a sandy beach neighbouring Abermenai Point. Several *O. chilensis* spat have also been identified on a *F. serratus* frond at Llanidan (see Appendix II). Dislodgement of large macroalgae often occurs in the Menai Strait during winter storms, whilst deliberate removal of macroalgae is also a common occurrence amongst some bait collectors, with the latter activity gaining increased popularity in the area during the last 20 years (B. Roberts and R. Sharp, pers. obs.). The net flow-through of water in a south-westerly direction (Rippeth *et al.* 2002) and the regular formation of a back-eddy at Abermenai Point throughout most of the flooding tide (see Morgan 2007a) may lead to an eventual breach of an inherent lag through the eventual accumulation of Chilean oyster rafts at Abermenai Point, and may go some way to explain the anomalously high densities at this site shown in Figures 2.3 and 2.7. Macroalgae are not the sole rafting vector for Chilean oysters on the SAC, as four adult *O. chilensis* were found attached to a water-logged stick near Castell Gwylan in 2004 (see Appendix II).

2.5.2 Potential effects of O. chilensis on the qualifying habitats of the SAC

Whilst the likely effects of the spread of *O. chilensis* on the qualifying habitats of the SAC are currently unknown, the influential role played by oysters in the regulation of local population and community dynamics through their habitat creation and modification abilities (termed 'ecosystem engineering' *sensu* Jones *et al.* 1994) are numerous (Ruesink *et al.* 2005). Oyster reefs may be involved in the protection and amelioration of neighbouring ecologically-important habitats such as saltmarshes and seagrass meadows (Peterson and Heck 1999; Meyer and Townsend 2000; Newell and Koch 2004). Their gregarious nature leads to the creation of structurally-complex, heterogeneous biogenic habitats that promote niche diversification and biodiversity (Dame and Patten 1981; Zimmerman *et al.* 1989; Kennedy 1996; Lehnert and Allen 2002; Dubois *et al.* 2006), often leading to changes in the trophic structure of the community (Newell 1988; Dubois *et al.* 2007; Newell *et al.* 2007). Due to their structural resilience, oyster shells persist on the sea bed long after their death, and as a result, the 'engineering' functions of oysters extend far beyond their own lifespan (Parras and Casadío 2006).

Within their native range, regeneration of biogenic reefs is thought to provide new habitats for the proliferation of *O. chilensis*. The resulting increase in habitat complexity is thought to promote stocks of the commercially-important blue cod, *Parapercis colias* (Forster in Bloch and Schneider 1801) (Cranfield *et al.* 2001), as well as macrobenthic biodiversity (Cranfield *et al.* 2004). A strong linear increase in both the number of individuals and species richness of benthic organisms with increasing oyster shell density has been observed within the Chilean oyster beds of the Menai Strait (see Appendix I). The increased complexity offered by higher oyster shell densities is shown to have a positive effect on the abundance of several species, including several polychaetes and marcoalgae, as well as *O. chilensis* juveniles. This may have important implications for the growth of the Chilean oyster population. As larvae are released at an advanced stage of development (pediveliger), the natural dispersal potential of the Chilean oyster is limited to the locality where they were released (Cranfield 1968; Stead 1971;

Westerskov 1980). Furthermore, it is likely that strong chemical signals from adult conspecifics, known to induce settlement behaviour in several other oyster species (e.g. Tamburri *et al.* 1992; Zimmer-Faust and Tamburri 1994), help maintain a strong stock-recruitment relationship. Further analysis is now required to see whether or not the positive increase in biodiversity with oyster density has implications on the trophic structure of the community, the ecosystem services provided and the quality and quantity of the qualifying habitats of the SAC and their associated flora and fauna (particularly “reefs”, as defined by CCW 2009).

2.5.3 Review of current key legislation concerning the introduction and spread of non-native species in the UK

The Convention on Biological Diversity 1992 (hereafter ‘CBD’) is routinely regarded as the most influential instrument regarding the conservation of biodiversity from the growing threats posed by NNS across all continents and concerning all transport vectors. Under Article 8(h), each Member State is obliged, as far as possible and as appropriate, to *“prevent the introduction of, control or eradicate, those alien species which threaten ecosystems, habitats or species”*. Whilst methods of implementation of Article 8(h) are not directly prescribed, subsequent Decisions communicated by the Conference of the Parties (COP) have aided in its transcription to regional and national legislation. Of particular significance is the introduction of non-binding Guiding Principles (GPs) (Annex I of the 6th COP, Decision VI/23), calling for a *“precautionary”* (GP 1) and *“three-stage hierarchical”* (i.e. *“prevention”, “detection / surveillance” and “control / eradication"*) (GP 2) approach to managing biological invasions, with strong encouragement for collaboration and information-sharing between Member States (GPs 8 and 9). With at least 45 global instruments and several more dealing, at least indirectly, with the control of NNS at regional and national levels (Fasham and Trumper 2001), a comprehensive account of NNS policy is well beyond the scope of this paper. Rather, we aim to highlight legislation concerning currently innocuous NNS that became established prior to the enactment of the relevant legislation. Where pertinent, we highlight the shortcomings within the current policy framework which has allowed for the spread of such species.

The UK’s commitment to Article 8(h) of the CBD is currently addressed through various European Directives and several national legislation and strategies (Table 2.3), most of which are often supplementary provisions, added to legislation directly concerning the protection of other particular interests (e.g. birds, shellfish movements, specific habitats, water quality). Responsibility for the implementation of each individual piece of legislation is thus devolved to several different governmental agencies, departments and statutory advisors to whom the nature of the legislative obligations concern, resulting in a rather conflicting and disjointed legislation framework concerning NNS. Whilst the emphasis of both legislation and management efforts is placed on the more successful and cost-effective prevention of NNS introductions (see Table 2.3), such strategies do not make provision for those currently innocuous NNS that became established prior to the enactment of legislation, and thus do not fully adopt the hierarchical approach indicated in GP 2 of the CBD. Furthermore, GP 1 states that a *"lack of scientific evidence should not be used as a legitimate reason for lack of action"*.

The EC Habitats Directive 1992 aids in the conservation of diversity amongst both species and habitats across the European Union, thus partially fulfilling each Member State's commitment to the objectives of the CBD. Article 22(b) relates to the safeguarding of various habitats and wildlife from the potentially detrimental effects of NNS, and is prescribed through the designation of SACs that are managed accordingly to protect and conserve those habitats and species identified as being of European importance (see Annex I and II of the Directive respectively). Under Regulation 35 of the Habitats Regulations 2010, each relevant Governmental Agency are required to provide conservation objectives for each respective SAC, as well as to assess and stipulate potentially detrimental activities that are of relevance to the objectives of the Directive. However, it appears that no provision is made for accidentally-introduced species nor indeed for those species that have already become established prior to enactment. Also of relevance are the anthropogenic activities within the SAC that are likely to be associated in facilitating the spread of *O. chilensis* (see above). Whilst recognized as potential targets for review under the UK Marine and Coastal Access Act 2009, 'bait collecting' (i.e. hand-collection of 'peeler crabs', lugworms (*Arenicola marina* L. 1758) or sword razor shell (*Ensis siliqua* (L. 1758)) and 'winkle picking' (i.e. hand-collection of *Littorina littorea*) are two examples of anthropogenic coastal activities that are currently subjected to minimal regulation under current UK legislation. Quantification of the importance of such unregulated activities to the transfer of NNS is now recommended as part of the assessment of currently unmanaged anthropogenic activities within SACs, as specified under Regulation 35 of the Habitats Regulations 2010. Whilst the recent formation of central depositories of information has likely improved public awareness concerning invasive species, the associated risks associated with their accidental transfer to new environments need to be fully considered if Statutory Instruments and other forms of control are to be created to help regulate human-mediated spread of NNS.

The Wildlife and Countryside Act 1981, along with its many amendments, is considered by many as offering some of the most powerful legislation regarding the introduction of NNS into the UK, although the lack of enforcement of this legislation since its ratification is contradictory to such views (Fasham and Trumper 2001). Excluding Scotland, where the Wildlife

Table 2.3 Summary of some of the key concerning non-native species in the UK, along with their respective relevance to the Chilean oyster (*Ostrea chilensis*) population in the Menai Strait and Conwy Bay Special Area of Conservation (North Wales, UK).

Legislative Instrument	Section of relevance to NNS and / or invasive species management	Does the provision cover NNS already-established prior to the enactment of the relevant legislation?
EC Habitats Directive 1992	Following the obligations stated under the Bern Convention 1979, the Directive concerns the conservation of several habitats of 'European importance' and their associated flora and fauna. Article 22b notes that each Member State must ensure that *"the deliberate introduction into the wild of any species which is not native to their territory is regulated so as not to prejudice natural habitats within their natural range or the wild native fauna and flora and, if they consider it necessary, prohibit such introduction".*	Like the ECC Birds Directive 1979, the Directive <u>focuses on the prevention of introduction</u>, thus the provision offered to species which have already become established prior to the enactment of this Directive is weak. Despite the habitat modification abilities of oysters, there remains no information on the modification abilities of *O. chilensis* upon the qualifying habitats within the SAC.
Shellfish and Specified Fish (Third Country Imports) Order 1992	The Order relates to the restriction of importation into GB of any shellfish or specified fish species from non-Member State Countries. Article 1 states that *"no person shall import into Great Britain from a third country any shellfish or specified fish except under the authority, and in accordance with the provisions, of a licence issued by the appropriate Minister".*	Again, the Order makes provision for the prevention of entry of selected species from outside political boundary, but <u>fails to address the prevention of movements of those NNS who have already become established within GB.</u>

Imports of Live Fish Act 1980	The Act aims to prevent the import, keeping or release of live fish and shellfish, along with their reproductive products, into the waters of England and Wales (except under licence). Article 1 (s1) forbids the *“release, in any part of England and Wales of live fish, or the live eggs of fish, of a species which is not native to England and Wales and which in the opinion of the Minister might compete with, displace, prey on or harm the habitat of any freshwater fish, shellfish or salmon in England and Wales”.*	The text appears to lack reference to those species that have already become established prior to its enactment. Furthermore, this Act is specific to the import and keeping of those NNS which are known to be harmful to the habitats of fish and shellfish. Whilst the habitat modification abilities of oysters in general are well-documented, no information is currently available on the ecosystem engineering potential of the Chilean oyster.
Wildlife and Countryside Act 1981	The Act is considered by many as offering some of the most powerful legislation regarding the introduction of NNS into the UK. Section 14 of the Act signifies that it is *“an offence to release (or allow to escape) into the wild animals "not ordinarily resident" or that are not regular visitors to Great Britain and other animals listed in Part I of Schedule 9, except under licence”*.	The Act makes no provision for those species introduced prior to the enactment of this legislation. Furthermore, the Chilean oyster is absent from Schedule 9, and is unlikely to be added to the list under the current consenting process. It therefore currently remains legal to transfer this species within the UK under this Act.

Marine and Coastal Access Act 2009 **Marine (Scotland) Act 2010**	Whilst no new or additional measures specifically relating to NNS are provided within these Acts, they provide the means for the creation of Conservation Orders that can be used to manage otherwise unregulated activities when this is necessary to further the conservation objectives of a particular Marine Conservation Zone or Marine Protected Area respectively.	Whilst, in principle, this potentially provides a useful additional tool to the management of all NNS, it does not clearly address the precautionary approach noted in GP 1 of the CBD. It is envisaged that a NNS would have to demonstrate invasiveness, either within the Protected Area or elsewhere, before any action is taken under this premise.
EC Plants Health Directive 2000	The Directive provides a legal framework for plant health within the EC, providing *"protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community".*	The Directive actively embraces the GPs of the CBD (see Unger, 2003), adopting a 'precautionary' approach to invasive species management and is one of the only legislative Instruments adequately addressing both the introduction of new NNS as well as the spread of all NNS, including those already established prior to its enactment. It is unfortunately only relevant to plant species and their associated 'pests'. Furthermore, implementation of the Directive at UK-level is devolved to the relevant governmental agencies within each of the 4 UK countries, meaning that adequately achieving its objectives thus requires substantial coordination.

and the Environment (Scotland) Act 2011 has provided several superseding amendments to the Act (see below), it remains *“an offence to release (or allow to escape) into the wild animals "not ordinarily resident" or that are not regular visitors to Great Britain and other animals listed in Part I of Schedule 9, except under licence”* under Section 14 of the Act. The term *“not ordinarily resident”* is taken to signify any species that is not resident in the wild in the UK, and thus Section 14(1)(a), as with many other UK legislation concerning NNS (see Table 2.3), is involved in the control and prevention of entry of NNS, and does not directly address those species that were introduced prior to the formulation of legislation, unless listed under Schedule 9. The minimum review period for additions to Schedule 9 is, at best, quinquenial, and there does not appear to be any mechanism for adding a species to the Schedule in the interim period. Furthermore, the consenting process for adding species to Schedule 9 appears to be heavily based on previous knowledge of taxa-specific invasions (e.g. evidence of previous invasive capabilities, likelihood of invasive behaviour based on life history characteristics; see Annex B of DEFRA 2009). In Scotland, however, Schedule 9 has been repealed under Article 17 (s8) of the Wildlife and the Environment (Scotland) Act 2011. Provision is instead provided by way of Orders. It appears that Section 14(1)(b) also makes provision for the anthropogenically-facilitated spread of a NNS to new areas outside its native range.

The Invasive Non-native Species Framework Strategy for Great Britain (DEFRA 2008) contains many promising aspects in relation to the development of legally-binding instruments aiding in the management of invasive species. The objectives of the Strategy are grounded in the GPs of the CBD, with sections 6 and 7 dedicated to both the ‘prevention of introduction’ and the ‘early detection, surveillance, monitoring and rapid response’ of NNS respectively. Section 7 also advocates the need for more rapid response assessments to identify, as well as regular, careful monitoring of even the most inconspicuous species, thereby increasing the efficacy of management decisions and strategies, with 7.1 and 7.3 specifically referring to those established NNS who are yet to demonstrate their invasive capabilities. With its main obligation aimed at achieving or maintaining *“good environmental status in the marine environment by 2020”* (see Article 1 (s1)), the EC Marine Strategy Framework Directive 2008 (transcribed to UK legislation through the Marine Strategy Regulations 2010) requires all Member States to provide, by 2012, *“an analysis of the essential features and characteristics, and current environmental status of those waters...”*, including *“an inventory of the temporal occurrence, abundance and spatial distribution of non-indigenous, exotic species”* (see Table 1 of Annex III of the Directive). Whilst it is appreciated that conducting frequent surveys that solely target a particular NNS would not be cost-effective, it may be possible to incorporate monitoring of the spread of NNS into present survey designs of the relevant conservation agency or otherwise (particularly within SACs, where qualifying habitat surveys are conducted under the premise of the ‘Common Standards Monitoring for Designated Sites’ (Williams 2006). The formation of central depositories of information will also encourage knowledge transfer between all of the various stakeholders, including governmental agencies and research institutes. It is hoped that these aspects can either be transcribed into legislation, either through major amendments to the current legislative framework or, more preferably, through provision stemming from the creation of comprehensive EU legislation, specifically intended for the management of non-native and / or invasive species and their many associated sectors of interest (e.g. aquaculture, climate change, fishing) (see below). Encouragingly, responsibility for the organization, development and implementation of the Invasive Non-native Species Framework Strategy for Great Britain has been allocated to a single coordinating body, namely the Great Britain Non-Native Species Mechanism (see Section 4 and Annex 1 of DEFRA 2008).

Four policy options have been proposed for consideration regarding the development of the EU Strategy on Invasive Species (Genovesi and Shine 2004). Table 2.3 aides in highlighting a minimum requirement for the targeted amendments to existing NNS legislation, particularly where the focus is placed solely on the introduction of new NNS. Expanding the provision to cover those NNS that have become established prior to the enactment of the relevant legislation would cover a broader range of potentially invasive species, as well as abide to the precautionary approach introduced in GP 1 of the CBD (*“Option B+*” of Genovesi and Shine 2004). This is not a novel suggestion (see Manchester and Bullock 2000), and it remains unknown why a revision of the legal provision concerning NNS in the UK has not been previously considered. Scotland has provided additional and upgraded provisions to several Acts of Parliament, including the Wildlife and Countryside Act 1981, through the ratification of the Wildlife and the Environment (Scotland) Act 2011. However, this strategy alone does not address the current complexity and lack of coherence and connectivity in the current legislation framework regarding invasive species. We advocate the opinions of Shine *et al.* (2010), who suggests the creation of a comprehensive and dedicated EU legal framework for managing invasive species (*“Option C”* of Genovesi and Shine 2004). For each Member State, the framework would provide clear, direct objectives for both the prevention of invasive species, as well as rapid risk assessment and prioritization techniques for the management of those currently innocuous NNS that have already become established. As demonstrated by the Invasive Non-native Species Framework Strategy for Great Britain (DEFRA 2008), responsibilities should be granted to a dedicated coordinating body, and a mechanism promoting effortless coherence and continuity with impending legislative instruments and other relevant sectors should also be created.

Conclusions

As far as the Chilean oyster population in the Menai Strait and Conwy Bay SAC is concerned, complete eradication of a species whose long-distance dispersal relative to its natural ability is very likely facilitated by multiple transport vectors would now undoubtedly prove impossible. Information is urgently required regarding the factors which promote the persistence and spread of this non-native oyster within the SAC and beyond, as well as the impacts of its increasing localized dominance on the native biodiversity and ecosystem function. Bearing in mind the profound ecosystem engineering abilities of oyster, it is therefore considered to be of prime importance to identify which factors are currently controlling the distribution and invasive abilities of the non-native *O. chilensis* population, how likely these factors are to change in the near future, and what implications this might have on the native communities within the Menai Strait and Conwy Bay SAC.

Additional provision would be enforced if the Chilean oyster was to be commercially cultured in the area in the future. The EC Regulation concerning the use of alien and locally-absent species in aquaculture 2007 provides a dedicated framework involving "*the introduction of alien species and translocation of locally absent species for their use in aquaculture within the EC*". The term *“introduction”* in this instance appears to cover the deliberate movement of a NNS to *“an environment outside its natural range for use in aquaculture”*, and is thus likely to include the intentional movements of those NNS that have already become established within the EU to areas beyond their natural dispersal abilities. It currently remains unclear how the Regulation will be transcribed to UK legislation. Further clarification for the inclusion of already established NNS within the legislation is advocated.

Appendix II: Anecdotal accounts of sightings of the Chilean oyster (*Ostrea chilensis*) in the Menai Strait

"I had another search for *Tiostrea* yesterday afternoon. The furthest east was near to Castell Gwylan and that was just a single individual. I could find none at Moel y Don. I found just a few near the old jetty below Porthamel so they seem to decline quite markedly to the east of the Llanidan lane to the shore where there were about 5 per square metre. There may be another mechanism aiding the spread as I found four on a water-logged stick."

Mr E. Ivor S. Rees – 12th October 2004

"Apart from the Brynsiencyn area, the only occurrence of *Tiostrea* that I'm aware of is near Port Penrhyn. The last time I visited Ballast Bank, I found a patch of ground just NW of the harbour wall where there were quite a few large *Crassostrea gigas* and what I thought were *Ostrea edulis* in various sizes up to about 9cms in length. Kim Mould (of 'Bangor Mussel Producers') suspected that they were *T. lutaria* and when I looked at them back here it seems he was right. At least, they are definitely not *O. edulis*, so I'm guessing that they're *T. lutaria* as I don't have any description of that species. Kim seems to think that they were transferred from the W end of the Strait by the 'Still Ostrea' with some Brynsiencyn mussels. As far as I know, no-one has any commercial interest in *Tiostrea*. Kim said he did take some of the large *C. gigas* for his own consumption and would eat the *T. lutaria* too if he found them!"

Mr Bill Cooke – 14th October 2004

"Found a couple of oysters at 10 m whilst diving off Plas Newydd. I have attached photos of the small one which I brought back"

Mr Paul Brazier – 31st July 2009

Figure VI A small Chilean oyster (*Ostrea chilensis*), collected live by Mr Paul Brazier at ~10 m below chart datum at Plas Newydd (North Wales, UK) on the 19th July 2009 (image by Mr Paul Brazier).

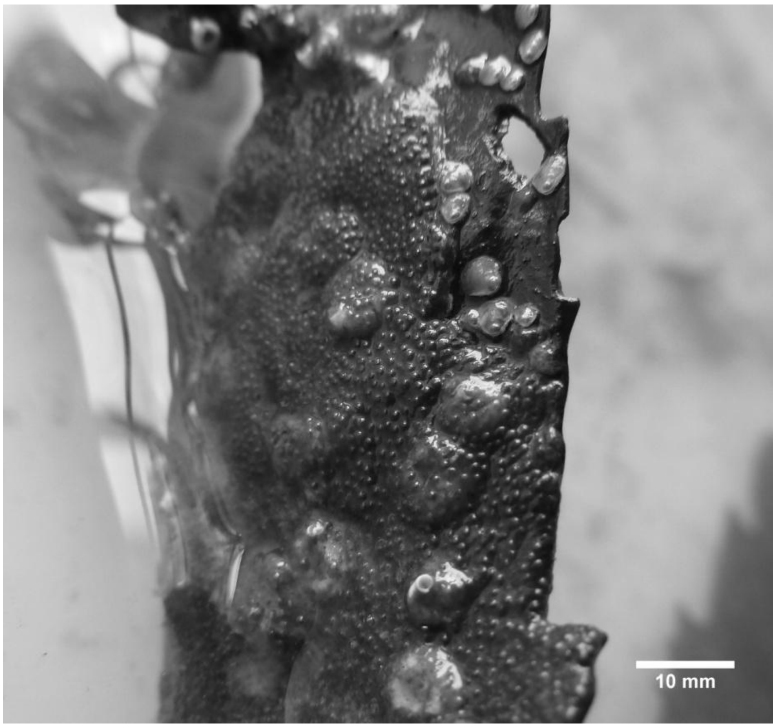


Figure VII Numerous Chilean oyster spat (*Ostrea chilensis*), newly settled on a piece of serrated wrack (*Fucus serratus*) and collected by Mr Paul Brazier at Llanidan (North Wales, UK) at approximately mean low water during the summer of 2010 (image by Mr Paul Brazier).

Chapter 3

Reproductive dynamics of the non-native Chilean oyster (*Ostrea chilensis* Philippi 1845) outside its native geographic range: past, present and future

3.1 Abstract

The geographic range expansions of many non-native species (NNS) are being facilitated as a result of a rapidly warming climate, often at the expense of native competitors. Understanding long-term changes in the reproductive dynamics of NNS is thus critical for the attainment of long-term conservation objectives. As well as providing comprehensive data on the reproduction of the Chilean oyster (*Ostrea chilensis*) outside its native geographic range (Menai Strait, North Wales, UK), this chapter demonstrates the importance of seasonal seawater temperature changes and food availability on the initiation, rate and magnitude of gametogenesis. Despite its narrow breeding season (June-July) and low annual numbers of brooding oysters ($\leq$4.6% of all oysters $\geq$60 mm shell length), high spatfall was observed each year (maximum mean monthly spat settlement = 2,570 spat m^{-2} y^{-1}), particularly following periods of high food concentrations (up to 14.2 μg L^{-1}) during early gametogenesis. Coupled with evidence of its highly-reduced natural dispersal capacity (<100 m), it is suggested that anthropogenically-mediated transport vectors have played a critical role in the recent spread of the *O. chilensis* population within the Menai Strait. Evidence is presented suggesting that a significant increase in mean annual seawater temperatures is likely to have contributed to the recent increase in the proliferation of this non-native oyster within the UK. Whilst further warming of the Earth's atmosphere is likely to further extend the breeding season, it remains to be seen whether or not future plankton dynamics will match or mismatch with the nutritional requirements of adult Chilean oysters and how this may affect the invasions success of this species in the near future.

3.1 Introduction

Oysters (family: *Ostreidae*) inhabit areas of the intertidal and shallow sublittoral of estuarine and marine environments, spanning between temperate and tropical latitudes (Carriker and Gaffney 1996). Historically, overfishing, disease and recruitment failure have led to the decimation of many commercial shellfish stocks worldwide, prompting considerable scientific endeavour into the culture of more suitable, alternative species with which to replenish native oyster beds. As a result, several oyster species have been deliberately introduced into areas beyond their native geographic range (see Walne 1974; Mann 1979). Often facilitated by human-mediated activities, movements of non-native species (NNS) across biogeographic boundaries have led to ecosystem-level changes with significant economic implications (Thomas *et al.* 2004). As a result, 'biological invasions' are currently identified as one of the most prevalent modifiers of global change (Vitousek *et al.* 1997). By instigating physical state changes in biotic and abiotic materials (thus altering the availability of resources to other species), non-native oysters can create, maintain and modify their habitat, leading to significant community and ecosystem level changes within their new environment (termed 'ecosystem engineering' *sensu* Jones *et al.* 1994). Their shells provide a hard substratum upon which fouling organisms may settle, often in areas of otherwise predominantly soft sediment. Gregarious behaviour promotes the formation of complex, three-dimensional benthic assemblages which offer a spatial refuge from predators and physical stresses (Coen *et al.* 2000) for a range of organisms including juveniles of commercially-important species (Lehnert and Allen 2002). Oysters are also proficient filter-feeders and play a key role in the translocation and transformation of large quantities of energy between the overlying water column and the benthos (Dame *et al.* 1980). By filtering large quantities of organic matter from the water oysters can function as important trophic links that provide a previously inaccessible source of energy to a range of benthic carnivorous predators and detritivors (Dame and Patten 1981). Biodeposition of faecal material can also modify the physical and chemical properties of the underlying sediment, and also initiate changes in the species assemblage composition and trophic pathways (Dubois *et al.* 2007).

The magnitude of any biological invasion is governed by the adaptivity and tolerance of the invader to a wide range of environmental factors (i.e. its invasiveness), the sensitivity of the invaded community to invasion stress (i.e. its invasibility), as well as the frequency and intensity of invader propagule release (i.e. propagule pressure). These determinants are becoming significantly compromised as a result of a rapidly warming climate, often favouring the proliferation of non-native species (NNS) at the expense of several native co-inhabitants (Dukes and Mooney 1999; Hellmann *et al*. 2008; Rahel and Olden 2008). Specifically, the reproductive dynamics of many temperate marine species is highly influenced by both sea temperature and the synchronicity between productivity and propagule development (Philippart *et al*. 2003). A warmer environment is likely to promote the establishment and spread of several NNS that were unable to proliferate under previous thermal regimes. Consequential phenological adaptations (e.g. augmented breeding season, earlier onset of recruitment, increased reproductive output) may further enhance invasive propagule pressure (Stachowicz *et al*. 2002; Sorte *et al*. 2010; Willis *et al*. 2010). Native species, on the other hand, are likely to be pushed closer to their upper thermal tolerance limits, with the increased physiological stress leading to a reduction in their competitive ability (Somero 2011). Understanding the reproductive dynamics of NNS is thus critical to the formation, prioritisation and successful execution of future management strategies, aimed at promoting the preservation of native biodiversity and ecosystem functioning. Such information can be particularly beneficial if obtained prior to the establishment of a NNS, when the prevention of spread through eradication is still a viable management option.

The Chilean oyster (*Ostrea chilensis* Philippi 1845) is indigenous to Chile and New Zealand, spanning a geographic range of 41-47°S and 34-47°S respectively (Buroker *et al*. 1983). The species is highly regarded as an oyster of both ecological and economic significance within its native range. Whilst infection by a haplosporidian parasite (*Bonamia exitiosa* Hine *et al*. 2001) has severely depleted fishing stocks in New Zealand during the last 25 years (Dinamani *et al*. 1987), over 8 million oysters was nonetheless harvested in 2009, equating to a retail value in excess of US$14.5 million. The increase in habitat complexity associated with dense *O. chilensis* beds is known to cause significant changes to the benthic macrofaunal community (Cranfield *et al*. 2004), as well as enhancing the commercially-important blue cod, *Parapercis colias* Forster 1801, stocks (Cranfield *et al*. 2001). Relative to other congeners, *O. chilensis* exhibits a highly extended brooding period, where the developing larvae remain in the mantle cavity for up to 8 weeks (Chaparro 1990). *O. chilensis* is a protandric hermaphrodite, maturing first as males before later developing into either simultaneous hermaphrodites or true females (Jeffs 1998). The larvae are predominantly released as pediveligers and will settle within a couple of hours, providing that a suitable substratum is available (Millar and Hollis 1963). Propagule dispersal is thus highly restricted and likely to be influenced by local currents and timing of release (Broekhuizen *et al*. 2011), although the possibility of earlier release as planktonic veliger larvae has also been proposed at lower latitudes (Cranfield and Michael 1989).

The Chilean oyster was introduced at Tal y Foel (Menai Strait, North Wales, UK) by the Ministry of Agriculture, Fisheries and Food (MAFF) during the early 1960s (Walne 1974) in an attempt to establish its potential as an alternative species with which to boost the diminishing native oyster (*Ostrea edulis* L. 1758) populations. Despite its highly reduced natural dispersal capacity and initial lack of spread during the first 30 years following its introduction (Richardson *et al*. 1993b), the Chilean oyster has recently shown a significant enhancement in its geographical extent within the now-designated Menai Strait and Conwy Bay Special Area of Conservation (SAC). A significant increase in the intensity of several local anthropogenic activities (e.g. bait collecting, mussel harvesting, yachting) and a lack of sufficient regulation under the current UK legislation framework have been suggested as possible reasons for this change (see Chapters 2 and 4), although the role of longer-term changes in key environmental parameters is currently unknown. Due to its status as a valuable fishery species and its potential as an important ecosystem engineer, the life history and reproductive dynamics of *O. chilensis* have been extensively studied throughout its native range (see Toro 1995; Jeffs and Creese 1996). Although the Chilean oyster is known to cause significant changes to species diversity with increasing densities in the Menai Strait (see Appendix I), information regarding the recently-observed proliferation of the UK Chilean oyster population is completely lacking. The present investigation thus provides comprehensive, quantitative information on the reproductive dynamics of the *O. chilensis* population within the Menai Strait and Conwy Bay SAC, with focus on both intra- and inter-annual spatial and temporal variation in the resulting spat recruitment patterns. As well as providing critical information for the effective management of this species outside its native geographic range, the present study demonstrates the value of critical environmental parameters, measured both across the entire native range of this species and within the SAC, as useful predictors of future invasion success of *O. chilensis* in a rapidly-changing climate. The data demonstrate how even the most innocuous NNS can become invasive if left unregulated for a considerable length of time.

3.2 Methods

3.2.1 Water temperature and chlorophyll-a concentration

Seawater temperature was monitored at 30 minute intervals during the entire study period (April 2009–October 2011) using three temperature loggers (Gemini Tinytag™ Splash 2 TG-410), each mounted on fixed structures at 0.8 m above chart datum at each of three locations in the Menai Strait, namely Abermenai Point, Mermaid and Plas Trefarthen (see Figure 3.1 for all site locations hereafter). Data collection and logger maintenance (including the removal of fouling organisms) were carried out at monthly intervals (when possible) during extreme low water spring tides (ELWS). Upon retrieval, the data were manually 'de-spiked' in order to remove anomalous values obtained during periods when the loggers were aerially exposed during ELWS. Monthly seawater chlorophyll-a concentrations were also determined at each site during the same period using the spectrophotometric method of Jeffrey and Humphrey (1975). 500 mL of

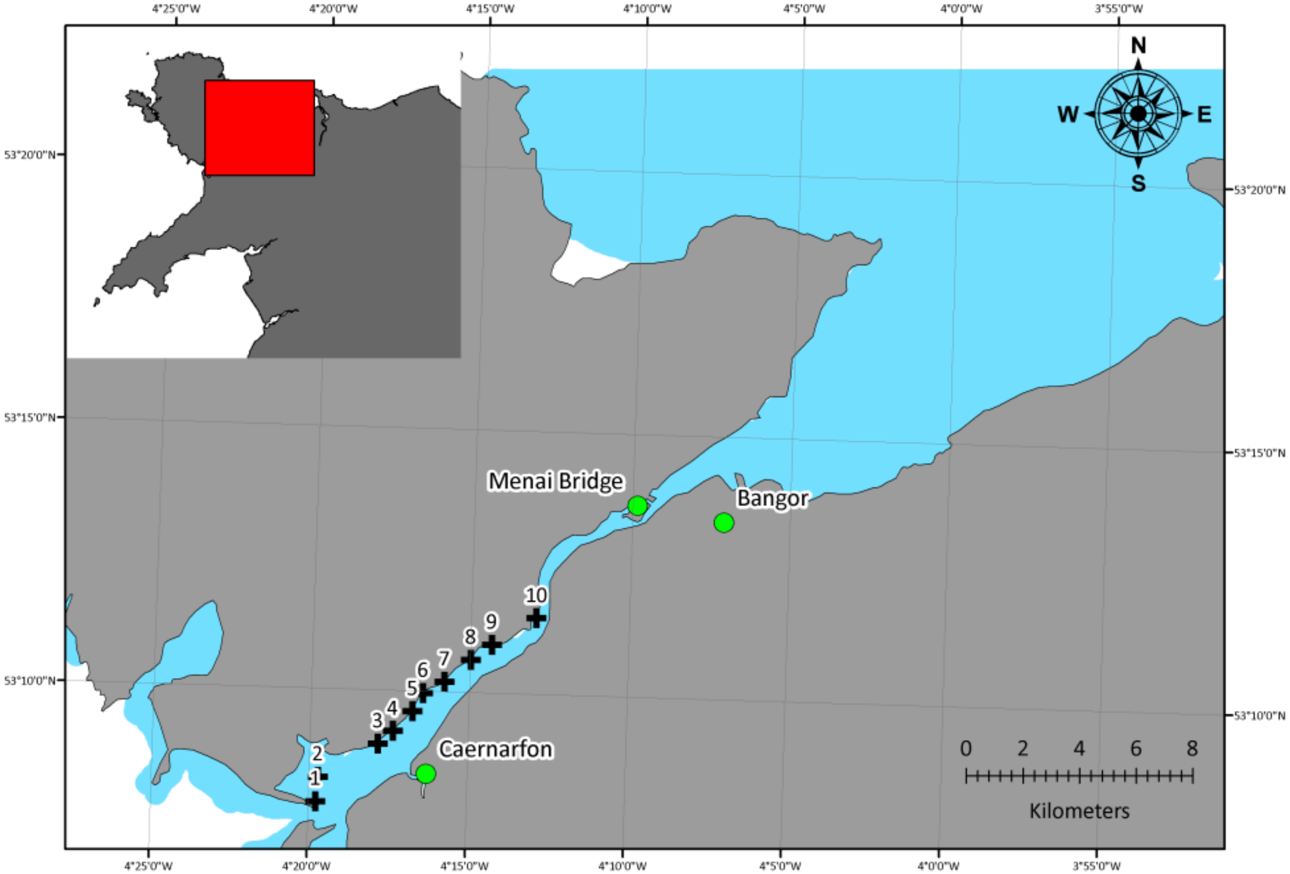


Figure 3.1 Map showing the Menai Strait and Conwy Bay Special Area of Conservation (blue), and the locations of the ten sites (1-10) where Chilean oyster (*Ostrea chilensis*) larval settlement was monitored. Site names: 1. Abermenai Point, 2. Traeth Melynog, 3. Stud Farm, 4. Cae Aur, 5. Mermaid, 6. Tal y Foel (site of original introduction), 7. Plas Trefarthen, 8. Llanidan, 9. Castell Gwylan, 10. Moel y Don. The data used to generate the SAC boundary are subject to Crown Copyright (reserved). Countryside Council for Wales, Licence NO. 100018813.

seawater was collected from ~50 cm below the surface at each site during ELWS. Samples were stored in opaque bottles and were always processed within 2 h following collection. Samples were filtered through 47 mm Whatman GF/C-type filter paper at 0.7 bar residual pressure and the chlorophyll-a extracted in 10 mL of acetone during a 24 h period of refrigeration. Each sample tube was centrifuged at 1000 rpm for 10 minutes and the absorbance of the resulting supernatant was measured at wavelengths of 630, 647, 664 and 750 nm using a spectrophotometer. Chlorophyll-a concentration was calculated using the following equation:

$$[\boldsymbol{chl-A}] = (11.85\,.\,(E_{664}-E_{750}) - 1.54\,.\,(E_{647}-E_{750}) - 0.08\,.\,(E_{630}-E_{750}) * \left(\frac{V_e}{LP\,.\,V_f}\right)$$

where E_n = absorbance at wavelength n (nm), LP = cuvette light-path (cm), V_e = extraction volume (mL) and V_f = filtered volume (L).

3.2.2 Adult brooding status and reproductive condition

Between April 2009 and October 2011, 15 small (40-50 mm shell length) and 15 large (60-70 mm shell length) oysters were collected monthly from Plas Trefarthen. To minimise the effect of any site-specific variation, all oysters were collected at 0.8 m above chart datum and from a restricted stretch of the shoreline (<0.3 km). All debris and epifaunal organisms were removed from the exterior surface of all specimens using a blunt knife and a hard-bristled brush. All oysters were transferred immediately to the laboratory, where both shell valves and their respective tissue sample were dried to constant weight at 60°C for 72 h in a drying oven and subsequently weighed to the nearest 0.01 g using a top-loading balance. Dried tissues were fully combusted at 500°C for 5 h in a muffle furnace and the ash-free dry weight (AFDW) of each tissue sample calculated. A condition index was calculated for each oyster using the following equation:

$$\boldsymbol{Condition\ Index} = 100\,.\left(\frac{Tissue\ AFDW}{Shell\ DW}\right)$$

The presence of oyster larvae within the mantle cavity was also noted where applicable. Estimates of the number of brooding larvae, mean larval size and stage of development were obtained by retaining each brood on a 100 µm sieve and washing before dilution in 100 mL of filtered seawater. Following re-suspension of the larvae using a perforated plunger, five replicate samples (500 µL each) were pipetted onto a haemocytometer. Mean larval density, size and life stage were determined using a compound microscope fitted with a calibrated eyepiece graticule and viewed at up to 40x magnification.

To ascertain the relationship between changes in adult oyster condition and gametogenesis, monthly assessments of gonad development in 5 mm^3 sections of gonad tissue taken from a further 15 small and 15 large oysters were undertaken between March and November 2010, based on the histological methods of Jeffs (1998). The gonad is packed around the digestive gland, so care was taken to ensure that samples were obtained from a localised region of the tissue to ensure consistency and comparability between individuals (see Jeffs 1998). Tissues were fixed for 36 h in Bouin's solution and preserved in 70% industrial methylated spirit until required. Following dehydration through a graded alcohol series (70-100% ethanol), the tissues were cleared in xylene and embedded in paraffin wax. 7 µm-thick microtome sections were stained and counter-stained with haematoxylin and eosin respectively and permanent slide mounts prepared. Each histological preparation was examined using a compound microscope at up to 40x magnification to determine the sex (male, female or hermaphrodite) and subsequently assigned to a particular gonadosomatic index (Table 3.1), indicative of their respective stages of development (see Figure 3.2).

3.2.3 Patterns of spat settlement

Spatial and temporal variations in spat settlement were assessed at 10 sites in the Menai Strait and Conwy Bay SAC. At each site, four replicate settlement panel arrays were placed at intervals of 10 m at 0.8 m above chart datum. Each array consisted of four replicate slate panels (18x15 cm each), with the centre of each panel positioned 20 cm away from the centre of its closest neighbouring panel. Slate is a natural material that is commonly found along the shores of the Menai Strait, where it is often fouled with several sessile epifauna, including *O. chilensis*.

Panel arrays were first deployed during March 2009. At monthly intervals, all panels were collected and replaced with fresh panels, lightly cleaned using a soft wire brush and rinsed in a light acid solution. Collected panels were carefully placed in a designated rack system which avoided contact between panels and immediately returned to the laboratory for analysis. Only the underside of each panel was examined, whilst a 1cm-thick border around the perimeter of the panel was also ignored to avoid potential edge effects. Each panel thus equated to a total area of 0.02 m^2, equivalent to 0.32 m^2 at each site. All spat (including dead specimens, distinguished by disarticulated shell valves with only the left valve remaining attached) were counted under a dissection microscope (6x magnification), giving an estimate of monthly settlement.

Table 3.1 Descriptions of the various gonadosomatic index (GSI) stages observed in the Chilean oyster (*Ostrea chilensis*) population from the Menai Strait and Conwy Bay SAC.

GSI	Description
0	*Resting or Spent* Total absent of any gametogenic products. Includes both immature oysters and spent oysters.
1	*Early Development* (see Figure 2a) Typified by onset of follicle formation (<25% of the entire histological section), containing early-stage gametogenic products. Ripe gametes (particularly ova) extremely rare.
2	*Late Development* Characterised by general increase in gonad mass (25-50% of the entire histological section). Reduction in stored food within the connective tissue. All stages of gametogenesis now present, with predominant stage of both male and female products varying between follicles.
3	*Fully Ripe* (see Figure 2b) Gonad mass >50% of the entire histological section. Ripe gametes (usually male) now predominant, although majority of follicles still contain small amounts of 1° and 2° spermatocytes and/or oocytes.
2	*Spawning* Although still relatively full, follicles are now undergoing an active discharge of gametes. Characterised by a general loss of late-stage gametogenic products into tubules.
1	*Resorption of Residual Gametes* (see Figure 2c) Follicles continue to reduce in size Follicles contain residual gametes undergoing cytolysis by phagocytotic amoebocytes, occurring in very high densities within the follicles and, less commonly, the connective tissue matrix.

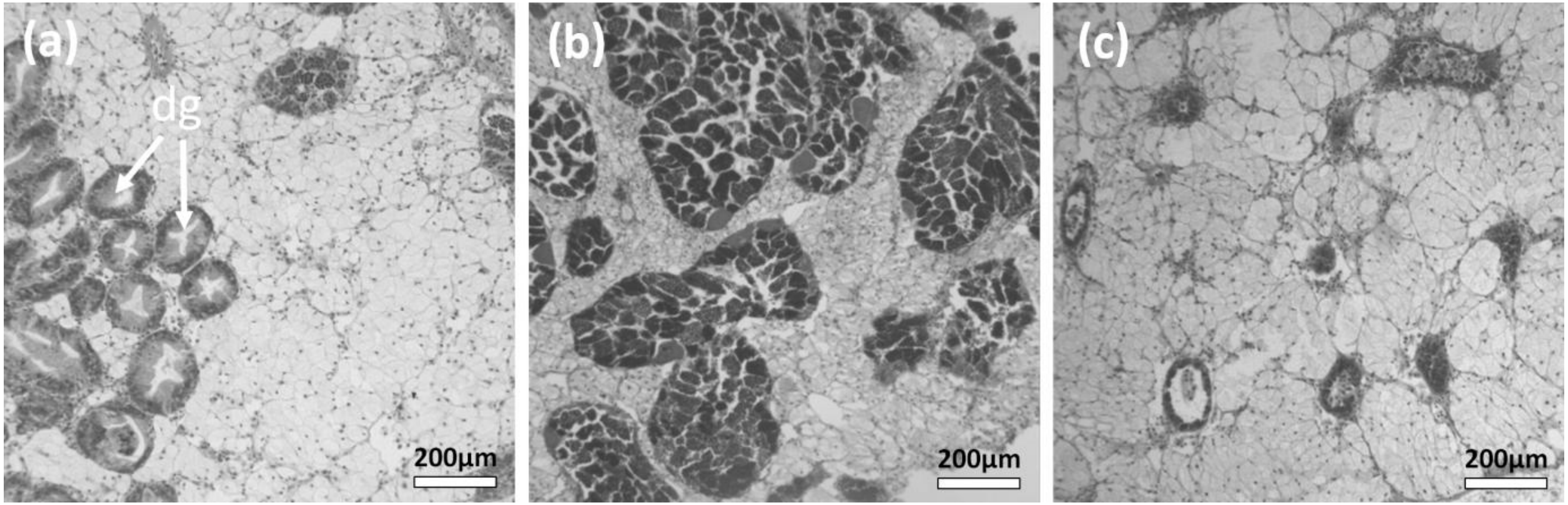


Figure 3.2 Photomicrographs (10x magnification) of histological sections of the reproductive tissue of Chilean oysters (*Ostrea chilensis*), showing (a) a male oyster showing early signs of gametogenesis (GSI stage 1, early development), (b) a large, ripe simultaneous hermaphrodite oyster (GSI stage 3, fully ripe), and (c) a near-spent individual showing empty follicles and the resorption of the remaining residual gametes (GSI stage I, resorption of residual). dg = digestive gland.

3.2.4 Larval dispersal

During June 2011, a transplantation experiment was conducted to mimic and quantify larval dispersal away from an established oyster bed. The experiment was designed to help determine whether site-specific spat settlement is a result of proximity to adult oysters or simply due to larval supply from more distant conspecifics. A total of 100 adult oysters (50-90 mm shell length) were transferred from Plas Trefarthen to the low shore (0.8 m above chart datum) of two sites (Mermaid and Traeth Melynog), where both adult oysters and spat settlement were rarely observed during 2009-2010 (see Chapter 2). The chosen sites were not, however, located towards the perimeters of the current distribution of *O. chilensis* within the Menai Strait, thus ensuring that the geographic range expansion of this NNS was not intentionally encouraged. Settlement panels were positioned both within and at specific distances away from the newly-transferred oyster patches (0, 20, 40 and 100 m). All panels were positioned at the same tidal height and only in one direction (towards the south-west), away from the transferred oyster patch. Spat settlement was estimated on each panel in July, which was the peak settlement period observed in the Menai Strait during both 2009 and 2010 (see below).

3.2.5 Data analysis

A 3-way mixed model ANOVA was used to compare inter- and intra-annual oyster condition of both small and large oysters. Inter-annual variability in condition indices was intended to be discussed in relation to specific environmental parameters (namely sea temperature and chlorophyll-a concentration) recorded during each particular year, thus Year (3 levels) was considered a fixed factor. Both Month (9 levels) and Size (2 levels) were considered random factors, with Month nested within Year. Due to the ordinal nature of the GSI, a non-parametric Scheirer-Ray-Hare test was used to assess whether or not any significant temporal differences in GSI could be observed between the two size classes of oyster. A 3-way mixed model ANOVA was used to compare peak spatfall densities between years and sites, as well as among settlement panel arrays within sites. Inter-annual variability in peak settlement was intended to be discussed in relation to specific environmental parameters (namely sea temperature and chlorophyll-a concentration) recorded during each particular year, thus Year (3 levels) was considered a fixed factor. Both Site (4 levels) and Array (4 levels) were considered random factors, with Array nested within Site. Spatial (site: 10 levels, random) and temporal (year: 3 levels, fixed) variability in mean total annual spatfall was compared using a non-parametric Scheirer-Ray-Hare test. A 2-way ANOVA was used to test for any differences in spat settlement with distance away from the introduced oyster patches (4 levels, fixed) and between sites (2 levels, random). All ANOVA statistical analyses were conducted using the software GMav5 for

Windows (University of Sydney, Australia; see Underwood and Chapman 1997), whilst all non-parametric and regression-based tests were conducted using Minitab (Version 15).

3.3 Results

3.3.1 Water temperature and chlorophyll-a concentration

Environmental parameters showed relatively little variability between locations, with site-specific differences in seawater temperatures generally smaller than the stated accuracy of the data loggers themselves. As a result, data were pooled together to give mean estimates for the Menai Strait as a whole. Seawater temperature generally followed a consistent annual seasonal cycle, with minimum (~4.5°C) and maximum (~18.5°C) temperatures recorded during the winter (December to February) and summer (June-August) months respectively (Figure 3.3a-c). Whilst the spring (March-May) of 2011 was unequivocally warm, the attainment of a maximum temperature was delayed by several weeks and also persisted for a shorter duration compared to both 2009 and 2010. Chlorophyll-a concentration generally fluctuated between ~0.5-2.5 µg L^{-1} for the majority of each year, although the timing and strength of the spring phytoplankton bloom showed inter-annual variability. A maximum peak of ~8.0 µg L^{-1} was observed during mid-March during all three years of observation with an additional and much greater peak in phytoplankton productivity (~17.0 µg L^{-1}) occurring nearly a month later during 2009. Smaller peaks in productivity (>4.0 µg L^{-1}) were also more commonly observed in 2009 (Figure 3.3a-c).

3.3.2 Adult reproductive condition and brooding status

Distinct temporal differences in condition were observed within years between small and large oysters (Size | Date (Year): $F_{33,1008} = 1.54$, $p = 0.027$). In 2009, both small and large oysters showed a similar temporal change in condition throughout, with a significant decline between May and June (Figure 3.3d) coinciding with observations of brooding females within the population (Figure 3.3g). Whilst a similar initial pattern was also observed in 2010 and 2011 (Figures 3.3e-f), the subsequent post-spawning recovery differed between small and large oysters (see SNK in Table 3.2). The condition of the small oysters continued to decline into August before showing signs of improvement towards October. The condition of large conspecifics, on the other hand, significantly increased soon after the brooding period, staying relatively stable until another period of reduced condition into October (Figures 3.3e-f). No evidence of brooding or spat settlement was observed following this second period of decline in condition (Figure 3.3g-i).

In *O. chilensis*, male and female gametes undergo five and three stages of gametogenic development respectively. Towards the spawning period, developing and ripe gametes can occur

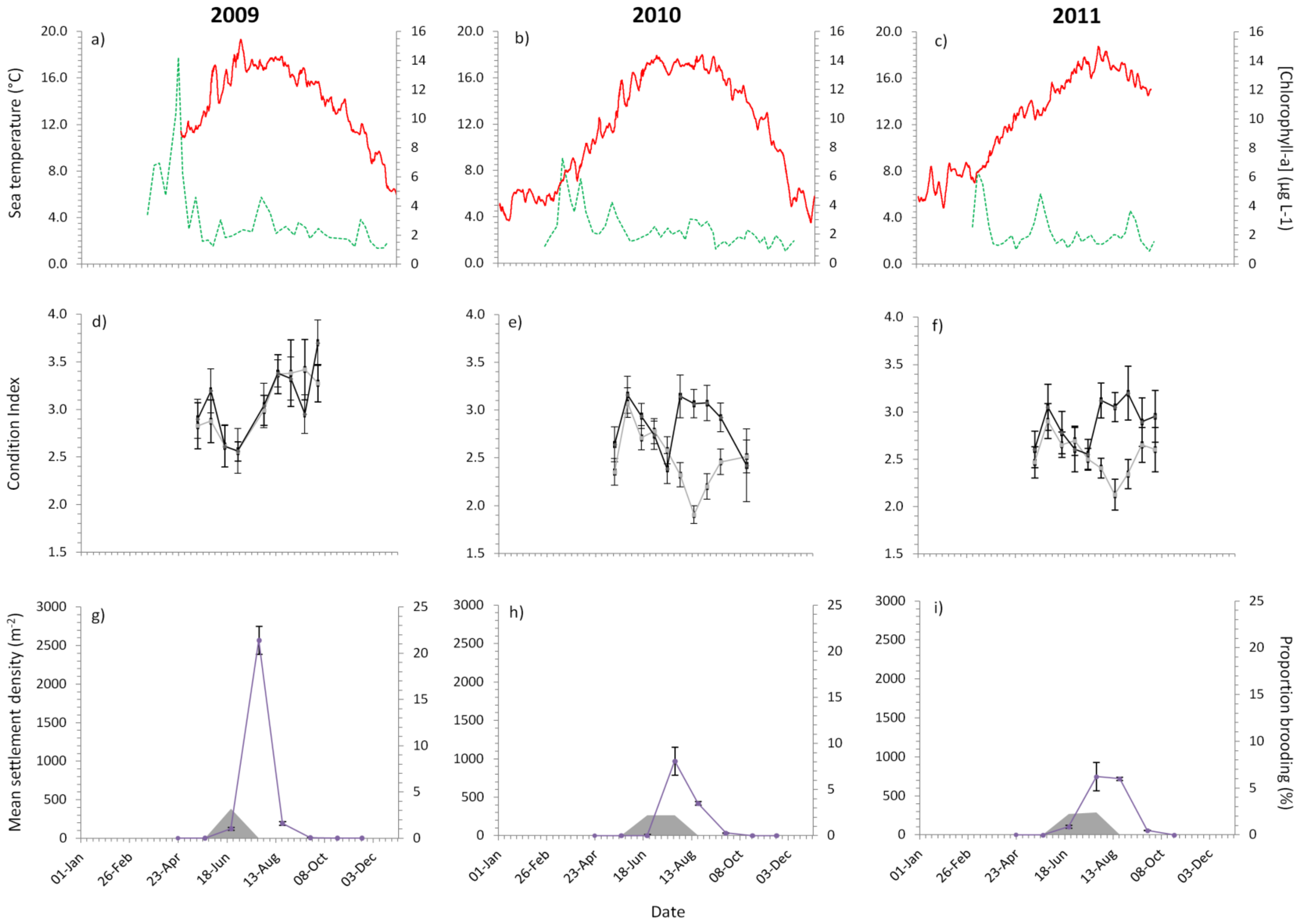


Figure 3.3 Inter-annual variability of seawater temperature (°C) (red line) and chlorophyll-a concentration (µg L^{-1}) (green line) (a-c), adult Chilean oyster (*Ostrea chilensis*) condition index ('small' or 40-50 mm shell length = grey line, 'large' or 60-70mm shell length = black line) (d-f), the proportion of brooding female oysters (%) within the population (>60mm shell length) (shaded area) and the mean monthly spat settlement (number of settlers m^{-2}) (solid line) (g-i) within the Menai Strait and Conwy Bay SAC (North Wales, UK). All error bars indicate ±1SE.

Table 3.2 3-way mixed model ANOVA examining the temporal (both intra- and inter-annual) variability in condition of adult Chilean oysters (*Ostrea chilensis*) from two distinct size classes (small: 40-50 mm, large: 60-70 mm shell length). ns = no significant difference.

Source of Variation	**df**	**MS**	**F**	**p**
Year	2	6.4	5.53	0.009
Date (Year)	33	1.2	3.08	<0.001
Size	1	11.1	19.17	<0.001
Year x Size	2	1.6	2.79	0.076
Size x Date (Year)	33	0.6	1.54	0.027
Residual	1008	0.4		
Total	1079			
Cochran's Test		C = 0.034, p>0.05		
Transformation		None		
		SNK Test	Size x Date (Year) (SE = 0.16)	
			Size (Date (Year)):	
			2009	ns
			2010	Large>Small from Aug-Oct
			2011	Large>Small from Aug-Oct

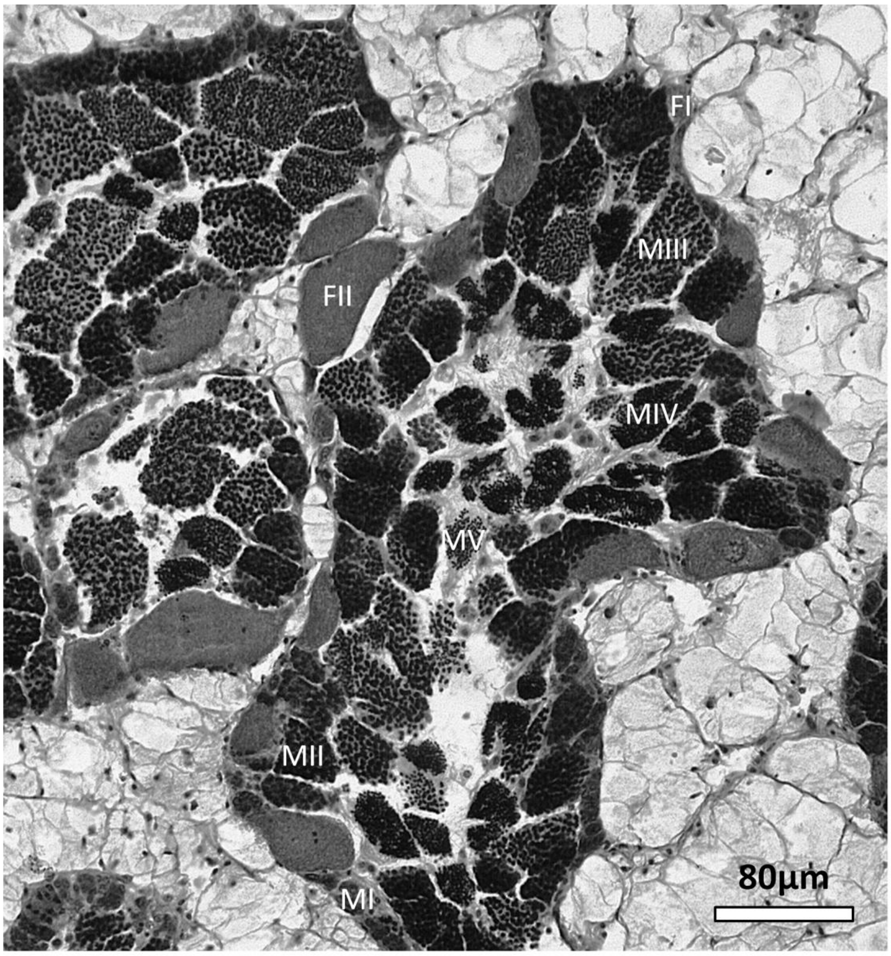


Figure 3.4 Transverse section of a near-ripe Chilean oyster (*Ostrea chilensis*) follicle, functioning as a simultaneous hermaphrodite and showing the various stages of gametogenesis. Codes: MI = spermatogonia, MII = 1° spermatocytes, MII = 2° spermatocytes, MIV = spermatids, MV = spermatozoa, FI = oogonia, FII = ovocytes. Note lack of ripe female gametes (i.e. FIII, see Figure 3.77bii). These ova would be extremely large (up to 250 µm diameter) and would occupy the majority of the follicle.

within individual follicles in this species (see Figure 3.4). Histological observations showed that small oysters within the SAC predominantly functioned as true males. Female reproductive products became more commonly observed within the follicles of larger conspecifics, with the concurrent presence of both male and female developing gametes within a single follicle confirming their functioning as simultaneous hermaphrodites (Figure 3.5). Several oysters from both size classes revealed signs of gametogenesis (i.e. GSI stage 1, early development) as early as March, indicating that gametogenesis within this population commences when sea temperature is ≤8°C. Evidence of spawning within the oyster population was observed in histological preparations from as early as mid-April, when seawater temperature approached 12°C. Peak spawning activity occurred during May, coinciding with the peak maximum GSI in both small and large oysters (Figure 3.6) and the appearance of brooding females during June-July (Figure 3.3g-i). In all three years of study, the numbers of brooding female oysters were very low (≤4.6% of all oysters ≥60 mm shell length throughout the whole year) and a clearly-defined, narrow period of brooding activity was also regularly observed (June-July) (see Figure 3.3g-i). No oysters were ever found to be brooding outside this period. The smallest brooding oyster measured 60.3mm shell length. Mean brood size was estimated to be 57,077±5,568 larvae per oyster (n = 6). In all but one brooding oyster, the larvae measured 290-330 µm shell length, with their light colour and presence of a ciliated velum characteristic of veliger larvae. The remaining oyster, collected during July 2010, contained larvae measuring 380-420 µm shell length. These larger larvae were generally darker in colour and had developed features characteristic of pediveliger larvae (see Chanley and Dinamani 1980). Release from the mantle cavity would thus have been imminent.

The decline in oyster condition index between May and July each year coincided with a significant reduction in GSI (pooled within size classes) (Scheirer-Ray-Hare 2-way ANOVA: $H_{8,253}$ = 154.7, p<0.001), as well as the start of both ripe gamete release (Figure 3.7a) and phagocytic digestion of residual gametes within the emptying follicles (Figures 3.7bi-bii). No significant difference was observed in GSI between the two size classes of oysters within months (Scheirer-Ray-Hare 2-way ANOVA: $H_{1,253}$ = 0.040, p = 0.840). However, no ripe ova were ever observed in small oysters throughout the study period. Furthermore, spawning in both small and large oysters appeared to occur prior to the full completion of development of the female gametes, suggesting that male gametes were released slightly earlier than female gametes within this population. GSI began to recover ~8-10 weeks following the peak spawning period in both size classes, although never to a level where a second spawning event would be possible. The breakdown and resorption of predominantly female gametes (i.e. GSI stage I, resorption of residual gametes) was occasionally observed in large oysters between August and November, always accompanied by numerous phagocytotic cells (Figure 3.7bi-bii). Whilst gamete resorption

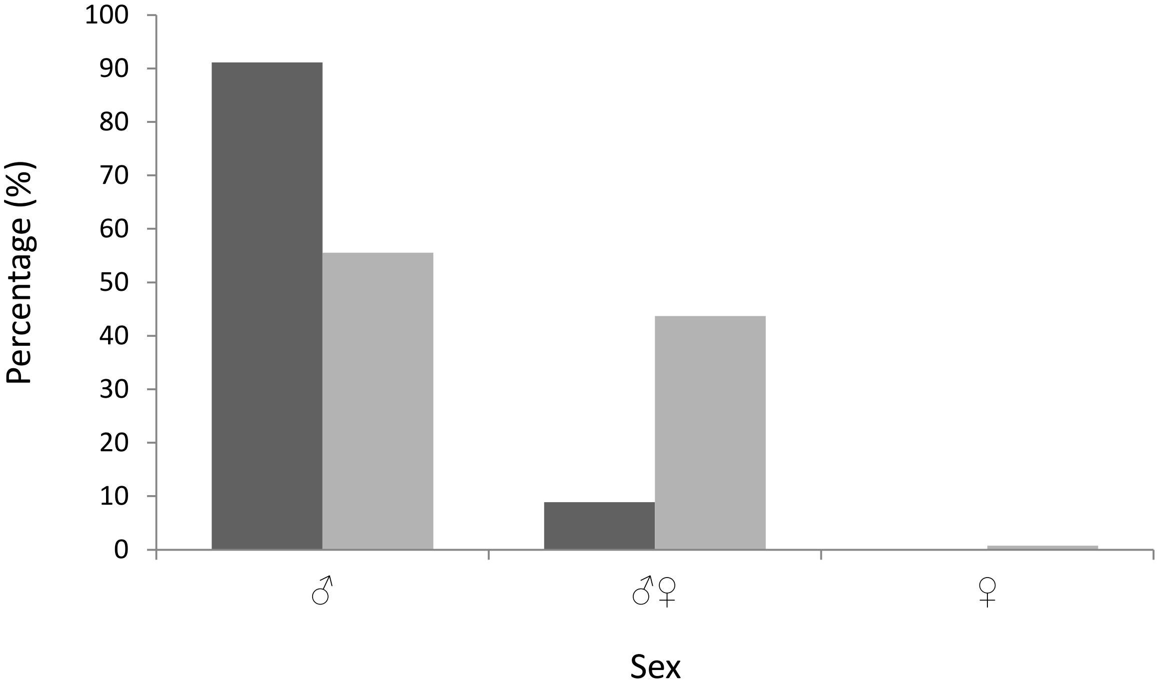


Figure 3.5 Relative percentages of Chilean oysters functioning as true males (♂), simultaneous hermaphrodites (♂♀) and true females (♀) within the Menai Strait and Conwy Bay SAC population. Bars: dark grey = small (40-50mm shell length), light grey = large (60-70mm shell length) oysters.

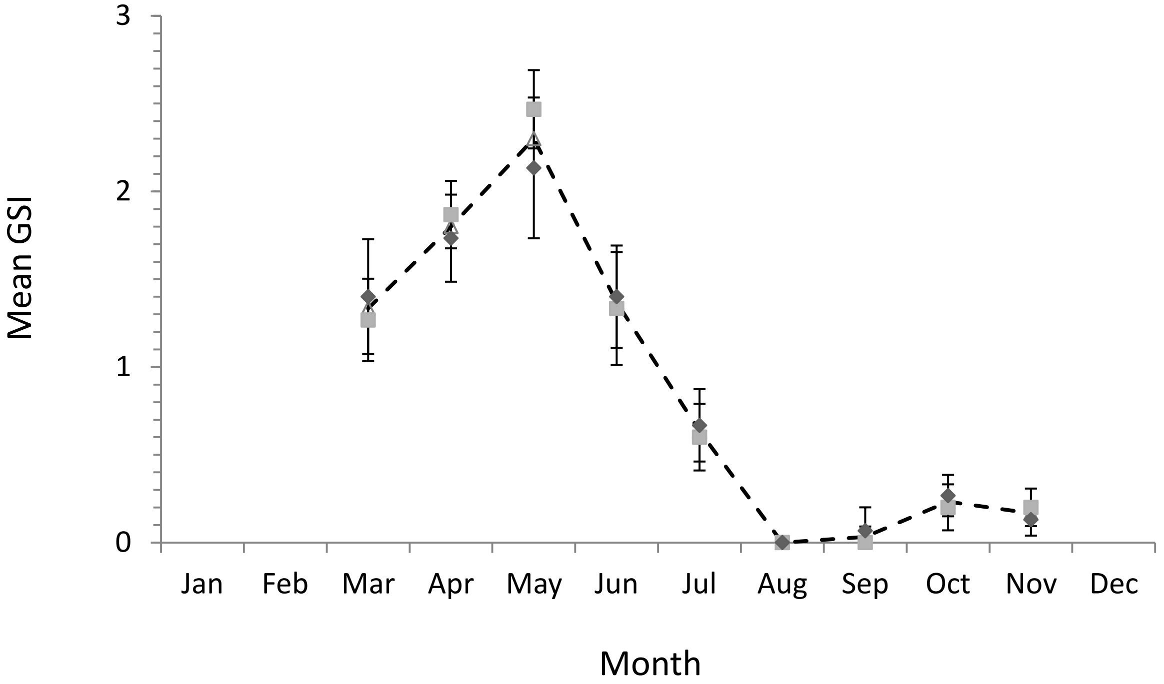


Figure 3.6 Seasonal change in mean (±SE) gonadosomatic index (GSI) of two distinct size classes of Chilean oyster (*Ostrea chilensis*) collected from the Menai Strait and Conwy Bay SAC population. Symbols: light grey squares = 'small' oysters (40-50mm shell length), dark grey diamonds = 'large' oysters (60-70mm shell length). See Table 3.1 for GSI details.

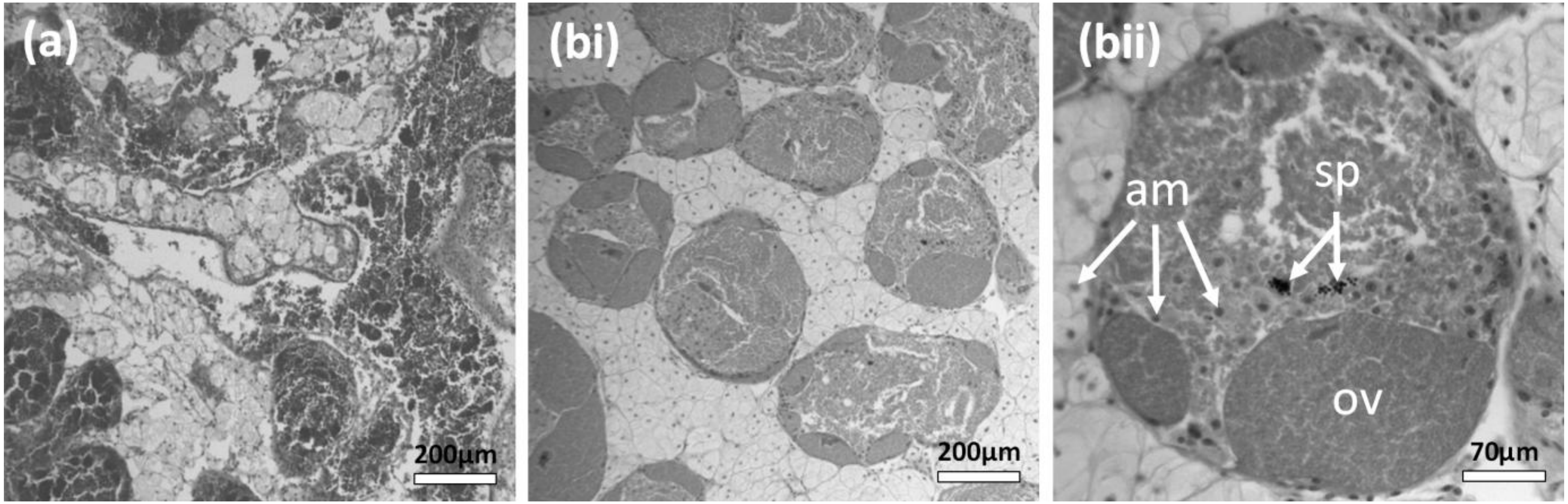


Figure 3.7 Photomicrographs (10x magnification) of histological sections of the reproductive tissue of Chilean oysters (*Ostrea chilensis*), showing (a) a male oyster releasing gametes through a cross-section of a tubule (GSI stage 2, spawning; June 2010), and (b) a large, hermaphrodite oyster showing degenerating gametes within the follicles at 10x (bi) and 40x (bii) magnification. ov = ovum, sp = spermatozoa. Note presence of numerous amoebocytes within the degenerating follicle.

was observed in both size classes during autumn (September-November), the occurrence of male and female gametes within the degenerating follicles differed between size classes, with large oysters containing varying amounts of residual sperm and large, ripe ova within the gonad/digestive gland complex; the latter was never observed in smaller conspecifics.

3.3.3 Patterns of spat settlement

Spat settlement was observed in all three years of study, with the period over which settlement occurred also relatively consistent between years. Spatfall was initially observed during June, peaking in July and progressively decreasing again between August and September. No larvae were settled between October and May (Figure 3.3g-i). However, whilst the general temporal pattern of spat settlement was relatively consistent between years, the magnitude of peak spatfall was extremely variable. Focusing on the four main sites of spat settlement only (namely Abermenai Point, Tal y Foel, Plas Trefarthen and Llanidan), mean peak settlement densities within sites were generally greater in 2009 (Year | Site: $F_{6,144} = 0.33$, $p<0.001$), although a degree of caution should be taken in interpreting the output of this ANOVA due to the lack of homogeneity of variances observed between treatments (see Table 3.3).

Incidentally, slightly warmer sea temperatures and the availability of nearly twice as much food during the spring phytoplankton bloom period were also observed during 2009 (Figure 3.3a-c). Interestingly, relative site-specific contributions to total mean annual settlement (Figure 3.8) were highly consistent each year (Scheirer-Ray-Hare 2-way ANOVA: $H_{6,11} = 0.64$, $p = 0.996$) and were positively correlated (Pearson correlation coefficient = 0.961, $p<0.001$) with local adult densities within each respective site (Figure 3.9). Furthermore, the magnitude of peak spat settlement was always greater at Plas Trefarthen (i.e. the site of highest mean adult oyster density; see Chapter 2) throughout the three years of study (see SNK in Table 3.3).

3.3.4 Larval dispersal

No larvae were observed to have settled >40 m away from the transferred adult oyster patch (Figure 3.10), hence observations of spatfall at 100 m away from the oyster patches were removed from any statistical analysis. A significant reduction (Distance: $F_{2,18} = 23.46$, $p = 0.041$) in mean spat settlement density was observed with increasing distance away from adult conspecifics (Table 3.4), with no significant differences observed among differing locations (Site: $F_{1,18} = 1.77$, $p = 0.200$).

Table 3.3 3-way mixed model ANOVA examining the spatial (both between and within sites) and temporal (both intra- and inter-annual) variability in the magnitude of peak spat settlement density in the Chilean oyster (*Ostrea chilensis*) within the Menai Strait and Conwy Bay SAC. ns = no significant difference.

Source of Variation	**df**	**MS**	**F**	**p**
Year	2	764861311.3	1.73	0.255
Site	3	317960908.1	287.49	<0.001
Array (Site)	12	4584338.9	1.27	0.240
Year x Site	6	441829209.2	134.03	<0.001
Year x Array (Site)	24	3296569.2	0.92	0.580
Residual	144	3596419.6		
Total	191			

Cochran's Test	C = 0.454, p <0.01
Transformation	None

SNK Test	Year x Site (SE = 453.9)	
	Year (Site):	
	Site 1	ns
	Site 2	2009>2010=2011
	Site 3	2009>2011>2010
	Site 4	2009>2010=2011
	Site (Year):	
	Year 1	Site 3>Site 4>Site 1=Site 2
	Year 2	Site 3>all others...
	Year 3	Site 3>all others...

Table 3.4 2-way ANOVA examining the difference in spat settlement density of the Chilean oyster (*Ostrea chilensis*) away from patches of adult oysters at two sites within the Menai Strait and Conwy Bay SAC.

Source of Variation	df	MS	F	p
Site	1	67.0	1.77	0.200
Distance	2	760.9	23.46	0.040
Site x Distance	2	32.4	0.86	0.441
Residual	18	37.8		
Total	23			
Cochran's Test		C = 0.475, p>0.05		
Transformation		Square Root		
		SNK Test	Distance (SE = 2.0)	
			Distance:	
			Across all sites	0 m>20 m=40 m

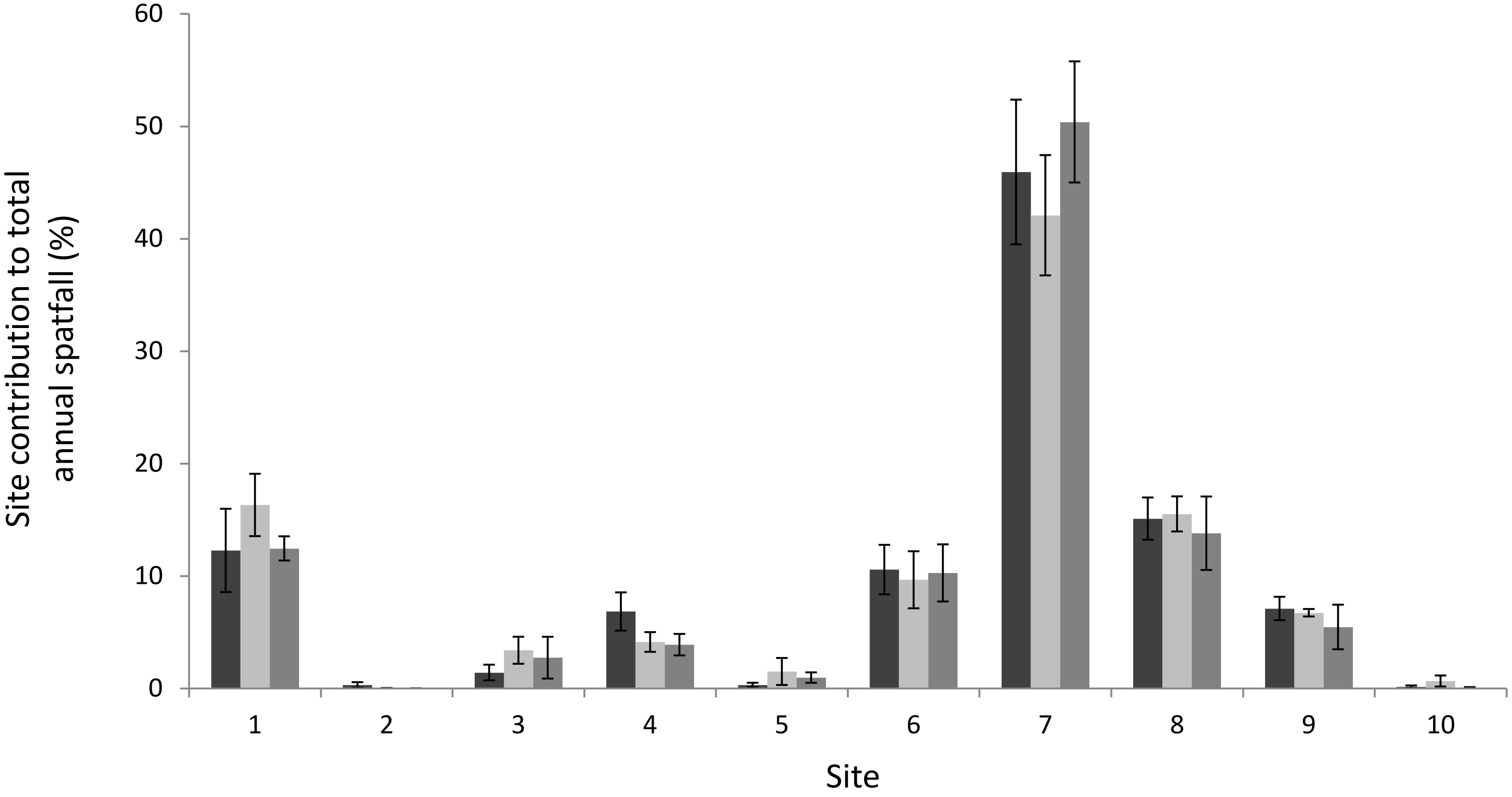


Figure 3.8 Inter-annual variability between mean (±SE) site contributions to the total annual settlement observed within the Menai Strait and Conwy Bay SAC during each respective year of study. For site codes, see Figure 3.1. Bars: black = 2009, light grey = 2010, dark grey = 2011.

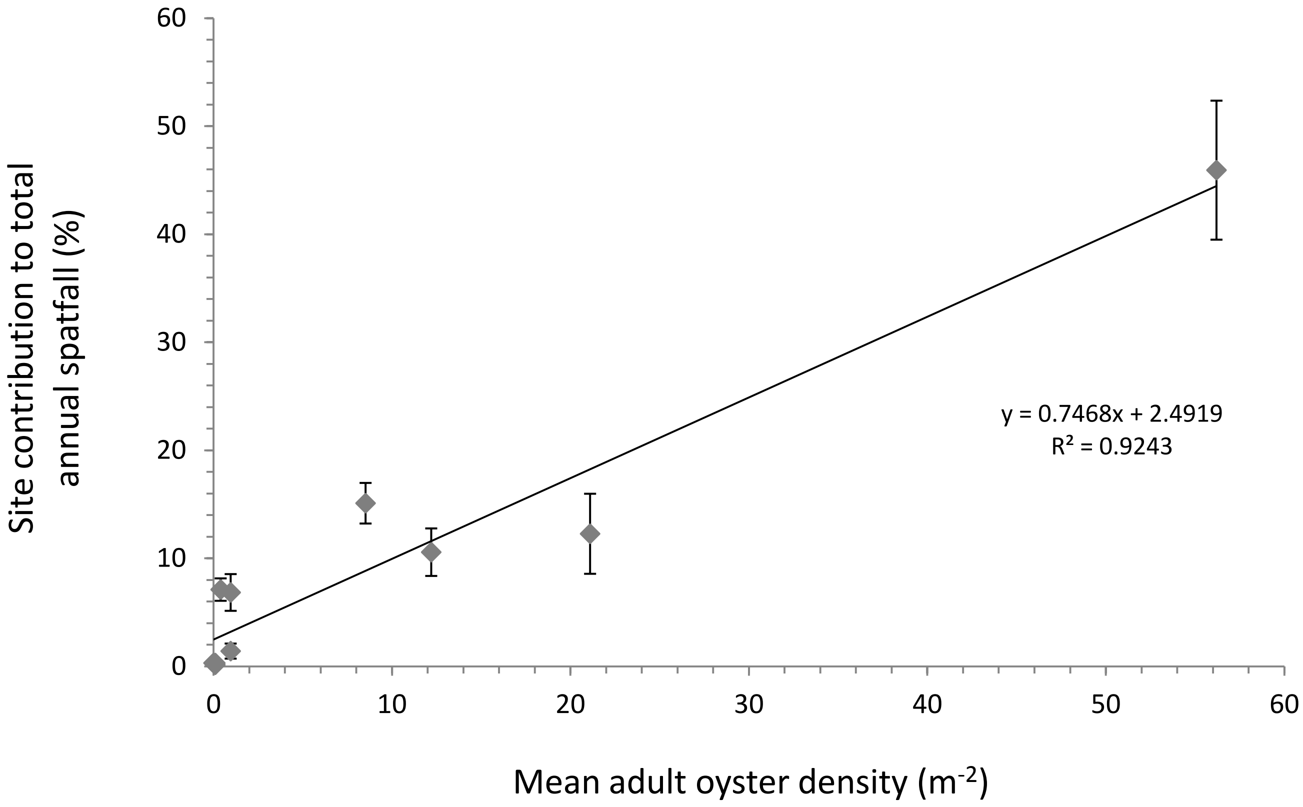


Figure 3.9 Relationship between mean (±SE) site contributions to total annual settlement observed and mean adult oyster density at each respective site within the Menai Strait and Conwy Bay SAC.

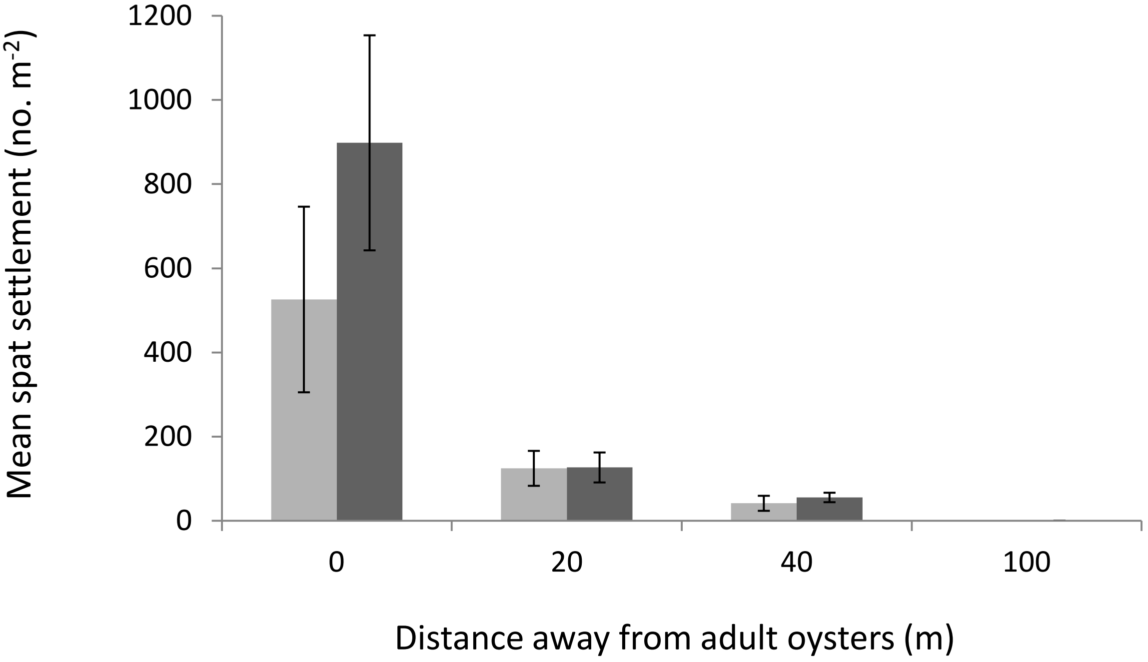


Figure 3.10 Change in mean (±SE) spat settlement with distance away from a transferred adult oyster patch (n = 100). Bars: light grey = Traeth Melynog, dark grey = Mermaid.

3.4 Discussion

This chapter presents the first-ever documentation of the reproductive dynamics of *O. chilensis* outside its native geographic range. Reproductive activity within the UK Chilean oyster population is highly restricted, with a narrow period of spat settlement (3-4 months) and equally low numbers of brooding females observed annually. Nonetheless, significant spatfall (maximum mean monthly spat settlement = 2,570 m^{-2}) with a strong stock-recruitment component is evident each year.

Seawater temperature has traditionally been regarded as the principal environmental parameter in determining both the onset and rate of gametogenesis of several marine invertebrates (Orton 1920; Coe 1931; Giese 1959), leading to the proposition of distinct differences in the reproductive dynamics of congeneric populations at different latitudes (Thorson 1950). In the northern hemisphere, short breeding periods restricted to the summer months and low numbers of large long-lived individuals are thus often indicative of populations at or close to their northernmost geographic extent. Equally, the duration of the breeding season is expected to increase at lower latitudes, occasionally resulting in continual recruitment throughout the year, with peak spawning activity occurring much earlier in the year than at higher latitudes (Lewis 1986). Independent studies of *O. chilensis* populations across its entire latitudinal extent reveal clear differences in the duration of the breeding season and are generally supportive of this hypothesis (Table 3.5). The UK Chilean oyster population (53°N) is most akin to the southernmost populations found in New Zealand (46°S) in terms of its reproductive dynamics, exhibiting a clear, unimodal periodicity in brooding activity, with evidence of spatfall restricted to the warmer summer months (Cranfield and Allen 1977; Westerskov 1980; Jeffs and Hickman 2000). Conversely, oysters inhabiting lower latitudes within their native range (36°S) are capable of brooding all year-round, with peak larval settlement correlating with periods of lower seawater temperatures (Jeffs et al. 1996, 1997). Both size and number of brooding females within the UK population is also analogous to those observed within several high latitude oyster populations in New Zealand (Hollis 1962; Cranfield and Allen 1977), although a lack of consistency across all localities at similar latitudes (see Table 3.5) is likely to be a product of the large degree of variability in growth between different populations, as well as between individuals within a single population (Toro et al. 1995).

It is generally accepted that climatic regimes influence the geographic distribution of species, partly through specific physiological temperature thresholds which determine their breeding potential and survival (Somero 2011). The establishment and invasion of the non-native Pacific oyster, *Crassostrea gigas* (Thunberg 1793), has been associated with increasing summer temperatures in regions of both the UK (Spencer *et al*. 1994) and Wadden Sea

(Diederich *et al*. 2005). Likewise, *s*everal studies have also reported a critical thermal limit for the initiation of the release of gametes in *O. chilensis*, although purported temperatures again vary considerably between localities (Solis 1967; Jeffs et al. 1996). In the UK, gamete release was observed as early as April-May, when seawater temperature approached 12°C. This is consistent with the observations from several studies from New Zealand (e.g. Jeffs et al. 1996; Brown et al. 2010), although much lower than that reported from Chilean laboratory culture trials (Chaparro 1990). Historical records show that the mean annual seawater temperature in the Menai Strait, estimated from mean monthly air temperature at RAF Valley meteorological station (Anglesey, North Wales, UK) (see supplementary material for details), has significantly increased since the introduction of *O. chilensis* during the early 1960s (Figure 3.11). During the first 30 years following the introduction of *O. chilensis* into the Menai Strait, only 38.7% of the mean annual sea temperatures were greater than the average mean annual temperature between 1962 and 2011 (i.e. 10.95°C), coinciding with observations from 1992 of a highly-restricted population distribution (0.4 km) with relatively low oyster densities (see Richardson et al. 1993). Conversely, 78.9% of the annual mean sea temperatures were >10.95°C between 1993 and 2011. The Chilean oyster is now found along >30 km of the Menai Strait coastline, with densities of up to 232 oysters m^{-2} (Morgan and Richardson 2012a), suggesting that temperature has played a crucial role in the spread of this NNS within the Menai Strait and Conwy Bay SAC. Whilst anthropogenically-mediated increases in greenhouse gas concentrations have led to an increase of 0.74±0.18°C in the global mean surface temperature since the beginning of the 20th century (IPCC 2007), a further increase of 1.5-3.0°C has been predicted towards the end of 2100 (IPCC 2012). Given that *O. chilensis* proliferates in generally warmer climes within its native range, anthropogenically-mediated warming of the oceans is likely to facilitate the future spread of this non-native oyster species by extending its breeding season. Such phenological changes have already been demonstrated for other phyla (Edwards and Richardson 2004), often favouring NNS at the expense of many native congeneric species (see Hellmann et al. 2008).

Despite its narrow breeding season, *O. chilensis* spatfall intensity was relatively strong during all years of study and was particularly pronounced following a period when food concentration during early gametogenesis was exceptionally high. Although the rate of gametogenesis within several bivalve populations has been linked with a specific range of seawater temperatures, the magnitude of gamete production is ultimately dependent on the availability of nutrients, obtained either through external food supply or from stored nutrient reserves (Seed 1976; Newell et al. 1982). Several trials involving the laboratory culture of *O. chilensis* have also highlighted the importance of a high food ration during the broodstock conditioning period in order to maximise offspring yields (e.g. Chaparro 1990; Wilson et al.

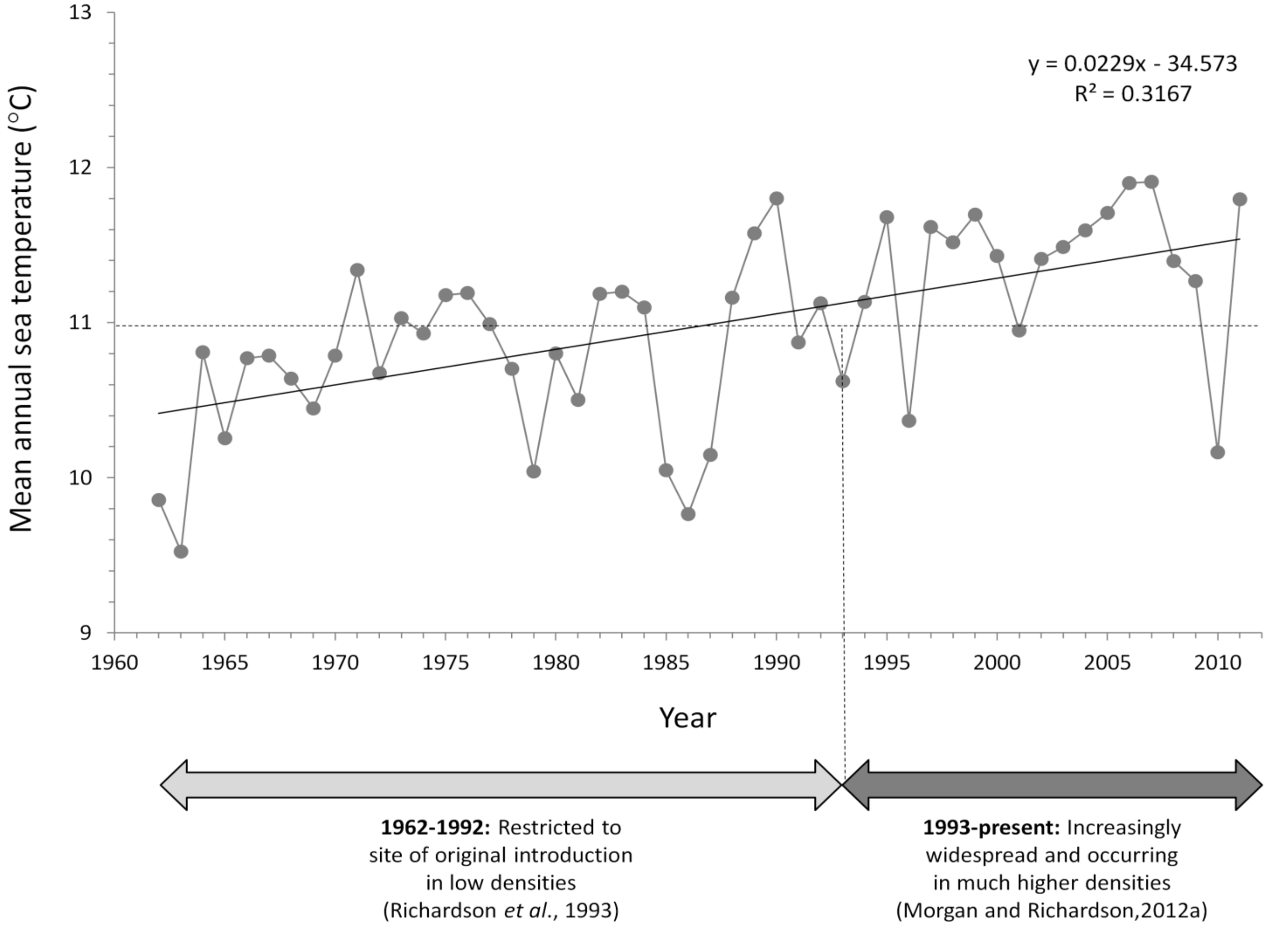


Figure 3.11 Mean annual sea temperatures (°C) in the Menai Strait during the last 50 years, estimated from air temperature observations from Valley metrological station (Anglesey, North Wales, UK) and known to be in direct correlation with sea temperatures in the south-eastern end of the Menai Strait (see Appendix V for more details). The periodicity of the temperature data is relative to the introduction of the Chilean oyster (*Ostrea chilensis*) into the area in 1962. Dotted horizontal line is equivalent to the average mean annual temperature between 1962 and 2011.

Table 3.5: Latitudinal variation in the reproductive dynamics of the Chilean oyster, *Ostrea chilensis*, both within and outside its native geographic range.

Geographic Location	**Latitude**	**Sea temperature range**	**Spat settlement period (no. of months)**	**Size of Smallest Brooder**	**Annual percentage of brooding (≥60 mm) oysters**	**Source**
Manukau Harbour New Zealand	36° 29′ S	11.0-24.0°C	All year round (12 months)	49mm	17%	Jeffs *et al*. (1996) Jeffs *et al*. (1997)
Hauraki Gulf New Zealand	36° 58′ S	13.0-23.0°C	All year round (12 months)	37mm	16%	Jeffs *et al*. (1996) Jeffs *et al*. (1997)
Tasman Bay New Zealand	41° 00′ S	11.0-18.0°C	Spring-Winter (7 months)	61mm	22.6%	Brown *et al*. (2010)
Quempillén, Chiloé Chile	41° 52′ S	9.0-19.0°C	n/a	37mm	n/a	Toro *et al*. (1995)
Otago Harbour New Zealand	45° 50′ S	7.0-17.0°C	Spring-Summer (5 months)	42mm	19.5-21.0%	Westerskov (1980)
Foveaux Strait New Zealand	46° 40′ S	8.5-16.0°C	Spring-Summer (5 months)	60mm	7-10%	Jeffs and Hickman (2000)
Menai Strait, Wales United Kingdom	53° 10′ N	4.5-18.5°C	Spring-Summer (2 months)	61mm	≤4.6%	This Chapter

1996). Chlorophyll-a concentrations between sites were relatively constant throughout the duration of this study. However, chlorophyll-a concentration towards the north-eastern end of the SAC (where currently *O. chilensis* are extremely rare) can be twice as high as those observed herein (see Simpson et al. 2007). Given a lack of regulation regarding accidental and deliberate transfers within the SAC (see Morgan and Richardson 2012a, 2012b), this is of critical importance to the future of this NNS.

Histological evidence of a rapid increase in gamete development following the spring phytoplankton bloom within the UK Chilean oyster population supports the theory that a strong reserve of nutrients is plays an important role in regulating the rate of gametogenic development in many nutrient-storing marine invertebrates (see Gabbott 1976, 1983). Several bivalve species are known to be reliant on stores of energy reserves (principally glycogen) when food supply is low. Size- and age-related differences in dry weight-related condition indices (often directly correlated with glycogen content; see Gabbott and Stephenson 1974) have previously been observed in *Ostrea edulis* (Walne 1970), and have been attributed to a higher metabolic demand in smaller, younger individuals (Holland and Hannant 1976). Due to the high spring peak plankton bloom and the relatively increased incidence of smaller peaks (including a peak of ~6 µg L^{-1} immediately following the spawning period), small oysters may have been able to recover at the same rate as larger conspecifics during 2009. Conversely, lower nutrient availability during both 2010 and 2011, particularly following the spawning period, may have hampered the recovery of small oysters due to their relatively higher metabolic demands. The post-spawning recovery in both small and large oysters coincides with histological observations of post-spawning gamete resorption. Interestingly, gamete resorption can occur in unfavourable environmental conditions, including periods when food reserves are low (Lubet et al. 1987). This leads to the recycling of gametes and the repartitioning of energy to satisfy other metabolic demands. Resorption of extremely large ova, present only in large *O. chilensis* in the Menai Strait population, is likely to aid in the post-spawning recovery of this oyster species under long periods of malnutrition.

Due to its extended brooding period and highly reduced planktonic larval stage, *O. chilensis* is unlikely to spread considerable distances away from adult conspecifics within the Menai Strait by natural dispersal alone. Supporting evidence of a strong stock-recruitment relationship and an extremely limited dispersal distance is demonstrated in this chapter. Gregarious settlement, common in several other oyster species (e.g. Bayne 1969; Tamburri et al. 1992, 2008), may further assist in promoting a strong stock-recruitment relationship. The previously documented recent spread of this NNS across >30 km of shoreline during the last 20 years is paradoxical with these findings, suggesting that other vectors of dispersal are in operation (see Morgan and Richardson 2012a, 2012b). Management experience relating to another NNS with a highly-reduced natural dispersal capacity, namely the invasive ascidian, *Didemnum vexillum* (Kott 2002), has shown that the identification and regulation of all transport vectors (thus inhibiting propagule pressure) is critical to the success of eradication efforts (see Holt and Cordingley 2011). Other vectors of dispersal have been proposed to explain the recent spread of this species outside its native range, including rafting (O'Foighil et al. 1999), bivalve culture (Morgan and Richardson 2012a) and periwinkle harvesting (Morgan and Richardson 2012b), although such events are often sporadic and difficult to quantify. Jeffs (1998) has suggested that the simultaneous development and release of spermatozoa and ova within mature hermaphrodites means that self-fertilisation is a strong possibility within this oyster species. However, evidence presented herein indicates that the timing of gametogenesis within large, hermaphroditic oysters may be slightly offset, with spermatozoa being released prior to the attainment of fully ripe ova within the same follicles. This, together with evidence from Chaparro (1990) indicating the requirement of a higher water temperature (>14°C) to initiate the release of female gametes in *O. chilensis*, would predicate against self-fertilisation in the species.

Conclusions

Seawater temperature is shown to be the primary determinant of the initiation of reproductive development within the UK's non-native Chilean oyster population, whilst food availability during the early period of gametogenesis is likely to determine the numbers of gametes produced. Whilst ocean warming as a result of global climate change is likely to extend the duration of the brooding season of this species, it remains to be seen whether or not future plankton dynamics will match or mismatch with the nutritional requirements of the broodstock (see Cushing 1990) and have positive or negative effects on the proliferation of this species within the designated SAC and beyond. The highly restricted natural larval dispersal of this species may allow relatively more time for intervention in the invasion process. However, the potential for self-fertilisation (albeit minimal) and the ever-increasing frequency of anthropogenically-mediated transfers of this species indicate that actions to mitigate the spread of this non-native oyster should not be disregarded. The observed contrast between the restricted breeding cycle and relatively high densities of both adult oysters and spat settlement suggests that the early post-settlement survival of this species may be relatively low. Scientific endeavour to aid in the management of this increasingly dominant non-native oyster population should thus be focused on two aspects: a) the early post-settlement mortality of Chilean oyster spat, with particular focus on intra- and inter-specific competition and predation, and b) the identification and regulation of all transport vectors (thus inhibiting propagule pressure).

Appendix III: Early post-settlement mortality and the role of predation

Preliminary data conducted during the course of this study period suggest that predation is unlikely to play a key role in the early post-settlement mortality of *O. chilensis*. Following natural settlement on slate panels, oyster spat (~4-day old) were transferred to one of three experimental sub-tidal cage set-ups (see Figure VIII) to test whether or not early post-settlement mortality differed when predators were excluded. Oyster spat survival was monitored from digital images (see Figure IX) of each plate at the following intervals: 1, 2, 3, 4, 7, 9, 11, 15 and 24 days.

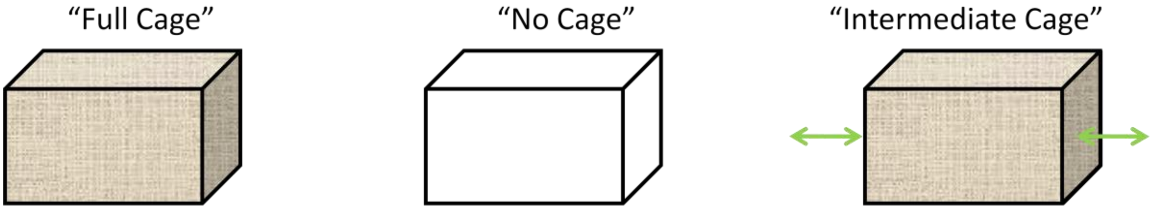


Figure VIII Illustration of three cage designs used to test the role of predation in shaping the distribution of *O. chilensis* in the Menai Strait (North Wales, UK). 'Full Cage': panels fully enclosed in a 500 μm mesh and held in shape by a PVC tubing framework, positioned inside the mesh. 'No Cage': PVC tubing framework only. 'Intermediate Cage': a form of procedural control, where panels were partly enclosed with 500 μm apart from two open ends which gave predators access to the panels. By positioning these open ends perpendicular to the main channel flow, the treatment would also account for any reduction in flow over the panels due to the presence of the mesh, mimicking the 'Full Cage' treatment.

Whilst yet to be statistically analysed, no obvious difference (relative to the observed variability) can be noted in mortality between any of the cage treatments (see Figure X), suggesting that predation does not play a key role in the structuring of the non-native *O. chilensis* in the Menai Strait. It is therefore possible that *O. chilensis* is 'released' from predation pressure in the Menai Strait due to the absence of natural predators (*sensu* "Enemy Release Hypothesis"). Increased intra-specific competition may account for some of the observed mortality, which formed a plateau at ~75% within all treatments. However, density was not considered a factor within the current design.

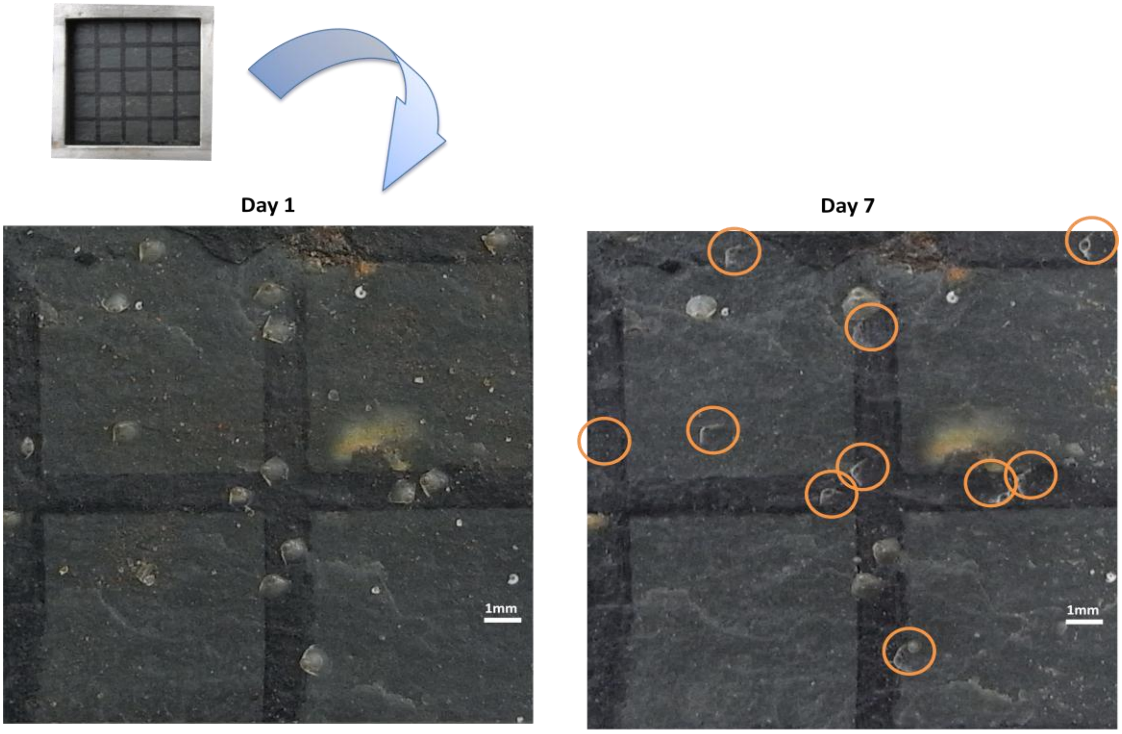


Figure IX Early post-settlement mortality of newly-settled *O. chilensis* (5 days old at 'Day 1') following a period of 7 days in the Menai Strait.

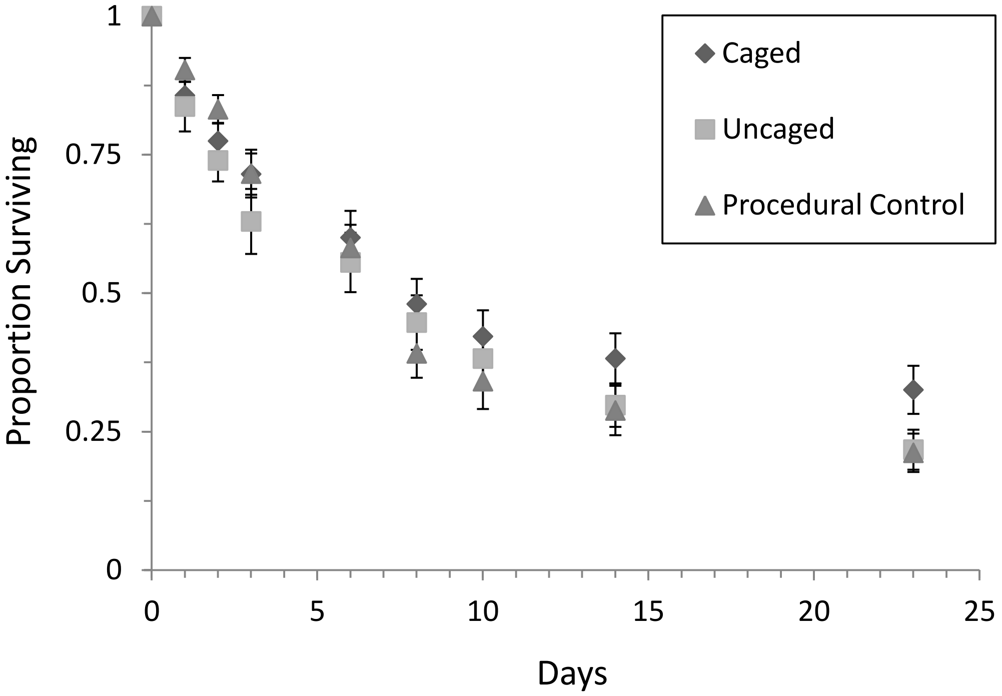
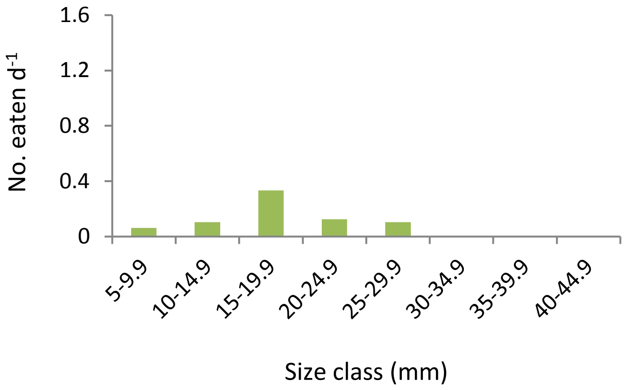


Figure X Survival rate of *O. chilensis* spat in the presence or absence of predators. Error bars indicate ±1SE.

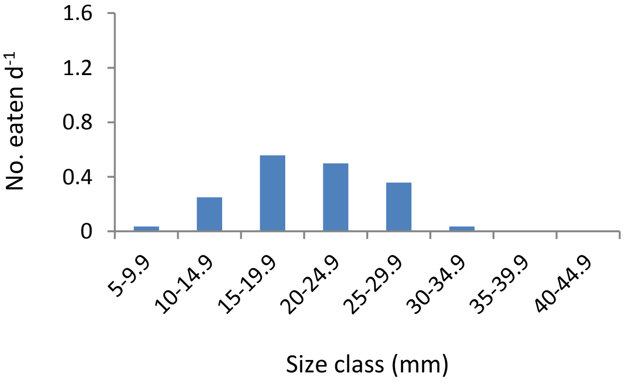
Appendix IV Shore crab (*Carcinus maenas*) predation on the Chilean oyster (*Ostrea chilensis*)

Preliminary data show that, in the absence of any other prey species, *C. maenas* can consume *O. chilensis* over a broad size range with mean size consumed increasing with crab size (Figure XI).

Small Crabs (35-45 mm carapace width):



Medium Crabs (50-60 mm carapace width):



Large Crabs (>70 mm carapace width):

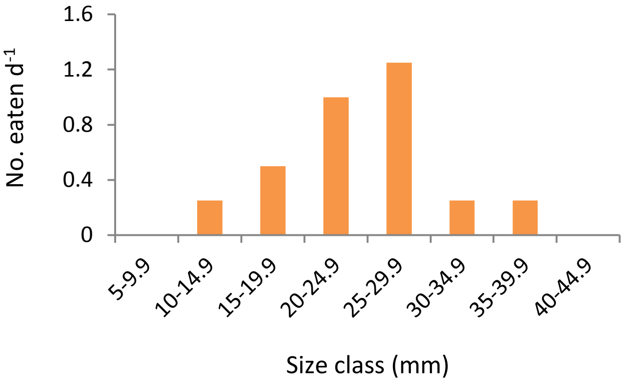


Figure XI: Size class (mm) preference (expressed as mean number eaten per day) of the shore crab, *Carcinus maenas*, feeding on Chilean oysters (*Ostrea chilensis*) when presented equal numbers of each respective size class.

A vast range of opening techniques were used to gain access to the oyster flesh, with energetically-unfavourable ‘chelal boring’ technique (see Elner and Hughes 1978) predominantly used for all but the smallest oysters (see Figure XII). However, when crabs were presented with either oysters or mussels of a preferred size class, the number of oysters consumed daily declined rapidly whilst the number of mussels consumed daily remained relatively stable (see Figure XIII).

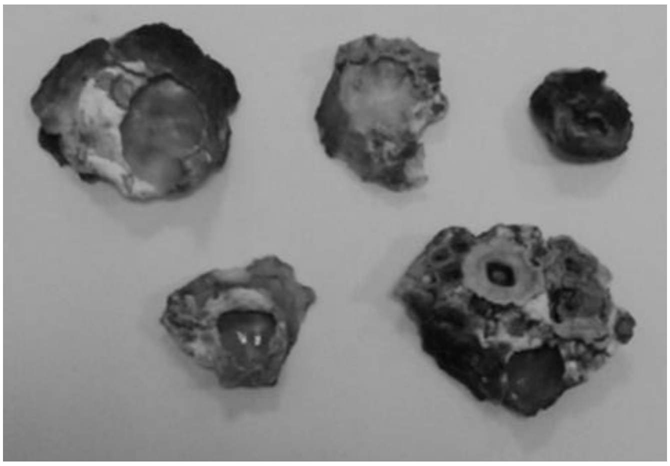


Figure XII Numerous dead *O. chilensis* showing shell damage following a 'chelal boring' attack by *C. maenas*. Note central hole in all specimens, where the continuous twisting action of the chela has eventually resulted in access to the oyster flesh.

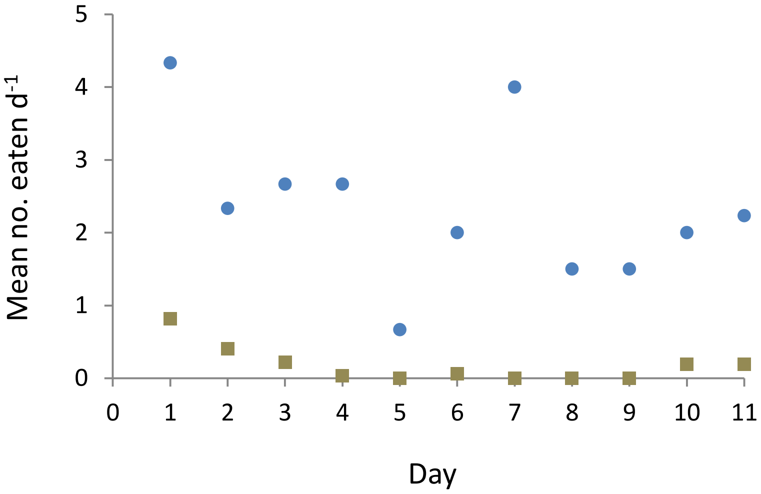


Figure XII Temporal variability in mean number of *O. chilensis* (squares) and *M. edulis* (circles) of a known preferred size range consumed daily when presented to isolated *C. maenas* (n = 6 each). Prey availability was kept constant by replacing eaten individuals immediately following consumption by a similar-sized conspecific.

These data suggest that the Chilean oyster gains a refuge against predation from even the largest shore crabs after 35 mm shell length (corresponding with approximately 2 years of growth in the Menai Strait; see Chapter 2). Furthermore, although smaller oysters can be eaten by shore crabs, it appears that they are also rejected based on a number of possible factors:

1. Mechanical difficulty in handling the oyster shell.
2. Learnt or otherwise acquired knowledge regarding the energetically-unfavourable nature of oysters in relation to the ease of access to the flesh.
3. Preference towards more accessible prey items, such as mussels.

Appendix V Estimation of historic sea surface temperatures from air temperatures recorded at RAF Valley meteorological station (North Wales, UK).

Walne (1958) identifies a near isometric linear relationship between mean monthly air temperatures recorded at RAF Valley (North Wales, UK) and mean monthly sea surface temperatures at Tal y Foel. This relationship would theoretically allow for a simple conversion of meteorological data in order to predict sea water temperature within the south-western end of the Menai Strait. However, it cannot be assumed that this relationship, observed over a period of only one year, holds true today or has indeed held true ever since the introduction of *O. chilensis* into the Menai Strait. Furthermore, thermal recording equipment has changed dramatically over the last 50 years, often highlighting the need for data calibration between long-term records.

In order to get a better estimate of the relationship between mean monthly air and sea surface temperatures within this region, sea temperature data were sourced from the scientific literature. A keyword search within 'Google Scholar' including the terms "mean monthly" AND "temperature" AND "Menai Strait" was used to identify potential sources of information. The raw data for each study were verified, extracted and correlated with mean monthly air temperatures from historic RAF Valley for each relevant month and year. Due to the relative consistency of the relationship across all data sets, the data were then pooled together to give an approximation of the relationship between local air and seawater temperatures over the last 50 years (see Figure XIV).

There was a highly significant correlation between pooled mean monthly air and seawater temperatures at RAF Valley and Tal y Foel respectively. Between 4 and 18°C, air temperature at Valley changes 0.93°C with every degree change of seawater temperature ($F_{1,114} = 1814.9$, $p<0.001$). Thus, historic mean monthly air temperatures recorded at RAF Valley were converted to estimated seawater temperatures of the Menai Strait during the last fifty years and then used to estimate the change in seawater temperature since the introduction of *O. chilensis* in 1962 (seen in Figure 3.11).

Historic seawater temperature data were extracted from Fry (1975), Utting (1988), Spencer (1990), Spencer (2002) and Evans *et al.* (2003), as well as Chapter 3.

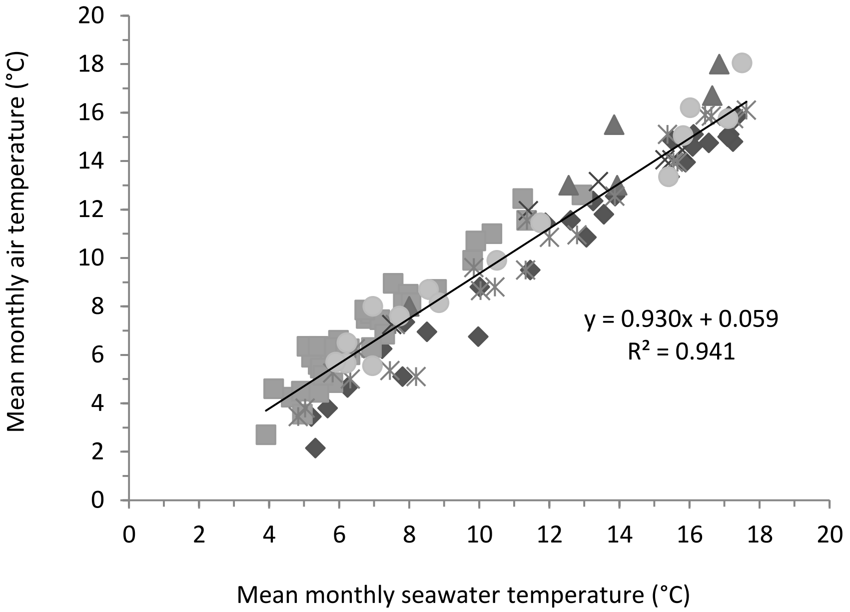
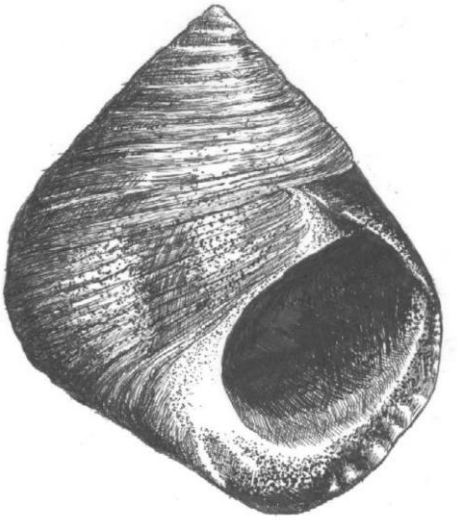


Figure XIV Relationship between mean monthly seawater and air temperature within the Menai Strait and RAF Valley respectively. Icons depict different sources of data (see text above for references).

Chapter 4

The potential role of an unregulated coastal anthropogenic activity in facilitating the spread of a non-native biofoulant



4.1 Abstract

Despite an exponential rise in anthropogenically-mediated transfers of non-native species during the last 150 years, several coastal anthropogenic activities remain unregulated under current legislation frameworks. This study investigates the potential role of commercial periwinkle (*Littorina littorea*) harvesting as an unregulated facilitator of both small- and large-scale geographic range expansion of an invasive oyster epibiont (*Ostrea chilensis*) within the Menai Strait (North Wales, UK) and beyond. The frequency of oyster-fouled periwinkles was greatest in areas of high adult oyster abundance and restricted to large, market-sized periwinkles (>20 mm shell height) inhabiting the low shore. Active efforts by commercial collectors to reject oyster-fouled periwinkles were found to be inadequate, with oysters of all sizes observed within collected hauls. Whilst the survival of fouled and unfouled periwinkles was comparable under post-collection refrigerated conditions, a significant decrease in both mobility and flesh content was associated with the presence of oyster epibionts. Survival of all but the smallest oyster epibionts under post-collection refrigerated conditions enhances the possibility of accidental non-native oyster transfers. Better interventions during both initial visual inspection and post-griddling stages are recommended, as well as the development of techniques that kill off all non-native epibionts, whilst leaving the freshness and marketability of the periwinkles uncompromised.

The following chapter has been published in the journal 'Biofouling' (2011 5-year impact factor = 4.488) and is thus subject to copyright by the publisher Taylor and Francis Ltd. Please consult the original journal article and cite as follows:

Morgan EH and Richardson CA. 2012. The potential role of an unregulated coastal anthropogenic activity in facilitating the spread of a non-native biofoulant. *Biofouling.* **28:** 743-753.

4.2 Introduction

Anthropogenically-mediated introductions of species into areas beyond their native geographic range have become progressively more frequent during the last 150 years (Hulme 2009). The successful proliferation of some of these 'non-native species' (hereafter 'NNS') has led to ecosystem-level changes within their new environment, often with major economic ramifications (Vitousek *et al.* 1997). The significance of such 'biological invasions' will ultimately be determined by the rate of secondary dispersal following successful establishment of a NNS population (Johnson *et al.* 2001). In its simplest form, the secondary spread of a NNS can be viewed as a single, unidirectional movement of propagules from the site of original introduction along an invasion 'front', with all suitable habitats behind the front being occupied by the invader (see Wilson *et al.* 2009). Based on this premise, the rate of spread would be expected to be generally greater in those NNS that exhibit high natural dispersal capacities, particularly in taxonomic groups where all subsequent phases of the life cycle are generally sessile or slow-moving (Mileikovsky 1971; Crisp 1978; Scheltema 1978; Strathmann 1985; Shanks *et al.* 2003). However, biological invasions are seldom this simple, and the geographic range expansion of even aplanic and anchiplanic NNS can be significantly augmented by other vectors, predominantly derived from anthropogenically-mediated activities such as ballast water transfers (Carlton 1985), hull fouling (Gollasch 2002) and accidental transfers associated with deliberate collection and movements of commercially-targeted species (Minchin 1996). Whilst the clarification and quantification of all potential mechanisms of dispersal is thus a major goal for those aiming to mitigate or prevent future biological invasions, several coastal anthropogenically-mediated mechanisms of dispersal and their potential to facilitate the spread of NNS remain completely overlooked.

The Chilean oyster, *Ostrea chilensis* Philippi 1845, is one example of a NNS that has recently managed to spread over relatively considerable distances despite its minimal natural dispersal capacity (Chapter 3). Native to both Chile and New Zealand, *O. chilensis* was deliberately introduced into the Menai Strait (North Wales, UK) at Tal y Foel (Figure 4.1) during the early 1960s as part of native oyster stock regeneration trials (see Walne 1974). Unlike other oyster species within the same genus, the progeny of *O. chilensis* are brooded throughout the entire larval development period within the female mantle cavity and are liberated as pediveligers that settle within minutes of their release (Millar and Hollis 1963; Cranfield 1968; Westerskov 1980), meaning that their natural dispersal capacity is highly reduced (see Broekhuizen *et al.* 2011). Although remaining relatively contained within its site of original introduction for over 30 years (see Richardson *et al.* 1993b), the Chilean oyster is now found

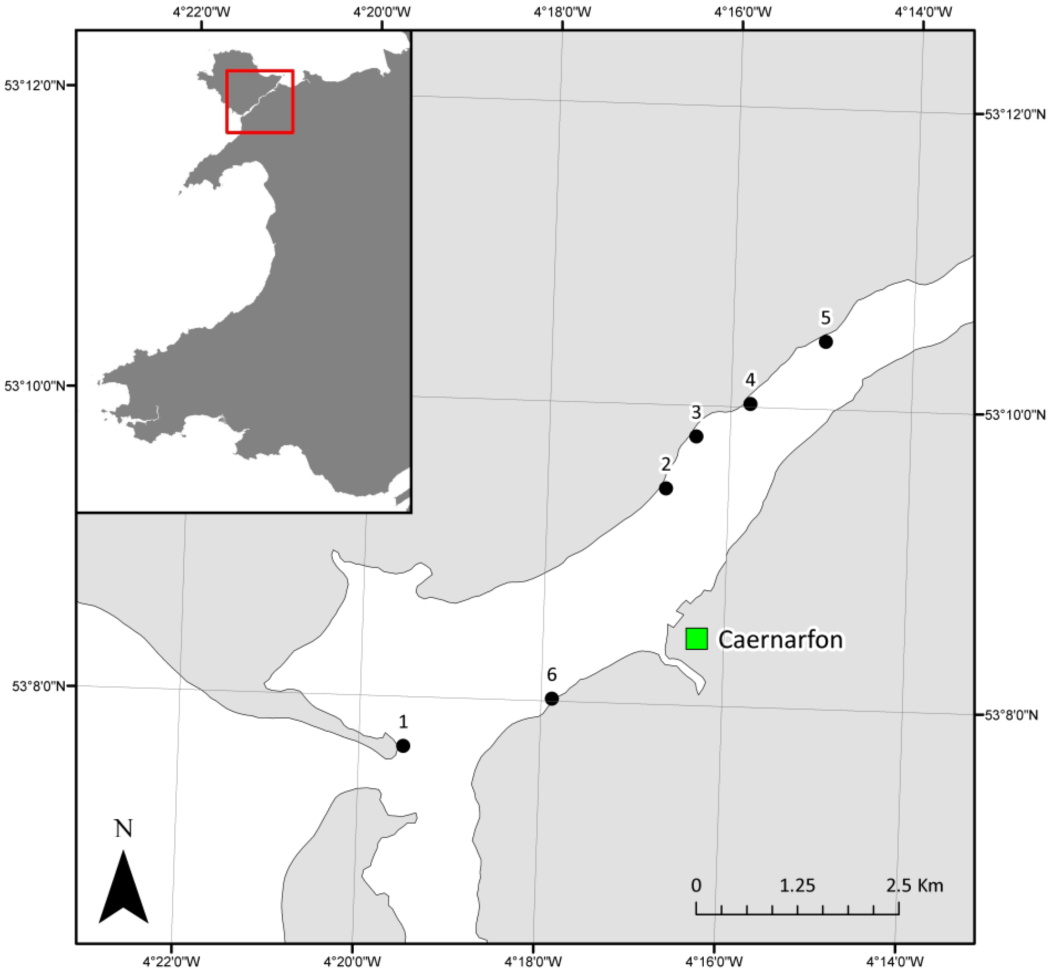


Figure 4.1 Map of the south-western end of the Menai Strait (North Wales, UK), showing site locations in relation to the town of Caernarfon (square symbol). Site codes: 1. Abermenai, 2. Mermaid, 3. Tal y Foel, 4. Plas Trefarthen, 5. Llanidan, 6. Caernarfon. Inset map shows location of the Menai Strait in relation to Wales.

along more than 30 km of the low foreshore of the Menai Strait, with several localised areas of exceptionally high densities of up to 232 oysters m^{-2} (Chapter 2).

The increase in hard surface area and structural complexity associated with increasing densities of *O. chilensis* has been shown to significantly alter species richness within the oyster bed (see Appendix I). However, the mechanisms that have facilitated the recent spread of this species away from its site of original introduction are yet to be identified. Following several recent observations of oyster-fouled common periwinkles, *Littorina littorea* L. 1758 (known locally as 'capped winkles'; Figure 4.2), the current study was designed to investigate the potential of the periwinkle industry as a transport vector which may facilitate the spread of *O. chilensis* both locally and across international boundaries.

Periwinkles generally predominate in the mid to low intertidal, and as a result, are gathered by hand during low spring tides. Current annual harvesting values for the UK are lacking, although a total of 1,027 T was declared in Ireland alone during 2009 (FAO FishStat, Version 1.0.1). This equates to a retail value in excess of £2.5 million, though capture figures are likely to be somewhat higher due to undeclared landings that stem from the 'underground' nature of the industry (Cummins *et al.* 2002). Whilst stocks are mainly targeted during the winter months (when consumption in south-western Europe is at its highest), periwinkle collection occurs all year round and often supplements the income of bait collectors and fishermen whose target species become unavailable during closed seasons and periods of unsuitable tides or weather conditions. Periwinkles from the UK are also regularly exported live to France, where they are used by oyster farmers as a method of mitigating algal fouling of cultured Pacific oysters, *Crassostrea gigas* (Thunberg 1793), and the mesh bags in which they are contained (see Enright *et al.* 1983; Cigarria *et al.* 1998; Carver *et al.* 2003).

Periwinkle collection is currently subject to minimal regulation throughout the world and is highly region-specific. Stock management is thus largely determined by the actions of the periwinkle collectors and wholesalers themselves. In most regions within the UK and Ireland, all harvested periwinkles are sold to a central wholesaler. Post-harvesting processing is often minimal, with little more than a quick visual inspection and weighing at the wholesale facility to ensure adequate consistency of commercially-sized periwinkles prior to international export (Cummins *et al.* 2002; pers. obs.). Some wholesalers may subsequently separate hauls into marketable and unmarketable size fractions using a griddling device. This process allows for the sorting of periwinkles into 'small', 'medium' and 'large' size classes (usually approximately <14mm, 14-20mm and >20mm respectively, although the exact definition of each size fraction varies with region). Periwinkles may then be re-bagged and kept in refrigerated conditions until sold (Cummins *et al.* 2002). The griddling process may also be used to facilitate

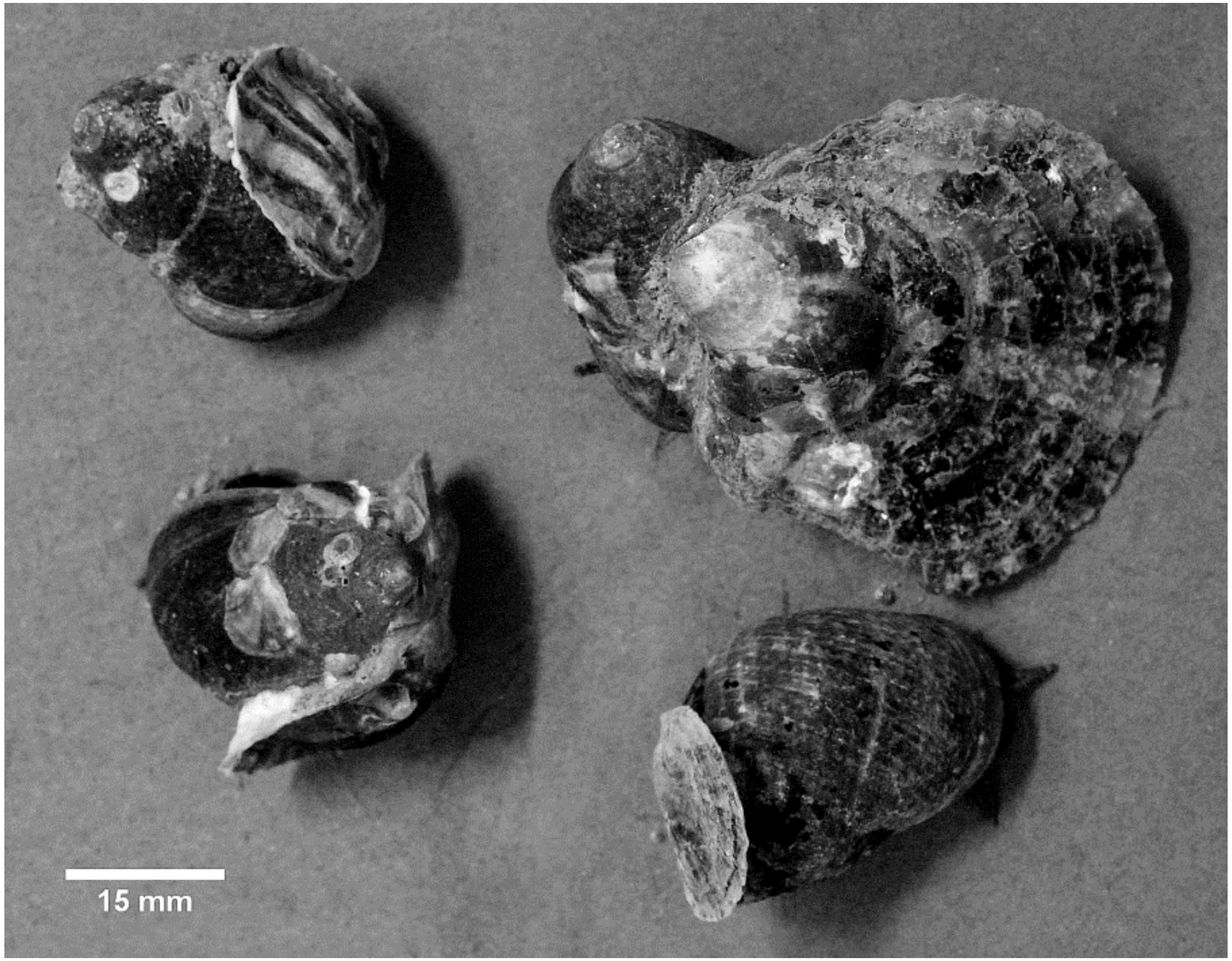


Figure 4.2 Common periwinkles (*Littorina littorea*) showing various degrees of fouling by the Chilean oyster (*Ostrea chilensis*), known locally as "gwichiaid hefo capiau" (Welsh for "winkles with caps" or "capped winkles").

the return of both ‘small’ and overly-fouled periwinkles to the shore for ongrowing (although not necessarily to the exact site of collection), thus maintaining local stocks; a practice known in some areas as ‘winkle farming’ (B. Roberts, pers. comm.). Such casual and unregulated movements of a target species, sometimes across international boundaries, can occasionally lead to the accidental transfer and/or spread of associated NNS (Minchin 1996; Eno *et al.* 1997).

The present study aimed to demonstrate the potential of the periwinkle industry as an unregulated coastal anthropogenic activity that may facilitate the geographic range expansion of an established non-native epibiont, whose natural dispersal capacity is highly limited (see Chapter 3). The significance of this increasingly-dominating oyster epibiont in relation to the marketability of harvested periwinkles was also explored. It is hoped that the findings of this chapter will help to highlight pressing issues with regards to this unmanaged fishery, promoting healthy cooperation between all stakeholders to protect both commercial and environmental interests within the Menai Strait, as well as in other areas where periwinkles are harvested worldwide. Specifically, the study aims to answer the following:

1. How does the occurrence of oyster-fouled periwinkles relate to adult *O. chilensis* densities in the Menai Strait and how likely are they to be collected by commercial periwinkle collectors?
2. What is the likely fate of oyster-fouled periwinkles once collected by commercial periwinkle collectors and sold to a central wholesaler?
3. Are there any significant differences between the quality and vigour of oyster-fouled and unfouled periwinkles?

4.3 Methods

4.3.1 Oyster fouling frequency and evidence of commercial collection

During December 2010, a total of six sites (>0.5 km apart) were surveyed within the Menai Strait (North Wales, UK; see Figure 4.1). Three sites (Abermenai, Llanidan, Plas Trefarthen) contained high mean densities of *O. chilensis* (>25 oysters m^{-2}) at low water, whilst the remaining three sites (Caernarfon, Mermaid, Stud Farm) contained significantly lower mean densities (<1 oyster m^{-2}) (Figure 4.1). Commercial periwinkle harvesting is known to be a regular activity within all sites throughout the year (pers. obs.). At each site, a total of sixty quadrats (0.25 m^2) were surveyed at low-, mid- and high-water (approximately 1, 3 and 5 m above chart datum and hereafter ‘HW’, ‘MW’ and ‘LW’ respectively), giving a total coverage of 45 m^2 at each site. All periwinkles within each quadrat were counted and their shell height (operculum lip margin to spire tip) measured to the nearest 1 mm using Vernier callipers. The number of periwinkles fouled by *O. chilensis* within each quadrat was noted, and the respective shell lengths (umbo to shell margin) of each epibiont was measured to the nearest 0.1 mm. The number of adult *O. chilensis* was also noted within every fifth quadrat, giving an estimation of mean adult oyster density at each site. Sub-samples of periwinkles collected by two independent commercial periwinkle collectors at two sites (Llanidan and Plas Trefarthen, LW only) were obtained to investigate whether or not the specimens collected using the survey technique were representative of those collected commercially for human consumption. Neither collector had any prior knowledge of the experimental design or the purpose of the study, thus ensuring that their collections were fully representative of a typical periwinkle haul in terms of periwinkle size range and the numbers of oyster-fouled specimens.

4.3.2 Survival of periwinkles and their oyster epibionts under refrigerated conditions

The impact of a refrigeration period (typically carried out at a wholesaler facility following collection) upon the survival of both target species (i.e. periwinkles) and the non-native epifoulant (i.e. oysters) was also empirically assessed. During June 2011, a total of ninety Chilean oyster shells (right, flat shell valves only) were deployed inner shell surface upwards on the shore at LW at Plas Trefarthen for two weeks to encourage natural *O. chilensis* spat settlement. All shells were subsequently retrieved and examined for the presence of *O. chilensis* spat. Twenty eight shells, with the highest densities of spat settlement on their inner surface, were selected (hereafter ‘spat shell plates’), and their respective densities manipulated to ensure approximately equal spat coverage (~2 spat cm^{-2}). Three additional groups of oysters (each containing 105 oysters), measuring 15-25, 40-50 and 65-75 mm shell length and representative of 1-, 2- and 3-year old oysters respectively, were additionally collected from the same locality. Chilean oysters within the Menai Strait population show great variation in their degree of attachment to hard substrata. Some adult oysters are found attached to stones, shells or adult conspecifics, whilst others have outgrown their site of attachment and form loose aggregations (pers. obs.). Newly-settled oyster spat, on the other hand, are always attached and cannot be stripped from the shell substrata without causing severe damage (Walne 1974; pers. obs.). In an attempt to standardise oyster attachment across all size classes, the left, cupped valve of each adult oyster was glued to a right shell valve using a small amount of non-toxic adhesive putty (Milliput^{TM}). The use of epoxy-based adhesives in this manner ensured that both the newly-settled spat and larger oysters were always in contact with a relatively similar area of shell substrata, whilst leaving their respective rates of survival uncompromised (e.g. Harper 1992; Macreadie *et al.* 2011). When not in use, all spat shell plates and oysters were stored in well-aerated flow-through seawater aquaria at 15°C.

Twenty-four spat shell plates and ninety oysters from each size class were transferred to a refrigerator held at 5°C, simulating the conditions that they might have experienced if they had been accidentally gathered by periwinkle collectors and transferred to a wholesaler facility. Following intervals of 2, 6, 12, 24, 48 and 72 h in the refrigerator, four spat shell plates and fifteen oysters from each size class were returned to labelled baskets within the maintenance aquarium. The remaining four spat shell plates and thirty oysters from each adult size class were kept fully submerged at 15°C throughout and acted as a control treatment. No attempt was made to determine the actual cause of mortality (i.e. due to the periodicity of aerial exposure or the actual refrigerated temperature, or equally, a combination of both), thus justifying the lack of adequate procedural control (e.g. whereby oysters were held in water at 5°C). Oyster mortality was estimated 72 h following re-immersion. Adductor muscle activity was assessed under a stereo microscope. An oysters was deemed to be dead if the shell valves were disarticulated from each other, leaving only the left valve attached (spat only), or if the shell valves remained agape following slight physical disturbance to the adductor muscle.

Similarly, the tolerance of fouled and unfouled periwinkles (n = 140 each) to refrigerated conditions was assessed during the same period to assess whether or not oyster epibionts had a significant effect on periwinkle survival. One hundred and twenty periwinkles from both treatment groups were transferred to a refrigerator held at 5°C, with 20 randomly-selected from each group returned to labelled cages within the maintenance aquarium after the following intervals: 2, 6, 12, 24, 48 and 72 h. The remaining twenty periwinkles from both treatment groups were kept fully submerged at 15°C throughout, again acting as a control treatment. Periwinkle mortality was estimated 72 h following re-immersion. A periwinkle was deemed to be dead if either its exposed foot showed no response to gentle physical disturbance using a metal seeker, or alternatively, if re-orientation had not been successfully completed following a period of 24 h.

4.3.3 Comparison of fitness and quality of fouled and unfouled oyster epibionts

During a field visit to Plas Trefarthen in June 2011, 'fouled' (n = 91) and 'unfouled' (n = 71) periwinkles of similar size (mean shell height = 25.4±0.1 mm) were collected and held in a 25 L flow-through aquarium until required. All fouled periwinkles had at least one oyster (mean shell length = 15.3±1.1 mm) attached to their shell surface with no other epibionts. Using a modification of the methods of Eschweiler and Buschbaum (2011), the relationship between the presence of oyster epibionts and periwinkle fitness was assessed by comparing the relative mobility and dry weight of fouled and unfouled *L. littorea*. The capacity of upturned periwinkles to re-orientate themselves back into an upright position was used as a proxy to assess the effect of oyster fouling on periwinkle mobility. Ten periwinkles from both fouled and unfouled collections were placed upside-down in one of four 5 L aquaria, each containing a layer (2 cm deep) of sand and topped-up with seawater held at ambient temperature (15°C). An additional five periwinkles from the fouled group, whose oyster epibionts had been manually removed prior to the commencement of the experiment, were also placed in each aquarium in a similar manner (thus leaving thirty-one fouled and unfouled periwinkles in the holding tank). The periwinkles were initially equally spaced so as not to influence each other following their return to an upright position. Following 90 minutes within the aquaria, the number of fully re-orientated periwinkles within each treatment was counted. The high degree of similarity in the response of both fouled and unfouled periwinkles within each of the four aquaria ($\chi^2 \leq 0.38$, $p \geq 0.944$) allowed for the pooling of periwinkles from each tank across each of the three treatments. This gave a more adequate degree of replication within each treatment and also allowed for the comparison of the ratios of re-orientated to upturned periwinkles in each treatment using a χ^2 test of independence. The remaining thirty-one periwinkles within both fouled and unfouled collections were boiled in tap water for 2 minutes. The flesh was then removed using a fine dissecting needle, placed in pre-weighed ceramic vials and dried to constant weight in a drying oven for three days at 65°C. Dry weights were determined to the nearest 0.001 g using a top-pan balance. The mean dry flesh weight of the periwinkles was used as a proxy for comparing the body condition of fouled and unfouled winkles, which were compared using a 2-sample t-test.

4.4 Results

4.4.1 Oyster fouling frequency and evidence of commercial collection

Periwinkles fouled by *O. chilensis* were observed at five of the six study sites (Figure 4.3; Appendix VI), with the proportion of oyster-fouled periwinkles greater at those sites containing higher adult oyster densities (Kruskal-Wallis H = 224.6, df = 5, $p<0.001$; Figure 4.3 inset). Periwinkles fouled by *O. chilensis* were found exclusively at LW and no oysters were ever found attached to periwinkles of <20 mm shell height (Figure 4.4). Whilst oyster fouling was more commonly observed on periwinkles ≥26.0 mm (Kruskal-Wallis H = 885.38, df = 4, $p<0.001$), periwinkle size-frequency at LW (pooled between all sites) followed a left-skewed, unimodal distribution, with a mean shell width of only 21.7 mm (Figure 4.4). This 'mismatch' may partly explain the relatively low fouling frequency (≤10.5% of all periwinkles at each site) observed throughout the study area (Figure 4.3; Appendix VI).

The size range of fouling oysters observed varied between sites, with generally greater range in size observed at sites containing high mean adult oyster densities. Whilst predominantly

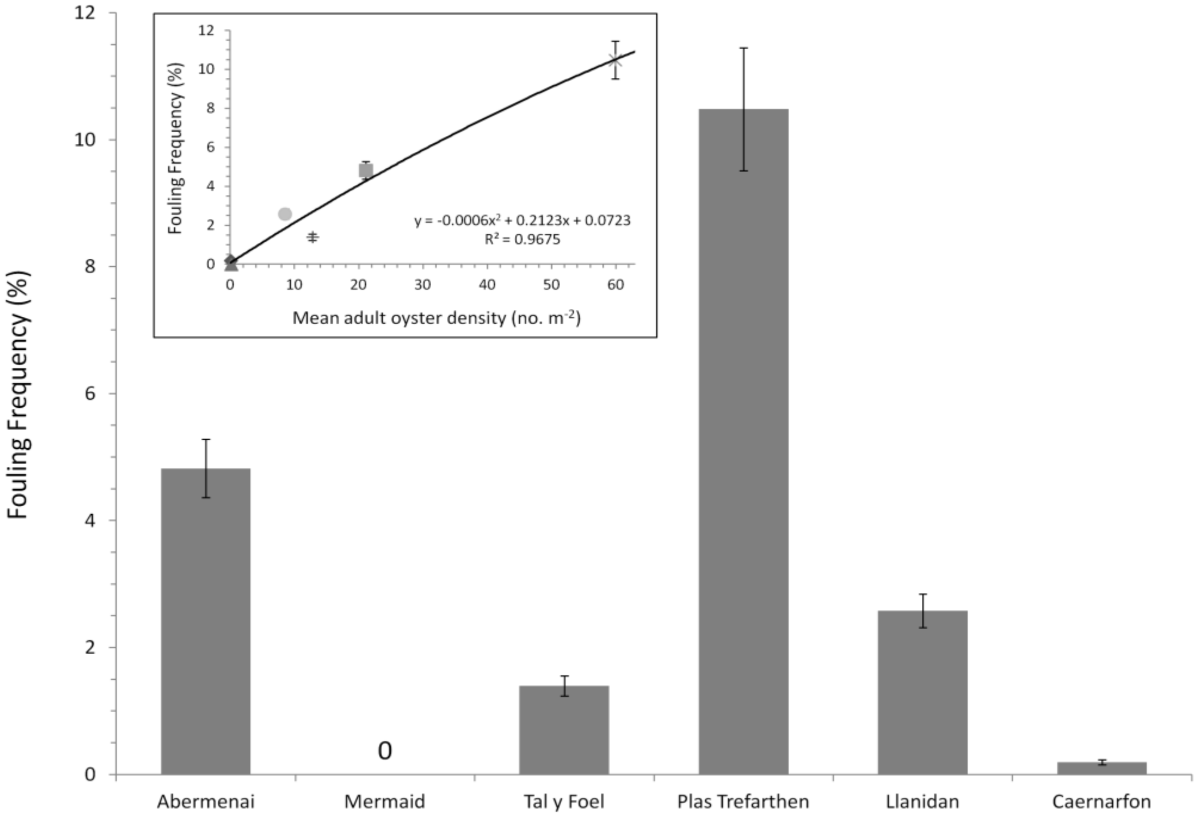


Figure 4.3 Mean percentage fouling frequency (±SE) of Chilean oysters (*Ostrea chilensis*), attached to common periwinkles (*Littorina littorea*) at each study site within the Menai Strait (North Wales, UK). Inset shows a highly positive correlation (second degree polynomial) between fouling frequency and mean adult oyster density (no. m^{-2}) within each site. Symbols: circle = Llanidan, cross = Plas Trefarthen, diamond = Caernarfon, plus = Tal y Foel, square = Abermenai, triangle = Mermaid.

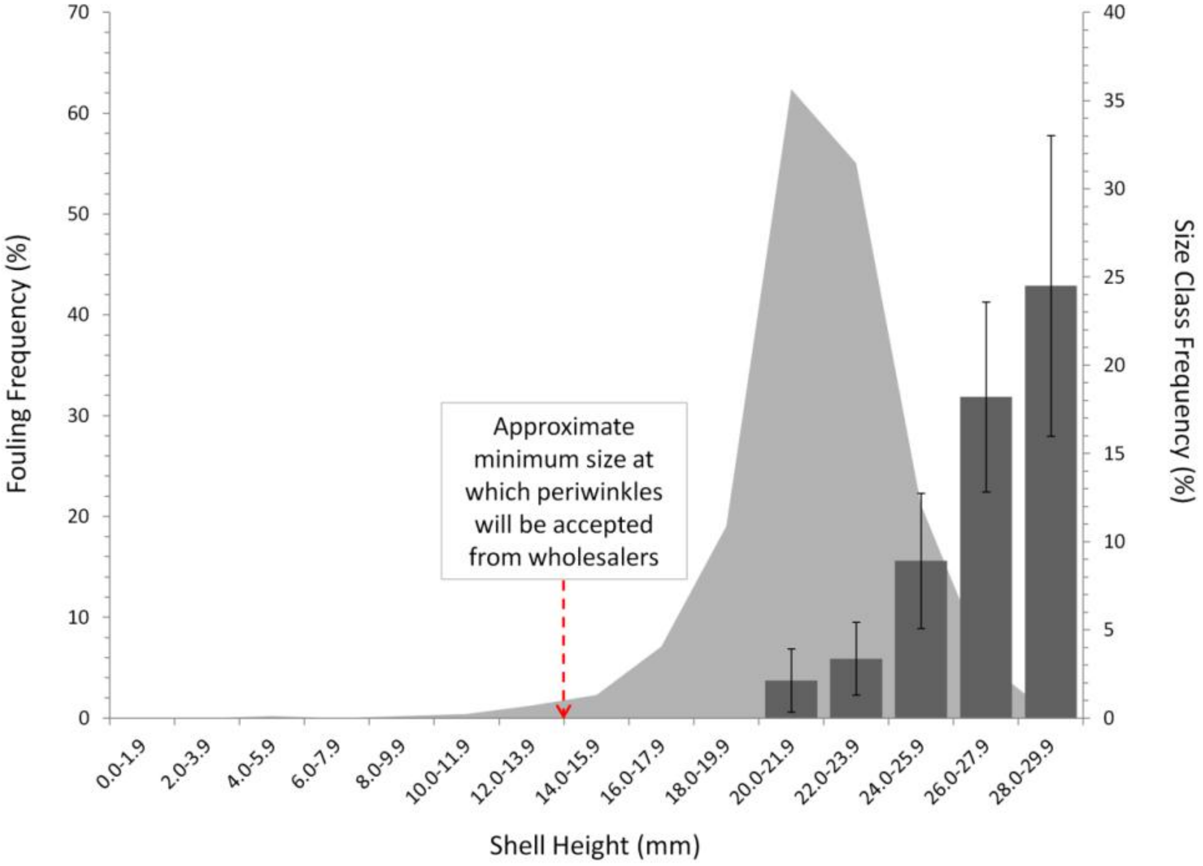


Figure 4.4 Size-specific mean percentage fouling frequency (±SE) (dark grey bars) of common periwinkles (*Littorina littorea*) (pooled across all sites), fouled by the Chilean oyster (*Ostrea chilensis*) in the Menai Strait (North Wales, UK). Data overlays size-class frequencies (%, grey silhouette) of periwinkles collected during a quantitative study at mean low water (pooled across all sites).

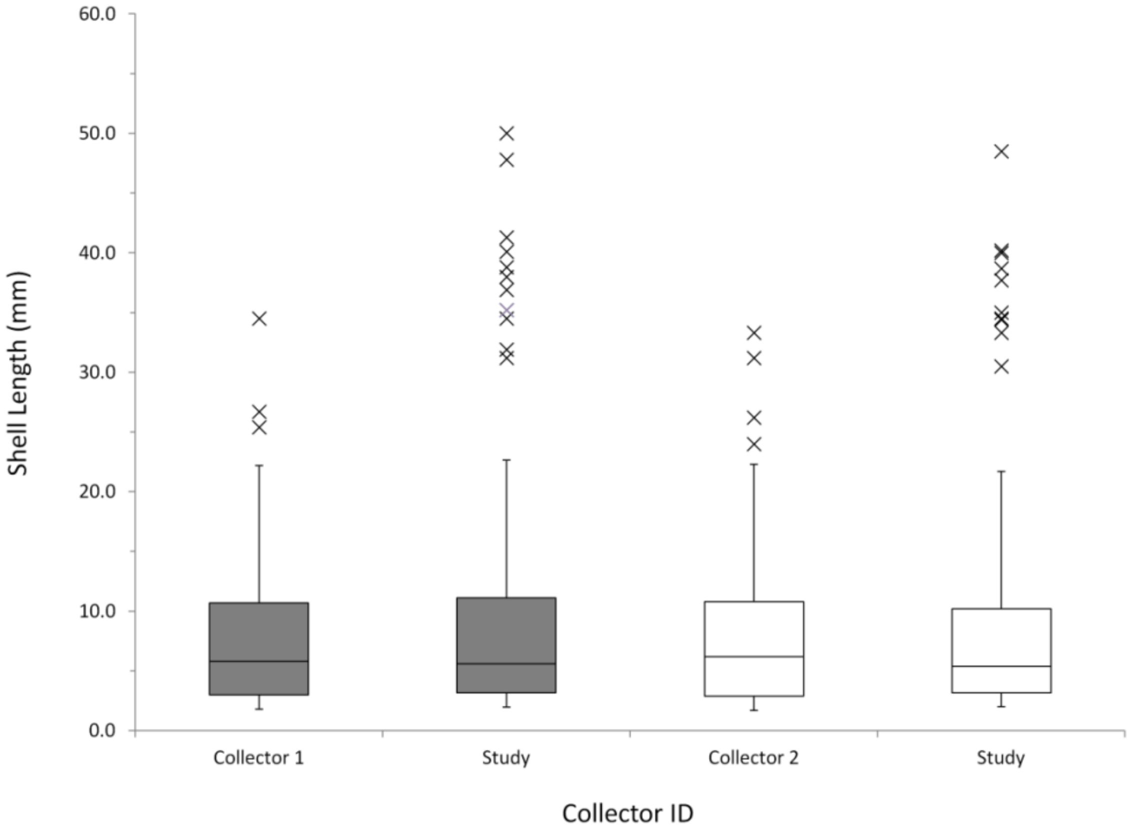


Figure 4.5 Comparative boxplots of the size distribution of epifouling Chilean oysters (*Ostrea chilensis*) collected by commercial periwinkle collectors (i.e. 'Collector 1', 'Collector 2') and by the author of this chapter (i.e. 'Study') at Abermenai Point (shaded boxes) and Plas Trefarthen (unshaded boxes) (Menai Strait, North Wales, UK).

fouled by juvenile oysters (<12 mm shell length) at all sites, periwinkles were also occasionally fouled by larger, mature oysters (up to 50 mm shell length) at both Plas Trefarthen and Abermenai (Figure 4.5). Neither the ratio of fouled to unfouled periwinkles ($\chi^2 \leq 0.186$, df = 1, $p \geq 0.666$) nor the median size of periwinkles (Mann-Whitney $W \leq 40780.5$, $p \geq 0.217$) differed significantly between those sub-sampled from independent periwinkle collectors and those collected directly from LW (Figure 4.5). However, using the modal class progression analysis of Bhattacharya (1967) (pooled across sites), three distinct oyster size-classes were identified in the samples collected at LW (4.0, 11.1, and 35.0 mm shell length), whilst only two size classes were detected in the sub-samples obtained from local periwinkle collectors (3.6 and 10.6 mm shell length). This suggests that active attempts to avoid the collection of periwinkles with oyster epibionts >25 mm are made by commercial collectors, although their efforts are not entirely infallible (see outliers in Figure 4.5).

4.4.2 Survival of periwinkles and their oyster epibionts under refrigerated conditions

The survival rate of oysters to varying periods of refrigeration showed a differing response with size (Log Rank $\chi^2 = 257.9$, df = 3, $p < 0.001$), with spat oysters showing greater vulnerability than all other size groups (Figure 4.6). Nearly all spat oysters (92.7%) died following an emersion period of only 6 h. The mortality rate of spat oysters could be fitted to a Gompertz model (see Figure 4.6 inset), resulting in an LT_{50} value of 3.2 h. Conversely, oyster mortality was negligible across all other size fractions and control treatments, with ≤2.8% mean mortality observed in all treatments. All oysters from all size class groups survived within the control treatment, confirming the significance of the refrigeration process upon their rate of mortality. Additionally, both fouled and unfouled periwinkles were able to survive refrigeration for up to 72 h, with no mortality observed within either treatment.

4.4.3 Comparison of fitness and quality of fouled and unfouled oyster epibionts

The presence of oyster epibionts was negatively associated with the ability of periwinkles to re-orientate themselves under submerged conditions ($\chi^2 = 13.572$, df = 2, $p = 0.001$). Whilst none of the fouled periwinkles were capable of re-orientation, 27.5% of their unfouled conspecifics were able to return to an upright position in under 90 minutes. Interestingly, only 10% of periwinkles whose epibionts had been manually removed prior to the commencement of the experiment were able to fully re-orientate themselves following the experimental treatment, suggesting that growing with an increasingly large epibiont may compromise their ability to re-orientate in some way. Unfortunately, further analysis to test for any significant difference between the re-orientation ability of unfouled and control periwinkles could not be carried out due to the small

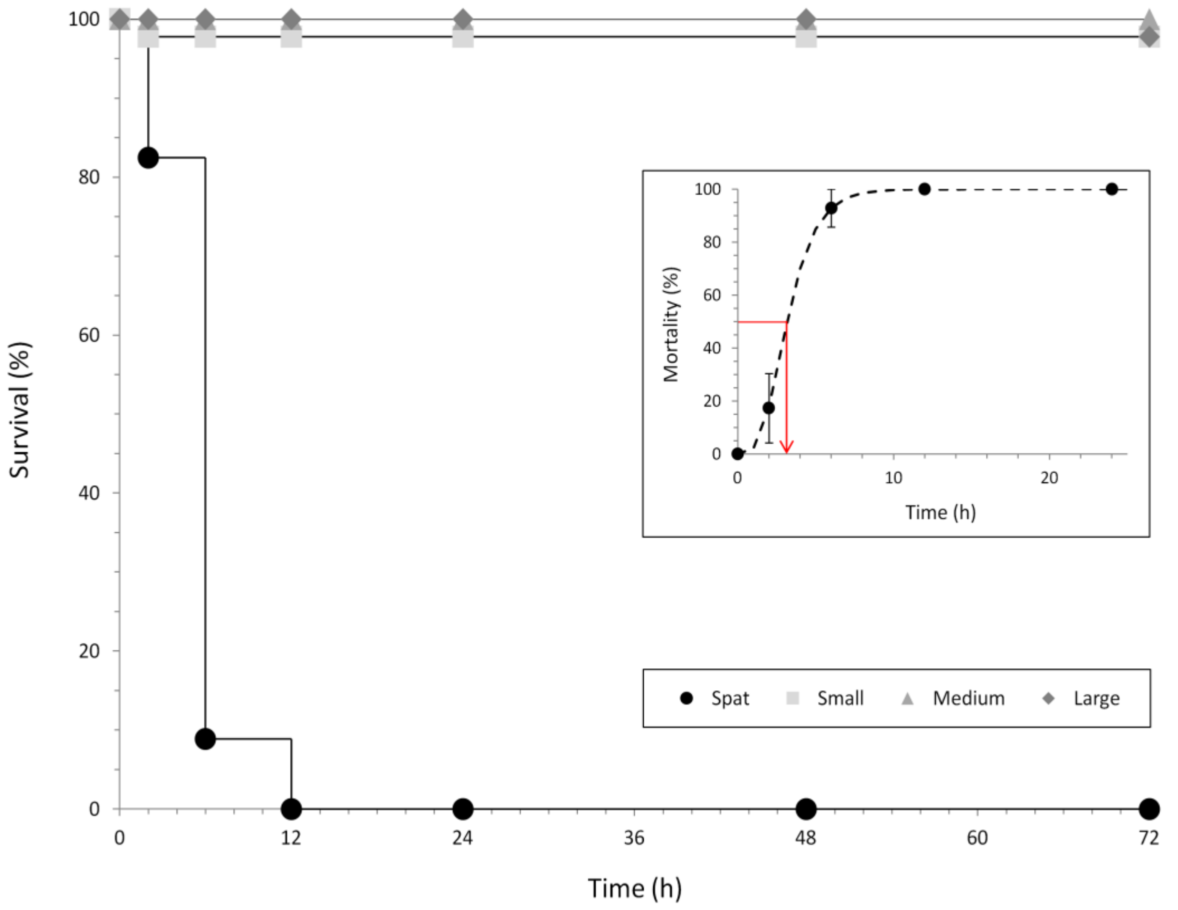


Figure 4.6 Kaplan-Meier curves of the survival of Chilean oyster (*Ostrea chilensis*) when exposed to varying durations of refrigerated conditions. Spat = <5mm, Small = 15-25mm, Medium = 40-50mm, Large = 65-75mm shell length. Inset shows a Gompertz model ($y = a.exp^{b.exp^{(ct)}}$, where a = 1.0, b = -8.5 and c = -0.8, R^2>0.999) fitted to the mean percentage mortality (±SE) of spat oysters over time, giving an LD_{50} = 3.2h (dotted arrow).

sample size of the 'control' group, thus giving >20% of all treatments with expected counts of <5 (see Yates *et al.* 2002). Fouled periwinkles (0.264±0.010 g) had a significantly poorer body condition than unfouled conspecifics (0.308±0.009 g) (t = -3.30, df = 60, p = 0.002).

4.5 Discussion

Periwinkle shells are often fouled by many native and non-native epibionts, including algae (Wahl 1996), barnacles (Buschbaum and Reise 1999), oysters (Eschweiler and Buschbaum 2011; present study) and spionid worms (Warner 1997). The common periwinkle has no known natural chemical, mechanical or physical defences to regulate epifouling intensity. It has been suggested that, at high densities (>400 periwinkles m^{-2}), epibionts may be directly removed by the 'bulldozing' and grazing activity of conspecifics (Wahl and Sönnichsen 1992; Wahl *et al.* 1998). However, periwinkle densities are probably never high enough within the Menai Strait (<100 periwinkles m^{-2}) to initiate sufficient 'bulldozing' activity. Moreover, the proportion of oyster-fouled periwinkles was significantly greater at sites containing higher adult oyster densities in the Menai Strait, suggesting that fouling frequency is related to epibiont propagule supply. Sessile and slow-moving benthic marine invertebrates rely on the dispersal of larval progeny as their foremost method of transport away from adult conspecifics. The duration spent in the water column as planktonic larvae thus serves as a major contributor to the distribution and reproductive dynamics of these species. Whilst the larvae of *L. littorea* spend several weeks in the water column (Fretter and Graham 1980) and are likely to be transported over vast distances, pediveliger larvae of *O. chilensis* are known to settle within minutes following release (Millar and Hollis 1963; Cranfield 1968; Westerskov 1980). Whilst periwinkle stock recruitment is likely to be affected by the actions of collectors and wholesalers in other neighbouring regions, the fouling of periwinkles by oysters is restricted to those areas where adult oysters are present.

Epibionts were only present on marketable, 'large' periwinkles (≥20 mm shell height) in the Menai Strait and were virtually exclusive to LW, echoing the findings of both Smith and Newell (1955) and Warner (1997) at other locations within the UK. Warner (1997) suggested that size-specific fouling frequency is simply a function of the time spent as a potential basibiont and the increased surface rugosity of older shells caused by shell erosion and abrasion. However, periostracum abrasion was not particularly obvious in large periwinkles in the Menai Strait populations and settlement appeared to occur equally on both newer (i.e. recently deposited) and older regions of the shell. Furthermore, small periwinkles were relatively uncommon at LW in the Menai Strait, with >93% of the total periwinkle population of ≥18 mm shell height (pooled across sites). Size-frequency distributions of gastropods along a vertical shore gradient can become disproportionate due to a combination of two factors; an unequal rate of mortality amongst distinct size classes (either over the whole or part of the intertidal range of the species in question), and the active migration of a particular size cohort, relative to all others (Vermeij 1972). Physical and biological factors may therefore inhibit the ability of smaller periwinkles to inhabit areas of the high and low shore respectively. The observed absence of small periwinkles at LW in the Menai Strait may be attributed to an increase in predation pressure imposed upon juvenile periwinkles. The green shore crab, *Carcinus maenas* (L. 1758) predates voraciously upon small periwinkles <9 mm in length, with successful attacks on periwinkles of 9-18 mm shell length taking five times longer but those >18 mm remaining unconsumed (Hadlock 1980). Considering the limited encroachment into the intertidal by *O. chilensis* in the Menai Strait (Chapter 2) and the prominence of large periwinkles at LW (this Chapter), the tendency of oysters to settle on larger periwinkles is, in this case, likely to be related to the intolerance of *O. chilensis* to the stresses of the intertidal zone (Stead 1971; Westerskov 1980) and the sheer lack of smaller periwinkles at LW. Whatever the mechanism that restricts fouling of all but the largest periwinkles, the likelihood of the accidental collection of oyster-fouled periwinkles by collectors becomes inadvertently increased by concentrating collection efforts at LW (where larger, more economically-valuable periwinkles are found).

Oysters are known to have a profound influence upon key ecological processes, including the maintenance of biodiversity through their habitat-modification abilities and their role in nutrient cycling and food-web dynamics through the translocation of energy from the overlying water column to the benthic environment (see Ruesink *et al.* 2005 for review; Chapter 1). Oysters are also vectors of many disease-causing organisms. The Chilean oyster is highly susceptible to infection by *Bonamia ostreae* (Pichot *et al.* 1980), which has previously decimated several European populations of the European native oyster, *Ostrea edulis* L. 1758 (e.g. Balouet *et al.* 1983; van Banning 1985). In 2011, *B. ostreae* was confirmed to be present within an area of the Menai Strait, resulting in significant shellfish movement restrictions into, out of and within the region by way of a Confirmed Designation Notice (issued under the Aquatic Animal Health (England and Wales) Regulations 2009). It is likely that this potential vector of spread of infection is also likely to remain undetected given the currently unregulated nature of the periwinkle fishery. Considering its ecosystem engineering potential and its status as a vector of a highly-infectious parasite, it is thus crucial that the dispersal capacity of *O. chilensis* is not facilitated by the relaying of oyster-fouled periwinkles to areas away from their original point of collection. This chapter is believed to be the first to investigate the potential role of commercial periwinkle harvesting as an unregulated anthropogenic activity that facilitates the geographic range expansion of a non-native epibiont across regional and international boundaries. The fate of the oyster epibionts is largely dependent on the overall degree of fouling within a locality and the actions of both the collector and the wholesaler during the collection and post-collection processes respectively (see Figure 4.7). Periwinkles are collected from numerous populations, each with a varying degree of oyster fouling. Bags containing heavily-fouled periwinkles are instantly rejected upon a brief visual inspection of a small sub-sample. The remainder are normally griddled and sorted into three distinct size classes, with small periwinkles (i.e. those <14 mm shell height) rejected due to their low market value (McKay and Fowler 1997). In both cases of rejection, the periwinkles are returned to the Menai Strait to supplement local stocks, although not necessarily to the same locality from which they were originally collected. The remaining periwinkles are usually sold to the European market within 72 h of collection during periods of peak demand to ensure maximum freshness of the marketable product. The fate of these marketable periwinkles and their epibionts is currently unknown, although it is believed that some may be sold on to French oyster farmers who use them as a method of biocontrol within culture bags (Cummins *et al.* 2002).

The presence of oyster epibionts had no significant effect on the survival of periwinkles under simulated commercial refrigerated conditions, meaning that fouling is unlikely to negatively affect periwinkle freshness and survival. However, fouled periwinkles are more likely to be unmarketable due to their unsightly appearance, as well as their liability to block the griddling mechanism and to add excess weight to collected hauls. Excessively-fouled periwinkles are routinely discarded by wholesalers, who may return them to areas within the collection catchment area (although not necessarily to their original origin) in an attempt to maintain local stock recruitment. A period of emersion is a suitable method of mitigation against the spread of non-native epibionts when the tolerance of the target species is greater than that of the fouling organisms (Katayama and Ikeda 1987). Stress tolerance can often vary with size and age of a fouling organism (e.g. Murphy 1983; Sukhotin *et al.* 2003; this Chapter). Additionally, the emersion period must not be too long so as to compromise the quality and freshness of the commercial product. Owing to the ability of marketable periwinkles and all but the smallest *O. chilensis* to survive out of water for at least three days, the current study disregarded emersion as a successful method of mitigation against the spread of the Chilean oyster. Furthermore, the ability of Chilean oysters to tolerate several days of exposure to cold, refrigerated air suggests that the practice of ‘winkle farming’ could easily augment the geographic spread of this NNS. It remains to be seen whether or not other NNS are being transferred during the periwinkle collection process and subsequent ‘winkle farming’ in other countries, where management of the fishery is equally lacking (e.g. Canada, Ireland).

To quantify and put into context the harvesting model depicted in Figure 4.7, consider that the average marketable 'medium' and 'large' periwinkle weigh approximately 4.0 and 7.0 g

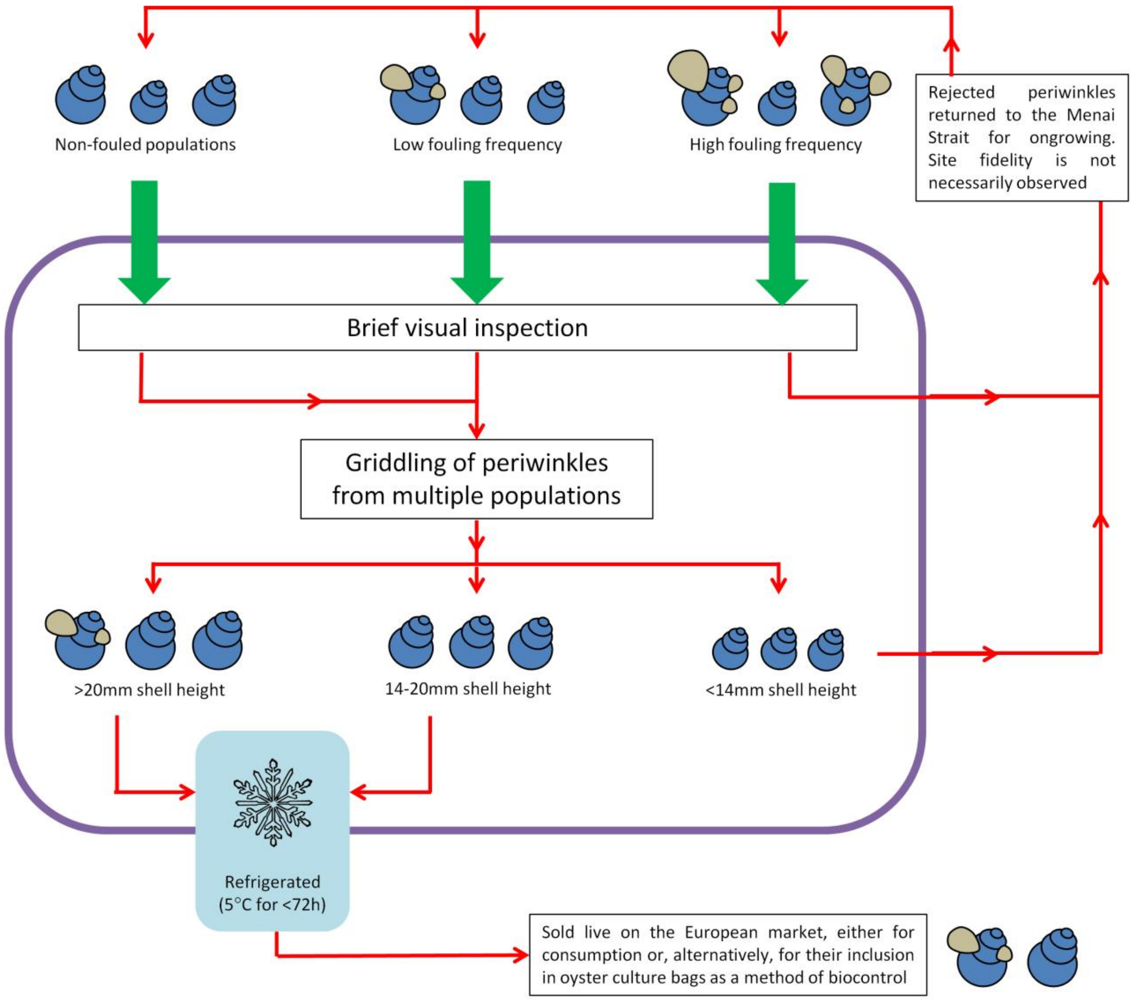


Figure 4.7 Schematic diagram depicting the typical commercial harvesting process of the common periwinkle (*Littorina littorea*) in the UK. Activities within the rounded-edged box represent those which occur within a typical wholesaler facility.

respectively. Consider also that a full collection bag is likely to hold up to 50 kg of periwinkles. The majority (82.5%) of periwinkles collected at LW in the Menai Strait are likely to be large (i.e. >20 mm shell height). Assuming a single haul with 10.5% of oyster-fouled periwinkles (i.e. the highest mean fouling frequency observed), a full bag is therefore likely to hold up to 354 large, oyster-fouled periwinkles. Rejection of these periwinkles upon visual inspection at the wholesaler facility would mean that several hundred oysters have the potential to be accidentally transferred to new localities within the Menai Strait with each bagful due to the process of 'winkle farming'. Should the periwinkles be accepted and griddled, it is likely that up to 20% of all fouling oysters will be ≥15 mm shell length, meaning that up to 71 oysters will survive the post-harvest refrigeration period per bag.

It is possible that the increase in Chilean oyster epibionts may have a negative impact on the periwinkle industry if transfers of this non-native oyster species both within and beyond the Menai Strait are left unregulated. Epifouling by several intertidal species is known to be concurrent with a reduction in the fitness of *L. littorea*, with both crawling speed (Buschbaum and Reise 1999; Eschweiler and Buschbaum 2011) and re-orientation (this Chapter) significantly lower in fouled periwinkles. Being active grazers of algal films, periwinkles are reliant upon correct orientation and locomotion for efficient feeding. Epibiont-induced increase in drag has been shown to decrease periwinkle growth (Wahl 1996), whilst laboratory studies have shown that the reproductive output of littorinid snails, manifested as a reduction in egg production and gonadosomatic index, decreased when epibionts were present (Buschbaum and Reise 1999; Chan and Chan 2005). It is likely that fouled periwinkles expend more energy in the development of foot muscle and possibly the deposition of shell material as opposed to reproductive and somatic growth (Wahl 1997).

Conclusions and recommendations

Considering the lack of adequate active avoidance of oyster-fouled periwinkles throughout the harvesting process, the industry should not be disregarded as a vector for transporting Chilean oysters across both local and international borders, particularly given the ability of all but the smallest oysters to survive in refrigerated conditions for several days. Whilst, in principle, the practice of ‘winkle farming’ is to be commended, care should be taken to return all periwinkles to the site where they were initially collected, thereby minimising the chances of facilitating the range expansion of *O. chilensis* and other NNS. Whilst collectors appear to actively avoid larger epibionts, the procedure is by no means flawless and smaller conspecifics that are capable of surviving the post-collection refrigeration period are, nonetheless, also collected accidentally. Given the sheer numbers of periwinkles collected, the manual removal of epibionts is unlikely to be a financially viable option that would provide a fail-safe method of inhibiting the accidental transfer of NNS. A significant reduction in periwinkle fitness and quality associated with fouled periwinkles support the findings of several others (e.g. Wahl 1997; Buschbaum and Reise 1999; Buschbaum 2000; Chan and Chan 2005; Eschweiler and Buschbaum 2011). It is suggested that raising awareness among bait collectors and wholesalers of NNS and their potentially damaging effects upon the industry and beyond may serve as a useful deterrent that discourages the collection of fouled periwinkles. Furthermore, this chapter highlights the inadequacy of the post-collection processing method as a mitigation measure to restrict the accidental NNS transfer. Better interventions during both initial visual inspection and post-griddling stages are recommended, as well as the development of techniques that kill off all non-native epibionts, whilst leaving the freshness and marketability of the periwinkles uncompromised.

Appendix VI: Population dynamics and oyster fouling frequency of the common periwinkle, *Littorina littorea*, in the Menai Strait (North Wales, UK)

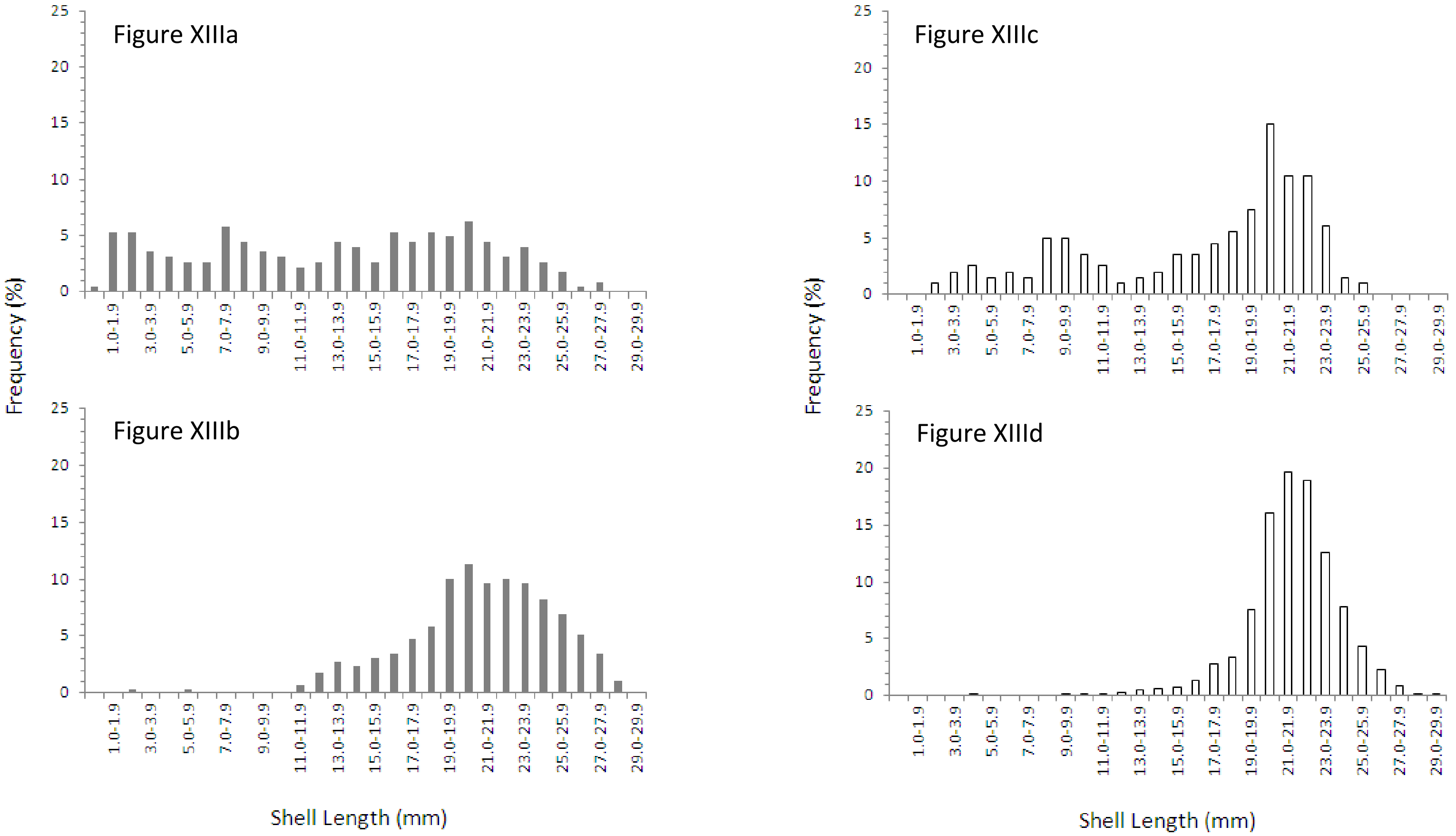


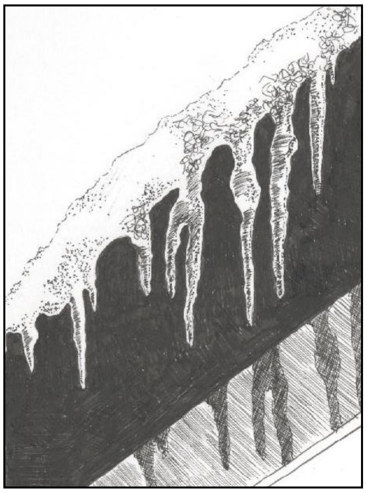
Figure XV Percentage size-frequency distributions of the common periwinkle, *Littorina littorea*, at mid- (Figures XIIIa-b) and low-shore (Figures XIIIc-d) in the Menai Strait (North Wales, UK) during June (closed bars) and December (open bars) 2010. Data for both shore levels pooled from six sites.

Table I Quantitative observations of common periwinkles (*Littorina littorea*) fouled by Chilean oysters (*Ostrea chilensis*) at 6 sites in the Menai Strait (North Wales, UK). Variability of all calculated means denoted in standard error units. MS = mid-shore, LS = low-shore.

		Abermenai Point		**Mermaid**		**Tal y Foel**		**Plas Trefarthen**		**Llanidan**		**Caernarfon**	
		Jul	**Dec**	**Jul**	**Dec**	**Jul**	**Dec**	**Jul**	**Dec**	**Jul**	**Dec**	**Jul**	**Dec**
Mean periwinkle density (no. m^{-2})	**MS:**	88.9 ± 15.6	43.1 ± 8.3	9.8 ± 2.2	3.3 ± 1.4	74.2 ± .11.6	14.1 ± 3.5	44.4 ± 8.6	35.0 ± 6.6	11.1 ± 2.9	2.1 ± 0.7	55.5 ± 8.3	38.8 ± 7.9
	LS:	65.6 ± 11.9	44.8 ± 9.2	60.5 ± 10.1	45.2 ± 9.4	44.3 ± 9.0	22.2 ± 7.0	82.4 ± 13.4	77.3 ± 12.3	32.1 ± 6.0	10.3 ± 2.0	80.0 ± 12.6	59.9 ± 10.0
Percentage of fouled periwinkles	**MS:**	13.1%		3.2%		4.1%		2.5%		10.0%		5.5%	
	LS:	33.3%		11.3%		LS: 24.8%		42.6%		21.7%		18.2%	
Percentage fouled by oysters	**MS:**	0%		0%		0%		0%		0%		0%	
	LS:	4.8%		0%		1.4%		0.5%		2.6%		0.2%	
Size of smallest periwinkle fouled by oysters		20 mm		n/a		26 mm		21 mm		25 mm		28 mm	
Size range of fouling oysters		3-15 mm		n/a		4-6 mm		3-17 mm		3-6 mm		7 mm	

Chapter 5

Acute cold winter temperature abnormalities and the proliferation of invasive species: an overlooked facet of global climate change?



5.1 Abstract

Successive cold winters during recent years have done little to convince climate change sceptics of the general warming of the Earth's atmosphere. Paradoxically, global warming is likely to be intricately linked to cold winter extremes in the Northern Hemisphere. Much uncertainty surrounds the significance of extreme climatic events, such as cold snaps, in mitigating the rate of change of geographic distributions, with the failure of their inclusion in modelled projections of future global biodiversity patterns suggested to be accountable for some of the large variability observed. Whilst unlikely to halt the northward migration of both native and non-native species, the predicted increase in the frequency and intensity of acute climatic extremes, particularly cold winter snaps, may well play a major role in suppressing the rate of invasiveness of non-native species within their respective new environments. Using the Chilean oyster (*Ostrea chilensis*) as a model species, this study investigates the potential effects of lethal and non-lethal climate change-induced cold winter temperature stress on the future success of a non-native species within its introduced range. By exposing various size classes of oysters (small: 25-35 mm, medium: 45-55 mm, large: 65-75 mm shell length) to a single, 2h period of freezing air temperatures (-2, -6 or -10°C, thus mimicking conditions potentially experienced at mean low water spring tides), oyster survival rate was shown to be significantly lower with decreasing air temperature (Kaplan-Meier Survival Analysis: $X^2 = 91.706$, $p < 0.001$). Conversely, native co-inhabitants showed increased vigour to freezing conditions. The blue mussel, *Mytilus edulis*, showed negligible mortality across all treatments, whilst mortality of the European oyster, *Ostrea edulis*, was confined to two back-to-back periods of air temperatures at -10°C. Small *O. chilensis* cooled and thawed as much as three and nine times quicker than their larger counterparts respectively, and were also subjected to significantly greater periods of extracellular ice formation. However, no significant difference was observed between oyster survival rates across size classes within each temperature treatment, suggesting that smaller, younger oysters are relatively more tolerant to freezing conditions than larger conspecifics ($X^2 \leq 2.00$, $p \geq 0.368$). Four weeks following a single 2h exposure period at -2°C, -6°C and -10°C, survival rates were 95%, 80% and 55% respectively. A case of 'strength in numbers' is presented, whereby small oysters, in the presence of several other conspecifics, are buffered against the effects of freezing air temperatures compared with those exposed to freezing temperatures in isolation. This has critical implications for the future invasion dynamics of this non-native oyster population within a designated SAC. Our findings are discussed in relation to the successful proliferation of this non-native species within a designated Special Area of Conservation and its role in modifying the native biodiversity.

5.2 Introduction

Climatic parameters, particularly temperature, are instrumental in shaping the geographic distribution of organisms (Hutchins 1947; Thorson 1950; Southward 1958; Southward *et al.* 2005; Hawkins *et al.* 2009), with the biogeographic boundaries of many species directly related to their physiological capacity to tolerate thermal extremes (Somero 2010). Anthropogenically-mediated increases in greenhouse gas concentrations have led to an increase of 0.74±0.18°C in the Earth's mean surface temperature since the early 1900s (IPCC 2007). Worryingly, native and non-native species are responding differently to a warming climate (Southward *et al.* 1995; Hawkins *et al.* 2003; Mieszkowska *et al.* 2005; Hiddink and ter Hofstede 2008). The breakdown of climatic barriers currently restricting the recruitment of transient non-natives can augment invasion frequency (Rahel and Olden 2008), whilst the generally broader thermal tolerance and larger dispersal capacity of established non-natives are likely to favour their proliferation at the expense of native co-inhabitants (Sorte *et al.* 2010). Phenological adaptations in response to a warming climate can also promote species invasiveness by increasing propagule pressure (Stachowicz *et al.* 2002; Ward and Masters 2007; Moore *et al.* 2011). Conversely, greater physiological stress pertaining from atmospheric warming can often be detrimental to the competitive resistance of native species (Lockwood and Somero 2011), facilitating the biotic homogenisation of habitats with severe global implications to the functioning of ecosystems and the multiple services which they provide (McKinney and Lockwood 1999; Olden *et al.* 2004; Helmuth *et al.* 2006).

Projections of future global climate change forecast a further 1.5-3.0°C increase in the global mean surface temperature by the end of the 21st century (IPCC 2007), punctuated by numerous climatic extremities (e.g. storms, hurricanes, heat waves, floods, droughts, cold snaps) (termed 'extreme climatic events' or 'ECEs') of increasing frequency and intensity (see IPCC 2012). Specifically, evidence is gathering which indicates an increasing prevalence of acute periods of exceptionally cold air temperatures (termed 'cold snaps') across large parts of the Northern hemisphere (Wang *et al.* 2010; Smith 2011; Liu *et al.* 2012). Several winters have been disrupted by periods of extreme sub-zero temperatures of record-breaking proportions, with devastating impacts on the structure and functioning of many native marine communities (e.g. 1962/63, Crisp 1964; 1978/79, Beukema 1979; 2009/10, Wethey *et al.* 2011). The impacts of cold snaps are of particular relevance to intertidal communities, which experience varying degrees of aerial exposure during each tidal cycle and are thus subjected to large variations in several abiotic factors on a daily basis. Highly mobile organisms (e.g. crabs, fish) are able to migrate to the more favourable subtidal zone with the ebbing tide and even those which fail to retreat in time are able to find refuge in less stressful microhabitats within the intertidal zone (e.g. sheltered crevices, rock-pools, beneath canopy-forming macroalgae). Sessile and slow-moving organisms are, by contrast, required to withstand periods of several hours of exposure to physical stressors such as desiccation, solar radiation and temperature extremes. The severity of the stress gradient is accentuated in areas where the timing of low water of spring tides (LWST) coincides with the hottest and coldest climatic conditions during the summer and winter months respectively (Helmuth *et al.* 2006).

Global warming is predicted to instigate species extinctions (Thomas *et al.* 2004) and poleward migrations (Parmesan and Yohe 2003) across several taxa. However, much uncertainty surrounds the significance of ECEs in mitigating the rate of change of geographic distributions, with the failure of their inclusion in modelled projections of future global biodiversity patterns likely to be accountable for some of the large variability observed (Pereira *et al.* 2010). This is of particular concern considering that maximum and minimum temperatures, as opposed to annual mean temperatures, are often of the greatest significance to the persistence and invasiveness of many non-native species (Stachowicz *et al.* 2002). Whilst unlikely to halt the poleward migration of non-native species indefinitely, future cold snaps have been hypothesised to act as a critical 'reset' mechanism which may impede the rate of biological invasions (Canning-Clode *et al.* 2011; Firth *et al.* 2011). Recent scientific endeavour within the field of ECEs has resulted in significant advancements in understanding of how cold snaps are likely to affect ecosystems and the services which they provide. Field observations showing correlations between cold winter temperatures and rates of mortality have been complemented by empirical testing of past, present and future climatic scenarios (Urian *et al.* 2010; Canning-Clode *et al.* 2011). Comparisons of the response of non-native species with their native ecological competitors have made subsequent predictions of community and ecosystem level changes more plausible (e.g. Lockwood and Somero 2011). Physiological stress is also likely to show divergence across the size/age gradient of both native and non-native congeneric species (e.g. Roy *et al.* 2002), and its incorporation into experimental design is known to be critical if more accurate predictions regarding future changes in invasion success are to be made (e.g. Urian *et al.* 2010). Whilst an increasing number of studies are beginning to highlight the importance of such parameters in order to make credible conclusions regarding the potential impacts of future cold snaps on biological invasions, no studies to date has taken all of these pertinent findings into consideration within their experimental design.

The intertidal zone of the Menai Strait and Conwy Bay Special Area of Conservation (SAC) (Figure 5.1) was identified as a suitable area to investigate the effects of cold snaps of increasing frequency and severity upon native and non-native species. Partly due to its historic status as an area supporting commercial fisheries and aquaculture growth trials of many bivalve molluscs, areas within the SAC (particularly the lower intertidal) support populations of several non-native species, including the Pacific oyster (*Crassostrea gigas* Thunberg 1793), the hard shell clam (*Mercenaria mercenaria* L. 1758) and the Chilean oyster (*Ostrea chilensis* Philippi 1845). These species co-exist with functionally-related natives, including commercially-important molluscs (e.g. the blue mussel, *Mytilus edulis* L. 1758) and rare, protected species (e.g. the native oyster, *Ostrea edulis* L. 1758). Non-native oysters may compete for resources such as food and space with many native species within their new environment and can also alter biodiversity and ecosystem functioning through habitat modification (e.g. Cranfield *et al.* 2001; Gutiérrez *et al.* 2003; Padilla 2010).

Ostrea chilensis is a flat oyster belonging to the family *Ostreidae*, and is native to both Chile and New Zealand, where it is a commercially-important species. It is a protandric hermaphrodite and the larvae are brooded within the female mantle cavity pending their release as pediveligers, which settle within minutes to hours following release (Millar and Hollis 1963). The species was deliberately introduced into the low intertidal at Tal y Foel (Menai Strait, North Wales, UK) by the Ministry of Agriculture, Fisheries and Food (MAFF) during the early 1960s to investigate its potential as an alternative culture species to replace the diminishing native oyster populations of the UK (see Walne 1974). Its initial spread away from the site of original introduction was unsurprisingly slow (averaging 13.3m y^{-1}) given its relatively low fecundity (Cranfield and Allen 1977), highly reduced pelagic larval phase (Millar and Hollis 1963) and the lack of suitable substratum flanking both sides of the oyster bed (see Richardson *et al.* 1993b). However, more recent evidence has shown a significant increase in both range expansion (averaging 0.6 km y^{-1}) and density (up to 232 oysters m^{-2}) (Chapter 2). This has led to significant changes to the local biodiversity of the communities associated with the oysters (see Appendix I) and, potentially, the qualifying habitats (see Annex I of the EC Habitats Directive) which warrant its current conservation status. Incidentally, LWST occurs between ~0400-0700h and ~1600-1900h (GMT) in the Menai Strait. The intertidal Chilean oyster population, occurring up to 2 m above chart datum within the SAC (Chapter 2), is thus subjected to both the coldest (am, winter) and warmest (pm, summer) annual air temperature extremes for up to 2 hours during each period of LWST.

Using the non-native *O. chilensis* as our model species, we investigated the significance of climate change-induced increases in the frequency and intensity of winter cold snaps as a potential mechanism controlling the spread and proliferation of an invasive species outside its native geographic range. Present observations and future projections of extreme cold snaps were mimicked in the laboratory. The resilience of *O. chilensis* to observed and future predicted acute cold snaps was then empirically compared with that of its native ecological co-inhabitants,

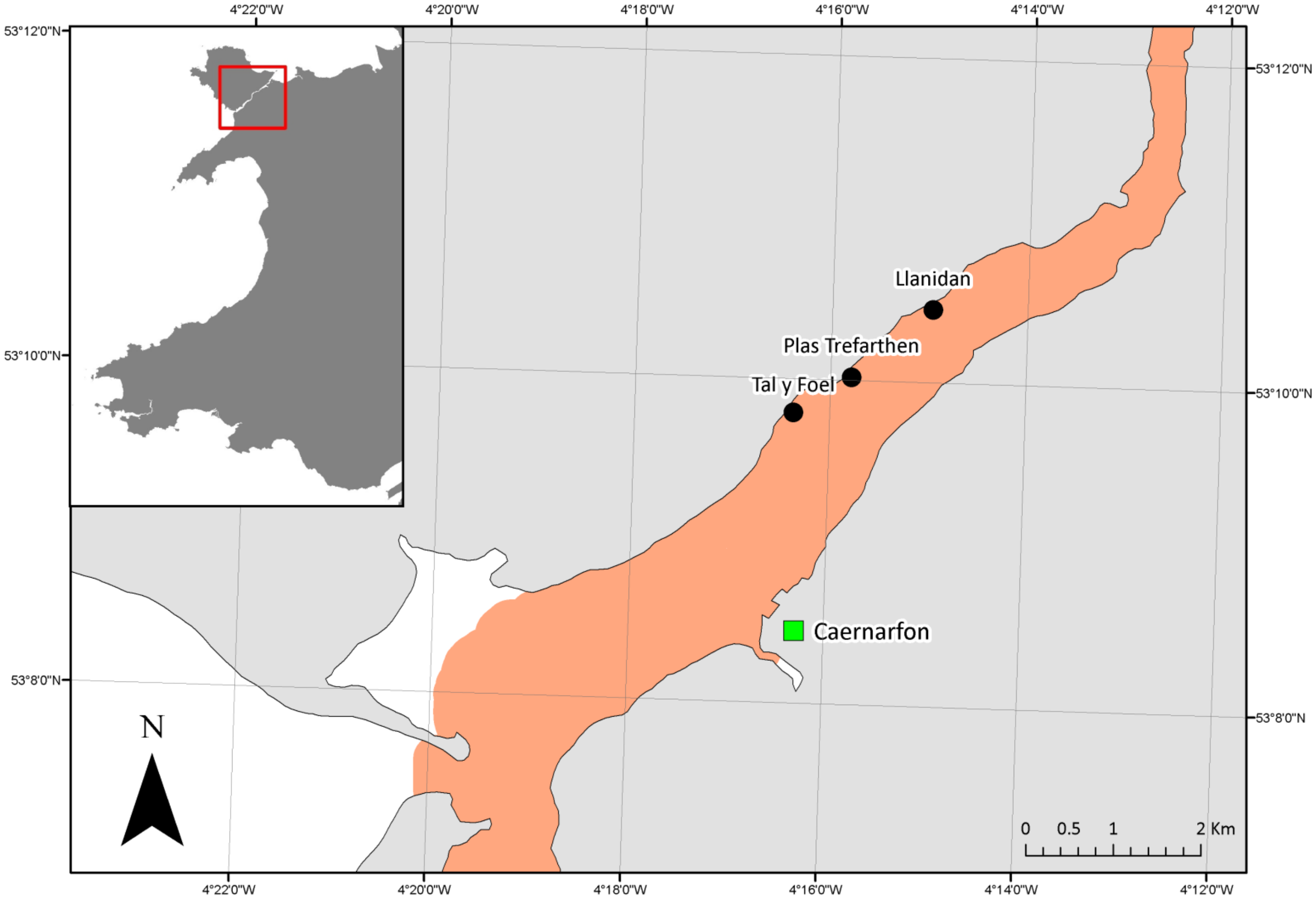


Figure 5.1 Map showing southern boundary of the Menai Strait and Conwy Bay Special Area of Conservation (SAC) (shaded in pink/orange) in relation to sites of collection and monitoring. Inset map shows general area of the entire SAC in relation to Wales (UK). Data used to generate SAC and SSSI boundaries is subject to Crown Copyright (reserved). Countryside Council for Wales, Licence No. 100018813.

namely *Mytilus edulis* and *Ostrea edulis*. Results from the laboratory experiments were complemented with real-time field observations of pre- and post-winter Chilean oyster and blue mussel densities within the SAC. Size-specific and density-dependent responses of small Chilean oysters, possibly experiencing their first winter, through to large, fully mature conspecifics to acute cold snaps were also investigated.

5.3 Methods

5.3.1 Field survey

Pre- (October 2010) and post-winter (March 2011) Chilean oyster and blue mussel abundances were determined at three sites (Llanidan, Plas Trefarthen and Tal y Foel) within the south-western end of the SAC (Figure 5.1), each known to harbour established populations of both bivalve species. *O. edulis* is extremely rare throughout the SAC and thus were not included in this part of the study. At each site, a 100 m transect parallel to the shore were surveyed at 0.8 m above chart datum. Five quadrats (0.1 m^2 each), with a distance of no more than 1 m between each quadrat, were placed at 20 m intervals along each transect line, giving a total coverage of 6 m^2 at each site. All live oysters and mussels were counted within each quadrat. Air temperature at each site was monitored every 0.5 h throughout the experimental period using a temperature logger (Gemini Tinytag™ Splash 2), housed within a Stevenson Screen and placed in an open location at <10 m above mean tidal level. Seawater temperature was also recorded using similar data loggers, affixed to solid structures at 0.8 m above chart datum at each of the three sites.

5.3.2 Animal collection and maintenance

Both *O. chilensis* and *M. edulis* were dredged from shallow subtidal populations (3.0 m below chart datum) at Plas Trefarthen (53°10'N 4°15'W) (North Wales, UK) during October 2011. Due to their rarity within the SAC, *O. edulis* were sourced from a commercial supplier (Rossmore Oysters Ltd.), who harvests a shallow subtidal population in Loch Ryan (54°55'N 05°10'W) (Scotland, UK). Despite inhabiting areas of slightly different latitudes, the collection of all species from the shallow subtidal ensured that any potential differences in their proficiency to tolerate freezing stemming from differential thermal exposures was minimal, although the likely confounding effects of environmental parameters between locations could not be eliminated. To test for any size-specific differences in tissue biomass between species, the allometric relationship between tissue dry weight and shell length was estimated for each of the three bivalve species (see Appendix VII). Thirty individuals across the size range available for each species were measured to the nearest 0.1 mm using Vernier callipers and all fouling organisms removed. The dry flesh weight of each shucked bivalve was determined following drying at 60°C for 72 h. No significant difference was observed between the slope of each length-weight relationship between species (Shell Length | Species: $F_{2,84} = 0.33$, $p = 0.717$). All remaining bivalves were thus measured to the nearest 1 mm and grouped into small (25-35 mm), medium (45-55 mm) and large (65-75 mm) size classes, equivalent to 0.07-0.20, 0.45-0.84 and 1.44-2.27 g dry flesh weight respectively. Only undamaged individuals that readily responded to physical disturbance (i.e. shell valves fully closing upon physical contact under submerged conditions) were used. Regrettably, insufficient numbers of small *O. edulis* were available, thus only two groups of native oysters (medium and large) were available for all laboratory experiments.

All bivalves were held in large, closed-system holding tanks containing fully-aerated seawater and maintained under an 8:16 h light:dark regime at a constant temperature of 5.0±0.1°C, equivalent to the typical ambient winter seawater temperature regime within the Menai Strait. Approximately 50% of the seawater within each holding tank was changed daily and a mixture of microalgal cultures (*Pavlova lutheri* (Droop) J.C. Green, *Rhinomonas reticulata* (I.A.N. Lucas) G. Novarino, *Tetraselmis chuii* Butcher) at approximately 1.0-3.0x10^6 cells mL^{-1}) was drip-fed into each holding tank. Following an acclimation period of 2 weeks, no bivalves had perished and thus all individuals were deemed adequate for use in all subsequent laboratory experiments.

5.3.3 Single acute exposure to freezing air temperatures under laboratory conditions

A total of 400 bivalves (see Table 5.1) were used to assess the size-specific survival of each species following a single, artificially-induced exposure to freezing air temperature (2 h duration). For all three species, each individual was allocated to one of five temperature treatments (three experimental and two controls), giving 10 individuals per available size class in each temperature treatment (Table 5.1). All bivalves across all three species were exposed to their respective treatment temperatures simultaneously.

Freezing air temperatures (-2, -6 and -10°C) were achieved using an external thermostat unit fitted to an ordinary house-hold upright freezer unit. A thermostatic probe (sensitive to within 1°C) was placed towards the centre of the freezer and mounted in a way so as not to be affected by the wire racks or cooling pipes within the walls of the freezer. Temperature stability was monitored using two temperature loggers, placed within the upper and lower freezing compartments respectively. An air temperature of 5°C was obtained using a standard, house-hold upright refrigerator and temperature stability was monitored in the same manner as for the freezing treatments. All treatments were thus conducted within enclosed units, standardising for any lack of air recirculation. Both 'Control' and 'Procedural Control' treatments were conducted

Table 5.1 Descriptive table showing details of each experimental treatment in which Chilean oysters (*Ostrea chilensis*), blue mussels (*Mytilus edulis*) and European flat oysters (*Ostrea edulis*) of up to three distinct size classes were exposed to various cold temperatures within enclosed household refrigerators and freezers, mimicking acute winter cold snaps. S = small (25-35 mm), M = medium (45-55 mm), L = large (65-75 mm shell length).

Treatment	**No. *O. chilensis***			**No. *M. edulis***			**No. *O. edulis***			**Description**
	S	**M**	**L**	**S**	**M**	**L**	**S**	**M**	**L**	
-2°C	10	10	10	10	10	10	n/a	10	10	Aerial exposure for 2 h at -2°C within freezer
-6°C	10	10	10	10	10	10	n/a	10	10	Aerial exposure for 2 h at -6°C within freezer
-10°C	10	10	10	10	10	10	n/a	10	10	Aerial exposure for 2 h at -10°C within freezer
Control	10	10	10	10	10	10	n/a	10	10	Submersion at 5°C within refrigerator
Procedural Control	10	10	10	10	10	10	n/a	10	10	Aerial exposure for 2 h at 5°C within refrigerator

conducted within a refrigerator, whilst all three freezing treatments were conducted within a freezer.

Following subjection to their respective temperature treatments for 2 h, all bivalves were returned to their holding tanks and mortality within each treatment group was assessed daily for a period of 28 days. An individual was considered to be dead when no response was shown to external physical disturbance and the adductor muscle was also fully relaxed. Pre-observations using time-lapse video showed that Chilean oysters that had previously been exposed to acute cold temperatures would often remain partially agape. The oysters were also slow to respond to any external physical disturbance but would, however, show signs of feeding behaviour if left submerged for a few hours. Such specimens were considered to be alive (albeit in a moribund state) and remained within the holding tanks until they showed no response. Comparisons of survival between treatments and between size classes were made using a Kaplan-Meier survival analysis and a log-rank test with Bonferroni correction (Kleinbaum and Klein 2012).

5.3.4 Increased frequency of freezing exposure under laboratory conditions

To assess the impact of cold snap frequency on native and non-native bivalves, the freezing exposure experiment (described above) was repeated with another 400 individuals, but with the addition of one additional period of exposure (2 h duration) to each respective temperature treatment, commenced 24 h following the initial exposure period. Between the two exposure periods, all bivalves were returned to the holding tanks and kept under ambient conditions as described above.

Due to the mixed semi-diurnal periodicity of the tides in the Menai Strait, this design could not provide (nor did it aim to achieve) an accurate representation of the natural conditions experienced by bivalve populations within the SAC. However, the timing of syzygy (i.e. the alignment between the sun, moon and the Earth) means that those organisms inhabiting the low intertidal within the SAC become emersed during both the coldest (during early winter mornings) and warmest (during summer afternoons) parts of each day during periods of LWST. The divergence between winter air and seawater temperatures are likely to be much lower when emersed during the warmest part of the day, thus bivalves are unlikely to undergo periods of thermal-related stress during this part of the tidal cycle. Restricting the experimental organisms to one emersion period per day also ensures that any significant morality can only be related to the period of exposure to freezing air temperatures.

5.3.5 Changes in tissue freezing rate with size, density and exposure temperature under laboratory conditions

The change in internal tissue temperature of both small and large *O. chilensis* and *M. edulis* was determined during a typical acute period of exposure (2 h duration) to current (-2 and -6°C) and predicted future (-10°C) freezing conditions. A hole (3.8 mm diameter) was drilled into the dorsal end of one shell valve (always the right, flat valve of oysters), taking care to avoid damaging the mantle tissue. Each hole was then plugged with a tapering PTFE plug, housing a thermocouple (type K) whose tip was always in direct contact with the exposed reproductive tissue. Dental wax (Majestic Drug Co. Inc.) was used to further ensure that the plugs remained air-tight. Plugged bivalves were then acclimated within the holding tank for a further three days and only healthy specimens (i.e. those that showed a closing response when touched) were used. Each bivalve was then independently subjected (i.e. one at a time) to one of three freezing temperature treatments (-2, -6 or -10°C) for a period of 2 h as described above, with the internal tissue temperature of each specimen measured every minute. Following 2 h under freezing conditions, bivalves were immediately returned to a water bath containing seawater held at 5°C. Measurements of the internal tissue temperature continued until the tissue had returned to ambient temperature. Total time spent frozen was estimated as the total number of minutes spent under the freezing point of seawater. By assessing the freezing and thawing rates of each bivalve individually (i.e. not in the presence of other conspecifics), the potential influence of neighbouring conspecifics was thus excluded whilst also mimicking areas of low oyster densities within the SAC, usually towards the edge of its non-native geographic range. For further comparative purposes, the effect of density on the rate of freezing was thus repeated for small Chilean oysters in the presence of numerous conspecifics across the entire size range, mimicking areas within the SAC where *O. chilensis* is by far the most numerous species within the low intertidal (Chapter 2).

5.4 Results

5.4.1 Field survey

Air temperatures were relatively similar between sites, with differences generally less than the stated accuracy of the data loggers. Site data were therefore pooled to give an average air temperature profile for the south-western end of the SAC. Air temperatures showed a high degree of variability throughout the winter of 2010-2011, with temperature differences of >8°C occasionally observed during individual days (Figure 5.2). The warmest temperatures (~13°C) were observed at ~1500 h, whilst the coldest temperatures (~-6°C) were observed at ~0600 h, with the latter coinciding with periods of MLWS. Sub-zero air temperatures were observed at

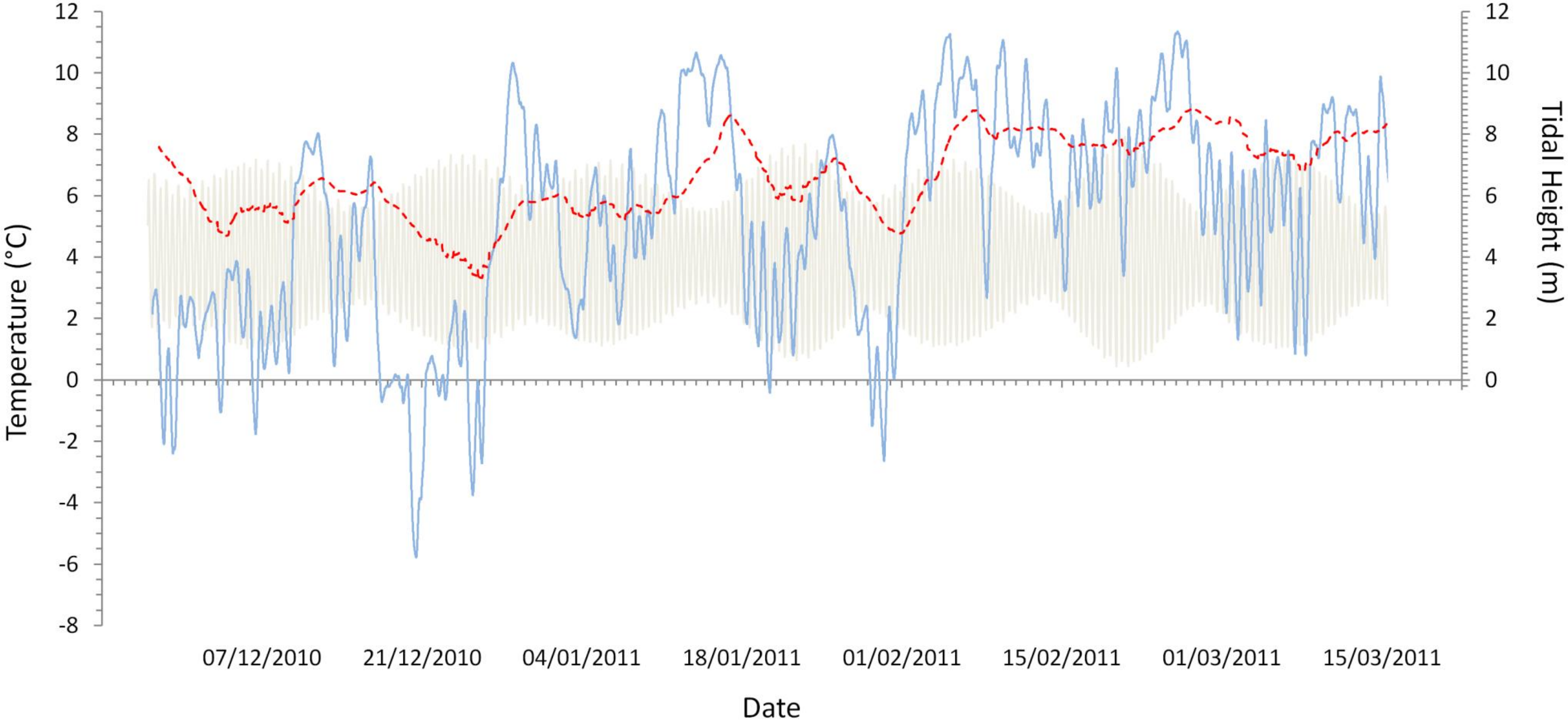


Figure 5.2 Mean winter air (blue line) and sea (red dotted line) temperatures (°C) recorded along the shore of the Menai Strait (Anglesey, North Wales, UK) during 2010-2011. Data overlay the change in tidal height (m above chart datum) in the area over the same period (grey line). Chilean oysters predominantly occupy areas ≤1 m above chart datum, thus showing how they were, in general, inundated by the tide during most of the coldest freezing temperatures observed.

some point during 30 days of winter. However, freezing air temperatures <-2°C were rarely observed during the survey period and temperatures as low as -6°C were only recorded during the early hours of 20th December 2010, when *O. chilensis* would have been inundated by the tide (see Figure 5.2). Considering that the majority of the *O. chilensis* population within the SAC inhabit areas ≤1 m above chart datum, it is suggested that oysters were exposed to freezing air temperatures during 2 of the 30 days only. In addition, oysters were never exposed to temperatures lower than -1°C (see Figure 5.2). Throughout the remaining 28 days where sub-zero air temperatures were recorded, *O. chilensis* was thus able to gain refuge in the shallow subtidal.

Changes in mean seawater temperature showed a distinct temporal lag in relation to changes in air temperature and varied between ~3°C and 8°C during the course of the study period. A mean seawater temperature of 4.6°C was recorded during the coldest day of the winter period, which was comparable to the temperature chosen for the 'Control' and 'Procedural Control' treatments in the laboratory experiments (i.e. 5°C). Neither *M. edulis* nor *O. chilensis* showed any significant decline in density following the winter period at both Tal y Foel (mussels: $t = -0.95$, $df = 58$, $p = 0.348$, oysters: $t = 0.99$, $df = 58$, $p = 0.326$) and Llanidan (mussels: $t = 0.48$, $df = 58$, $p = 0.634$, oysters: $t = 0.58$, $df = 58$, $p = 0.567$). Due to a period of stock manipulation by a commercial mussel farmer at Plas Trefarthen, an estimation of mean post-winter mussel density for this site was not possible. Pre- and post-winter oyster densities at this site again were not significant different ($t = 1.88$, $df = 58$, $p = 0.064$) (see Figure 5.3).

5.4.2 Size-specific response to acute periods of freezing air temperatures under laboratory conditions

No significant difference was observed between the survival rate of small, medium and large *O. chilensis* within each temperature treatment (Kaplan-Meier Survival Analysis: $\chi^2 \leq 2.00$, $p \geq 0.368$). *O. chilensis* size-classes were therefore pooled across each treatment and their survival rate at each treatment temperature compared using a log-rank test (Figure 5.4). Survival rate decreased significantly with decreasing freezing air temperautre ($\chi^2 = 98.87$, $df = 4$, $p<0.001$), with median time until death estimated at 27.6±0.4, 25.5±0.7 and 18.9±1.4 days at -2, -6 and -10°C respectively. Four weeks following a single 2 h exposure period at -2°C, -6°C and -10°C, *O. chilensis* survival was observed to be 97%, 84% and 55% respectively. Similarly, no significant difference was observed between the survival rate of small, medium and large Chilean oysters at each temperature treatment when cold snap frequency was effectively doubled (Kaplan-Meier Survival Analysis: $\chi^2 \leq 0.592$, $p \geq 0.744$). Again, pooling oysters across each respective treatment revealed that survival rate decreased significantly with decreasing freezing air temperautre (χ^2 =

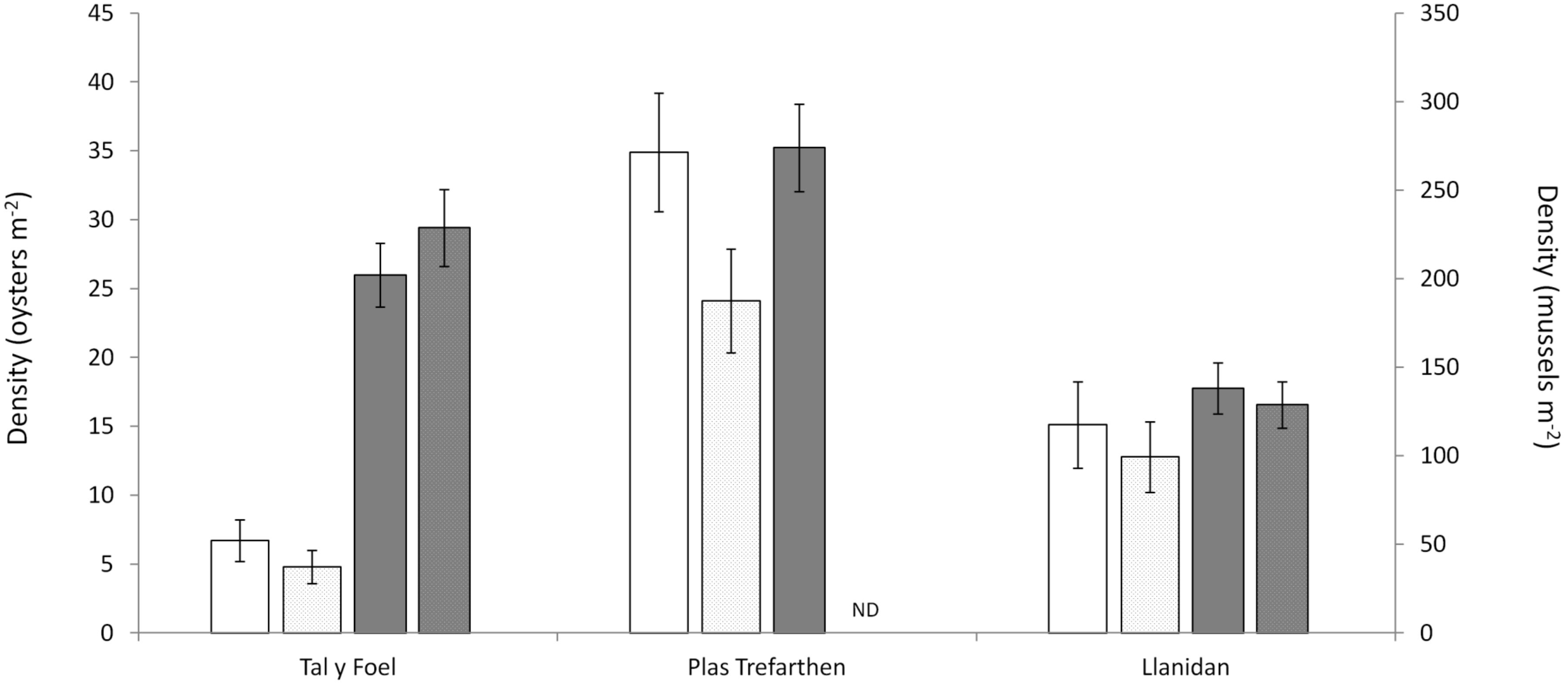


Figure 5.3 Pre- (solid bars) and post-winter (dotted bars) mean (±SE) densities (m^{-2}) of non-native Chilean oysters (*Ostrea chilensis*) (white) and native blue mussels (*Mytilus edulis*) (dark grey), during winter 2010-2011 at three sites located within the Menai Strait and Conwy Bay SAC (North Wales, UK). ND = no data available at due to unexpected harvesting of mussel population at this location.

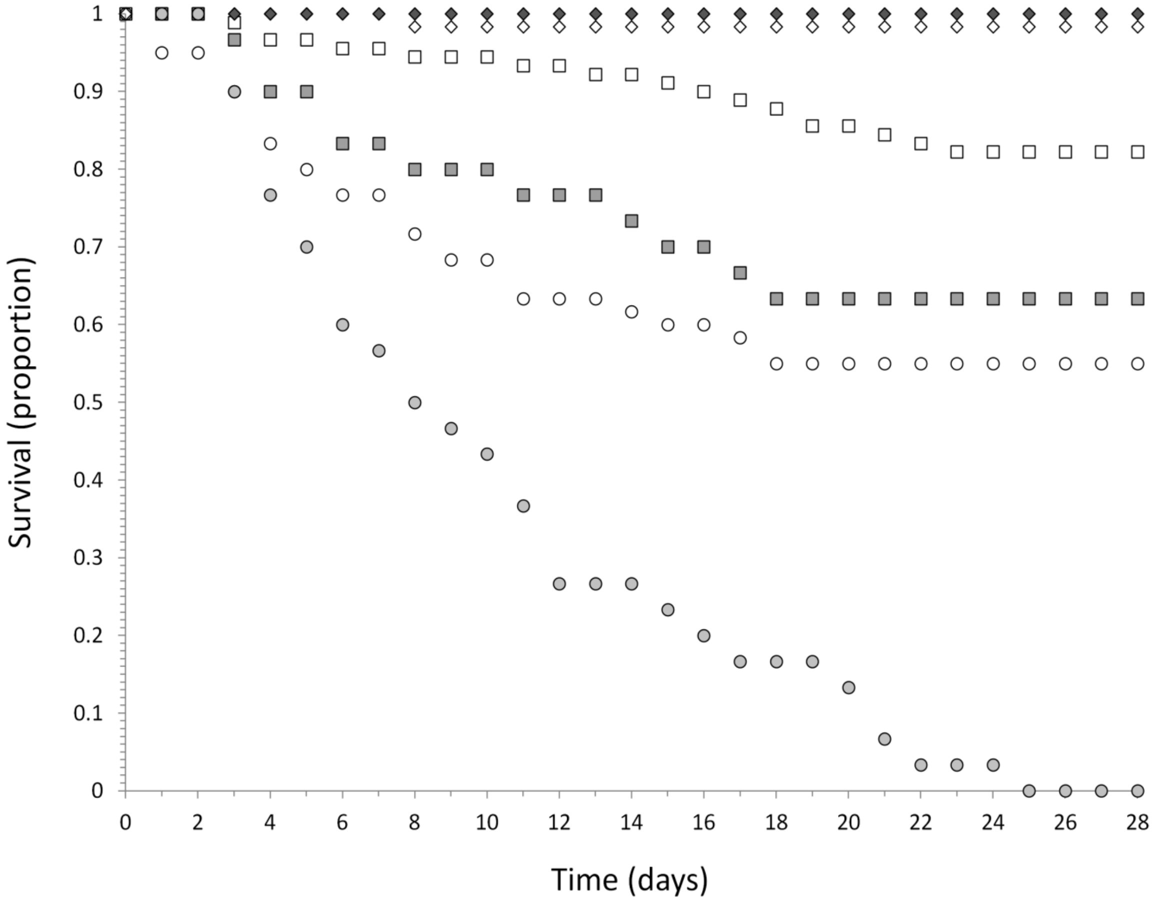


Figure 5.4 Pooled proportion of Chilean oysters (*Ostrea chilensis*) surviving after exposure to air temperatures of -2, -6 and -10°C for 120 minutes, either during one or two consecutive days. All oysters survived both control and intermediate control treatments and have hence been removed from the figure to improve clarity. Symbols: open diamond = -2°C, single period; closed diamond = -2°C, double period; open square = -6°C, single period; closed square = -6°C, double period; open circle = -10°C, singe period; closed circle = -10°C, double period.

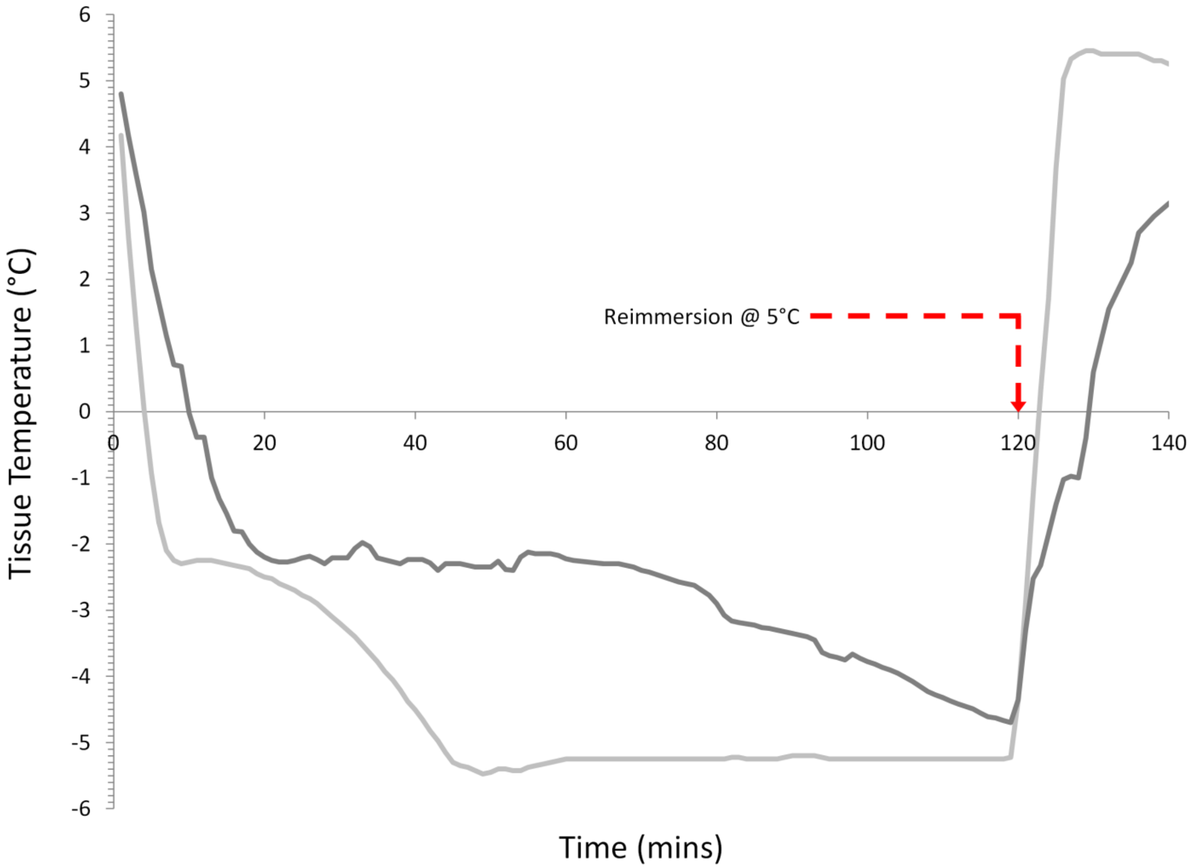


Figure 5.5 Change in tissue temperature of small (light grey) and large (dark grey) Chilean oysters (*Ostrea chilensis*), exposed to an aerial temperature of -6°C for 120 minutes and subsequentlly reimmersed in seawater held at 5°C (depicted by dashed arrow). Lines: light grey = small oysters (40-50mm shell length), dark grey = large oysters (60-70mm shell length). Similar patterns were observed for *M. edulis* when frozen at -6°C and for both species when frozen at -10°C, although freezing rates at the latter temperature were considerably greater (see Table 5.2).

164.27, df = 4, p<0.001), with median time until death estimated at 20.9±1.9 and 9.3±1.2 for -6 and -10°C treatments respectively (Figure 5.5). Whilst no *O. chilensis* had died in the -2°C temperature treatment, 63% of all oysters survived at -6°C, whilst none survived at -10°C. Critically, oyster mortality across both control and procedural control treatments was negligible, confirming that the freezing air temperature was the only factor responsible for the decreasing survival rate.

Native co-inhabitants (pooled across respective size classes) showed differential responses to freezing conditions. *M. edulis* showed negligible mortality (<2%) across all treatments (χ^2 = 3.584, df = 4, p = 0.465), whilst significant *O. edulis* mortality was confined to periods of two consecutive periods of exposure to -10°C only ($\chi^2 \geq 18.965$, p<0.001). Median time until death in this instance was estimated to be 22.1±1.1 days, with only 62% oysters surviving.

5.4.3. Changes in tissue freezing rate with size, density and exposure temperature under laboratory conditions

The typical pattern of change in tissue temperature observed in small and large *O. chilensis* when individually exposed to freezing temperatures is shown in Figure 5.5. Following an initial sharp decrease, tissue temperature underwent a period of stabilisation at ~-2°C due to the counteractive effect of heat of fusion release during phase transition of the extracellular fluid to form ice. The subsequent decline indicates the rate at which ice is formed in the visceral tissue (i.e. 'freezing rate'), eventually culminating in thermal equilibrium with the external air temperature. Given that seawater in the Menai Strait (salinity ~33) freezes at ~1.9°C, internal tissue temperatures of both small and large *O. chilensis* were unlikely to reach the critical point at which they would freeze. Observations at -2°C were thus omitted from further analysis.

At both -6°C and -10°C, small *O. chilensis* froze and thawed significantly quicker than both large conspecifics and *M. edulis* of similar biomass (Table 5.2a-d). A larger distinction between thawing rates was observed between small and large *O. chilensis* frozen at -6°C (approximately seven-times quicker) compared to -10°C (approxiamtely two-times quicker) (Figure 5.6). Ice crystals were present within the tissues of small oysters for a significantly longer period than in large oysters during both temperature treatments (Table 5.2e-f). Whilst a similar relationship was observed between the different size classes of *M. edulis*, mussels cooled and thawed relatively slower than non-native oysters of similar biomass, meaning that mussels are exposed to ice crystal formation for significatly less time than oysters (pooled across size classes) (Table 5.2e-f). Gaping behaviour also differed during periods of aerial exposure. Whilst commonly-observed in *O. chilensis*, particularly large oysters, such behaviour was seldom

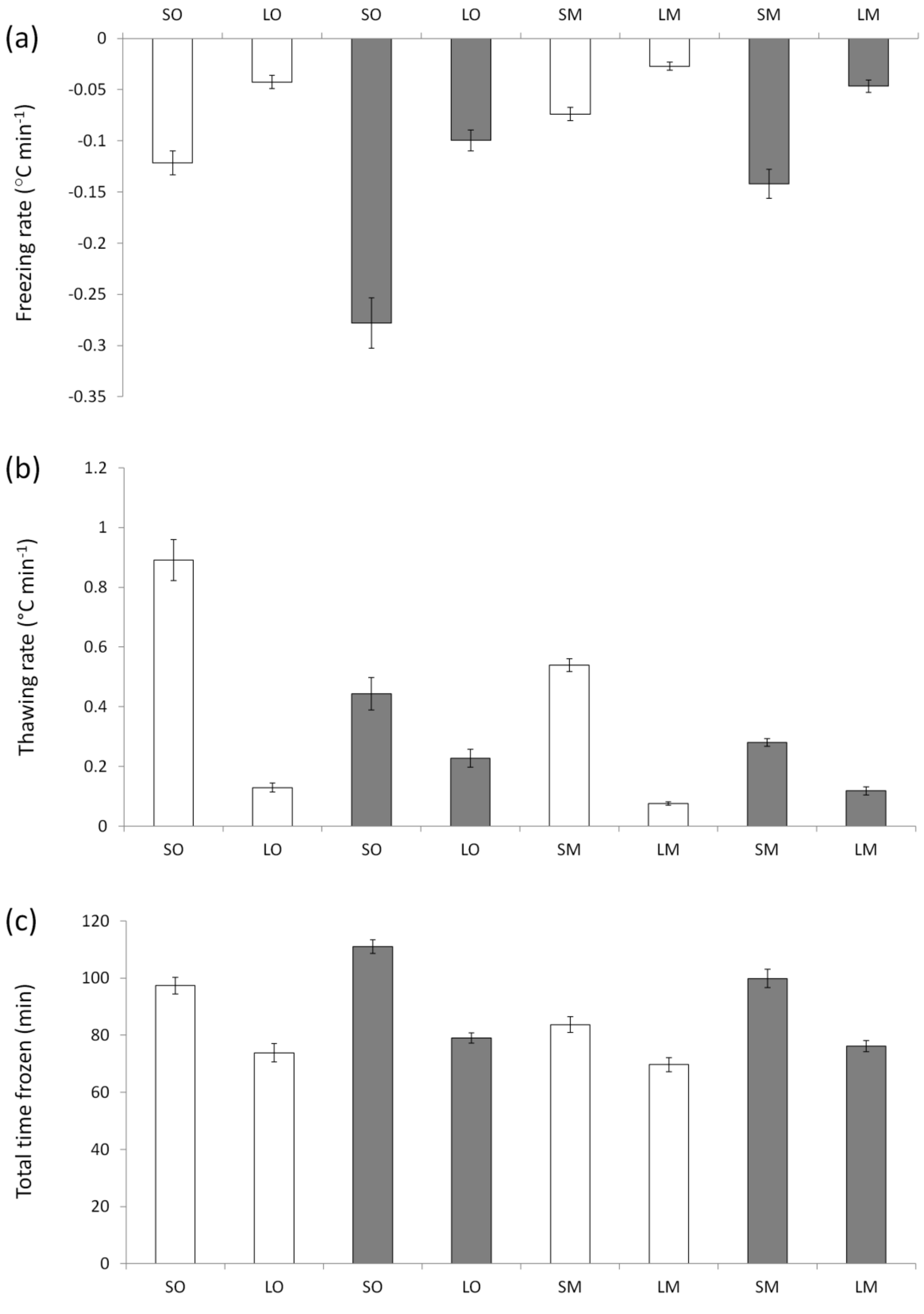


Figure 5.6 Mean (±SE) change in internal tissue temperature of small (25-35 mm shell length) and large (60-70 mm shell length) Chilean oysters (*Ostrea chilensis*) and blue mussels (*Mytilus edulis*), individually exposed to an aerial temperature of -6°C or -10°C for 120 minutes and immediately followed by a period of immersion in seawater held at 5°C. Figures (a), (b) and (c) refer to freezing rate (°C min^{-1}), thawing rate (°C min^{-1}) and total time where the tissues were frozen (mins) respectively. SO = small oysters, LO = large oysters, SM = small mussels, LM = large mussels.

Table 5.2 Fully-crossed ANOVAs examining freezing and thawing rates (°C min^{-1}) of small (35-45 mm shell length) and large (65-75 mm shell length) Chilean oysters (*Ostrea chilensis*) and blue mussels (*Mytilus edulis*) , as well as the total time for which tissues remain frozen (mins) following a 2 h period at -6 or -10°C.

(a) -6°C Freezing rates

Source of Variation	df	MS	F	p
Species	1	0.0057	15.74	<0.001
Size	1	0.0231	63.62	<0.001
Species x Size	1	0.0017	4.70	0.0483
Residual	20	0.0004		
Total	23			
Cochran's Test		C = 0.578, p<0.05		
Transformation		None		
		SNK Test	Species x Size (SE = 0.008)	
			Sp(Si):	
			Small	Oysters<Mussels
			Large	ND

(b) -10°C Freezing rates

Source of Variation	df	MS	F	p
Species	1	0.054	37.67	<0.001
Size	1	0.113	79.00	<0.001
Species x Size	1	0.010	7.30	0.014
Residual	20	0.001		
Total	23			
Cochran's Test		C = 0.637, p<0.05		
Transformation		None		
		SNK Test	Species x Site (SE = 0.02)	
			Sp(Si):	
			Small	Oysters<Mussels
			Large	Oysters<Mussels

(c) -6°C Thawing rates

Source of Variation	df	MS	F	p
Species	1	1.6188	40.97	<0.001
Size	1	23.0260	621.09	<0.001
Species x Size	1	0.0008	0.02	0.888
Residual	20	0.0371		
Total	23			
Cochran's Test		C = 0.463, p>0.05		
Transformation		Log$_{10}$		
		SNK Test	Species (SE = 0.06)	
			Species:	
			Across all Sizes	Mussels<Oysters
			Size (SE = 0.06)	
			Size:	
			Across all Species	Large<Small

(d) -10°C Thawing rates

Source of Variation	df	MS	F	p
Species	1	72.430	23.37	<0.001
Size	1	3.744	50.75	<0.001
Species x Size	1	0.065	0.88	0.359
Residual	20	0.074		
Total	23			
Cochran's Test		C = 0.426, p>0.05		
Transformation		Log$_{10}$		
		SNK Test	Species (SE = 0.08)	
			Species:	
			Across all Sizes	Mussels<Oysters
			Size (SE = 0.08)	
			Size:	
			Across all Species	Large<Small

(e) -6°C Total time frozen

Source of Variation	df	MS	F	p
Species	1	477.042	9.71	0.005
Size	1	2109.375	42.92	<0.001
Species x Size	1	135.375	2.75	0.113
Residual	20	49.142		
Total	23			
Cochran's Test		C = 0.320, p>0.05		
Transformation		None		
		SNK Test	Species (SE = 2.02)	
			Species:	
			Across all Sizes	Oysters>Mussels
			Size (SE = 2.02)	
			Size:	
			Across all Species	Small>Large

(f) -10°C Total time frozen

Source of Variation	df	MS	F	p
Species	1	294.00	8.65	0.008
Size	1	4648.17	136.78	<0.001
Species x Size	1	104.17	3.07	0.096
Residual	20	33.98		
Total	23			
Cochran's Test		C = 0.457, p>0.05		
Transformation		None		
		SNK Test	Species (SE = 1.68)	
			Species:	
			Across all Sizes	Oysters>Mussels
			Size (SE = 1.68)	
			Size:	
			Across all Species	Small>Large

observed in mussels. Critically, small oysters showed a significant, approximately three-fold reduction ($F_{1,11} \geq 35.38$, $p<0.001$) in freezing rate when positioned among similar-sized conspecifics as opposed to when they were measured in isolation (Figure 5.6).

5.5 Discussion

Severely cold winter temperatures are known to cause mass mortalities within many temperate intertidal populations (e.g. Crisp 1964; Firth *et al.* 2011; Wethey *et al.* 2011). This chapter lends support to the hypothesis that native and non-native competitors may differ in their response to cooler air and seawater temperatures associated with future climatic change, although these responses may not always be favourable to the invading species. Whilst unlikely to halt the poleward migration of both native and non-native taxa, the predicted increase in both the frequency and intensity of acute periods of extreme freezing temperatures may operate as a critical 'reset' mechanism which inhibits the rate of poleward spread of introduced species. Canning-Clode *et al.* (2011) suggest that the survival of the non-native green porcelain crab, *Petrolithses armatus* (Gibbes 1850), in the warm Atlantic waters of the south-eastern United States is severely hampered by periods of exceptionally cold winter seawater temperatures. The northern geographic range limit of the invasive lionfish (*Pterois* spp.) within the same geographic area is also thought to be determined by temperature (Kimball *et al.* 2004). However, for mobile species such as these, their survival during periods of extremely cold winters is partly determined by their ability to migrate to areas of more favourable temperature at a rate that is quicker than that of the cooling environment (see Hiddink and ter Hofstede 2008; Burrows *et al.* 2011). This chapter presents a relatively easier and perhaps more pertinent alternative by investigating the impact of present and future acute freezing events on sessile habitat-modifying species that predominate in the intertidal zone, where the magnitude of temperature aberrations is likely to be much greater.

Our study provides strong evidence to suggest that current sub-zero winter air temperatures may not be quite cold enough to significantly hamper the persistence of the non-native *Ostrea chilensis* population in the Menai Strait, with only 18% of the intertidal population expected to perish when exposed to a single 2 h period at -6°C (i.e. the coldest air temperature observed). Due to its aggregated distribution and its rarity at tidal heights >1 m above chart datum, it is also proposed that *O. chilensis* may have avoided prolonged exposure to freezing air temperatures during the relatively cold winter of 2010-2011 simply due to the mismatch between periods of extreme LWST and the coldest freezing air temperatures. However, Chilean oysters are likely to experience much higher rates of mortality in the near future if forecasted increases in the frequency and intensity of cold winter temperature aberrations in the Northern

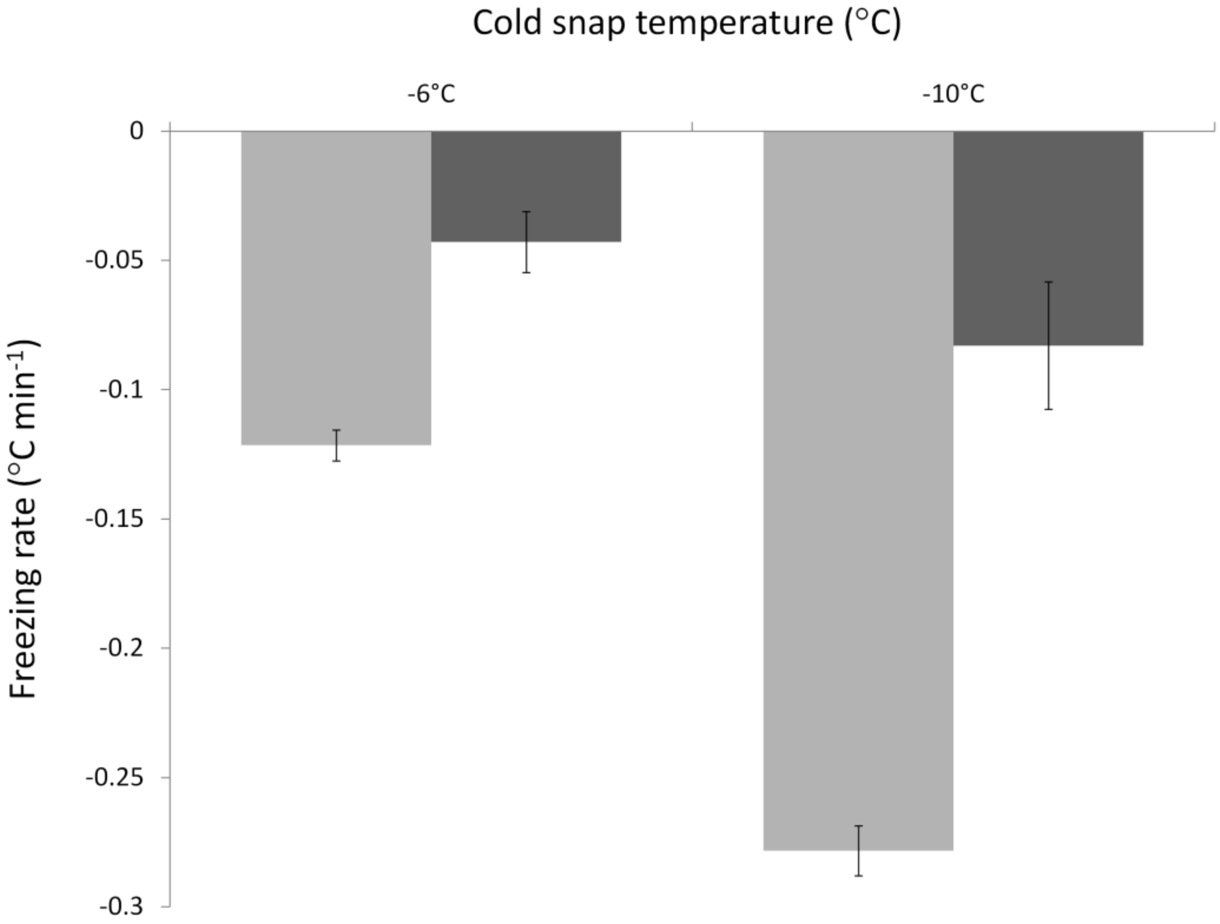


Figure 5.7 Differential tissue freezing rates of small (25-35mm) Chilean oysters exposed to sub-zero cold snap temperatures (°C) in isolation (light grey bars) or in the presence of conspecifics (dark grey bars). Error bars = ±1SE.

Hemisphere (see Wang *et al.* 2010; Liu *et al.* 2012) are correct. At -6°C, oyster mortality was nearly twice as high when cold snap frequency was effectively doubled. Furthermore, whilst nearly half of the Chilean oysters are expected to perish following an exposure to a single 2 h period at -10°C, two consecutive daily periods of -10°C is very likely to lead to a rapid loss of the entire intertidal *O. chilensis* population, even in areas of high oyster density. This chapter highlights the need for a more long-term assessment of survival following periods of freezing stress. Mortality across all treatments (where mortality was significant) was not observed until approximately 3 days following cold snap exposure. Ibing and Theede (1975) also showed how a mortality response following exposure to freezing conditions can be delayed for several days. With the frequency and intensity of cold snaps increased, the periodicity of significant mortality (i.e. the time up to MTTF) also increased. *O. chilensis* mortality was observed even after 3 weeks following exposure to freezing air temperatures, suggesting that long-term monitoring of native and non-native intertidal populations is required following cold snap periods.

The seminal work of Southward (1958) demonstrated how the thermal tolerance of intertidal organisms are often closely-related to the extent of both their geographic range and their occupied positions along the intertidal gradient. Consideration of the geographic distribution of each species investigated in the current chapter is in agreement with this concept, indicating that *O. chilensis* is not as well-adapted to deal with periods of sub-zero temperatures compared with two of its new ecological competitors. The Chilean oyster spans between 36-46°S latitude in the Southern Hemisphere (Toro 1995; Jeffs *et al.* 1996), but is confined to higher latitudes (53°N) beyond its native geographic range (Chapter 2). Despite this clear latitudinal difference, the interaction between the atmospheric circulation and oceanic heat exchange gives the UK its mild climate relative to several US states which occur on the same latitude (Seager *et al.* 2002). This results in a climatic similarity of 70% between the native and non-native range of *O. chilensis* ('CLIMATCH', Bureau of Rural Sciences 2009). Critically, however, harsh winter ECEs are limited in both Chile and New Zealand, where sub-zero temperatures in coastal regions are likely to be restricted to the poleward fringe of the Chilean oysters' native geographic distribution.

Suitable habitat for *Ostrea chilensis* occurs down to far deeper depths in the coastal waters of New Zealand (see Cranfield *et al.* 2001) than in the Menai Strait, meaning that subtidal populations are more likely to predominate within its native geographic extent. In contrast, *O. chilensis* within the Menai Strait generally occupies a narrow band from the low intertidal (2 m above chart datum) into the shallow subtidal (<8 m below chart datum), with the highest densities concentrated around 0.5 m below chart datum (Chapter 2). Information regarding the freeze tolerance of *O. chilensis* is scant and restricted to anecdotal evidence in Walne (1974), who notes that the Chilean oyster is unable to cope with periods of *"quite moderate frost"*, although no experimental data are provided to support this statement. By contrast, *M. edulis* showed greater resilience to freezing conditions than *O. chilensis*. In north-western Europe, the geographic range of *M. edulis* extends from the Franco-Spanish border (~42°N) as far north as the Svalbard Archipelago (~78°N) (Christiansen 1965). Mussels are also found along a much wider extent of the vertical shore gradient than both oyster species, with open coast populations predominating in the intertidal and reproductively-active individuals found throughout the entire eulittoral zone (Seed 1969). The freeze tolerance of mussels has been extensively studied and they have been shown to withstand exposure to temperatures of -10°C for at least 24 h (Williams 1970). Mussels were therefore not expected to perish during the current study, although their inclusion served as a useful proxy to determine whether or not any mortality may have been introduced due to the artificial freezing conditions. Significant losses of native oysters were restricted to those exposed to potential future cold winter temperatures, although mortality was never greater than 38%. In terms of its native geographic range, *O. edulis* occurs as far north as the Norwegian Sea (~62°N) (Alcaraz and Dominguez 1985). This species predominantly populates areas towards the low-water mark of the intertidal and into the shallow sublittoral, although it is extremely tolerant to periods of tidal emersion (Hummel *et al.* 1988). Native oysters can therefore be considered to be relatively well-adapted to periods of freezing temperatures.

Whilst information regarding the freeze tolerance of *O. edulis* is currently lacking, juvenile *O. edulis* are known to survive for several weeks in seawater maintained at 3°C, even in the absence of an exogenous food source (Child and Laing 1998). Shell valve gaping and high mortalities were reported in UK *O. edulis* populations during the extremely harsh and extended winter of 1962-63 (Crisp 1964). Mortality was attributed to the limited functioning of the adductor muscle, resulting in gaping, which in turn compromised the oyster's ability to deal with sediment loading. Observations of gaping behaviour were largely restricted to *O. chilensis* during the current study. *Ostrea chilensis* responded to physical stimuli and were thus deemed to be alive until the functionality of the adductor muscle was completely lost. It is likely that the rate of decline in the survival of *O. chilensis* will be even greater in the Menai Strait than in the laboratory since under field conditions silt concentration will be much higher and the combination of silt loading and low temperatures would act synergistically and increase oyster mortality.

Gaping behaviour has been documented in several bivalve species during periods of physiological stress (Davenport and Wong 1994) and is commonly cited as a mechanism for increasing aerobic respiration (Moon *et al.* 1970; Bayne *et al.* 1976; Nicastro *et al.* 2008). Many bivalves can utilise anaerobic metabolic pathways during periods of both short-term (e.g. during tidal emersion, predator attacks) and long-term stress (e.g. during cold winters, exposure to noxious environments). The American oyster, *Crassostrea virginica* (Gmelin 1791), is known to be reliant on the coupled fermentation of glycogen and asparate during such periods, with succinate and alanine accumulated as the metabolic end products (de Zwaan and Wijsman 1976). No information is currently available on the anaerobic metabolic pathways of *O. chilensis* and no attempts were made to control for any inter-specific variability in gaping-induced rates of evaporative cooling during the current investigation. Whilst gaping may potentially allow for optimum metabolic functioning during periods of tidal emersion, such behaviour will also inevitably lead to a significant level of water loss, although not always resulting in a significantly greater rate of mortality (see Lent 1969; Bayne *et al.* 1976). Differential behaviour during periods of tidal emersion has been shown to cause niche separation between indigenous (*Perna perna* L. 1758) and invasive (*Mytilus galloprovincialis* Lamarck 1819) mussels (Nicastro *et al.* 2010). Further studies would be required to test this hypothesis under cold (as opposed to warm) thermal extremities.

The influence of thawing rate on the tolerance of organisms to freezing temperatures may, on occasion, be dependent upon the rate of freezing. In some cases, a critical freezing rate is evident, above which a slower thawing rate will lead to significantly more physical damage to the organism than if the tissues were thawed more quickly (Malek and Bewley 1978). Alternatively, a slower thawing rate is more beneficial when the rate of freezing is below the critical value. Clear size-specific differences were observed in freezing and thawing rates of both oysters and mussels when individually exposed to extreme sub-zero air temperatures, with smaller bivalves freezing and thawing much quicker than larger conspecifics. The rate of freezing is known to be a critical component in determining both the degree of freeze tolerance in intertidal organisms and whether or not ice formation will occur within the tissues (Murphy and Johnson 1980). Smaller organisms have a higher surface area to volume ratio, meaning that cold air can act on more of their surface relative to their volume per unit time. The endothermic phase transition of seawater from liquid to solid (i.e. latent heat of fusion) will also be prolonged in larger individuals, meaning that their tissues will be buffered against the effects of freezing air temperatures for a longer period, thus delaying the onset of tissue ice formation (see Williams 1970).

The lack of any significant difference in the survival rates across size classes when bivalves were exposed to freezing temperatures in numbers analogous to the high densities observed at several areas within the SAC. Smaller, younger Chilean oysters are thus either more capable of tolerating freezing than larger conspecifics or are somehow offered some kind of protection from freezing air temperatures by their larger conspecifics within the oyster matrix. Our observations of a significant reduction in the freezing rate of small oysters when located within a dense patch of conspecifics as opposed to when exposed in isolation suggests that 'strength in numbers' may become critical to the long-term survival and dominance of this NNS in a rapidly-changing climate. Previous work has demonstrated the significance of position (both between shores and within patches of conspecifics on a single shore) on body temperature. For example both Helmuth (1999) and Denny *et al.* (2011) demonstrated how mussels occupying the edge of a patch can differ in their body temperatures by as much as compared to those occupying within the matrix, although the direction of this relationship is likely determined by experimental conditions (e.g. wind direction and strength, patch size). Furthermore, inhabiting an area within the matrix increases the thermal inertia of the oyster patch, thus mitigating any rapid temporal changes in key physical environmental parameters. This has critical implications for the future invasion dynamics of *O. chilensis* outside its native range. *Ostrea chilensis* locally forms dense patches of up to 232 individuals m^{-2} within the Menai Strait, whilst also occurring as single individuals in areas towards the edge of its geographic range (Chapter 2), possibly due to small-scale, anthropogenically-mediated transfers (Chapter 4).

Conclusions

Our limited understanding of the potential impacts of ECEs on both native and non-native species remains limited partly due to our neglecting of the 'increasing variability' element of climate change (see Lloret *et al.* 2012). Whilst further anthropogenically-mediated increase in global atmospheric temperature will continue to facilitate the poleward migrations of many native and non-native species alike, ECEs such as cold snaps are likely to play a critical role in alleviating the rate of biological invasions, particularly for sessile, epibenthic marine organisms that inhabit the intertidal zones. Size, density and behaviour (e.g. gaping, gregariousness) are all important parameters which can alleviate the stresses exhibited during cold snaps and need to be carefully considered when investigating the potential responses of species to a rapidly-changing climate. Particular focus on the impacts of invasive habitat-modifiers is justified. In the case of non-native habitat modifiers like *O. chilensis*, it is also important to consider their lasting effect on the native ecosystem, even following significant mortality events. Whilst the translocation of energy from the pelagic to the benthic food web is restricted to live oysters, the ecosystem engineering capacity of oysters through their habitat modifying abilities is preserved well beyond the lifetime of the animal.

Appendix VII Consistency in size-specific biomass between *O. chilensis*, *M. edulis* and *O. edulis.*

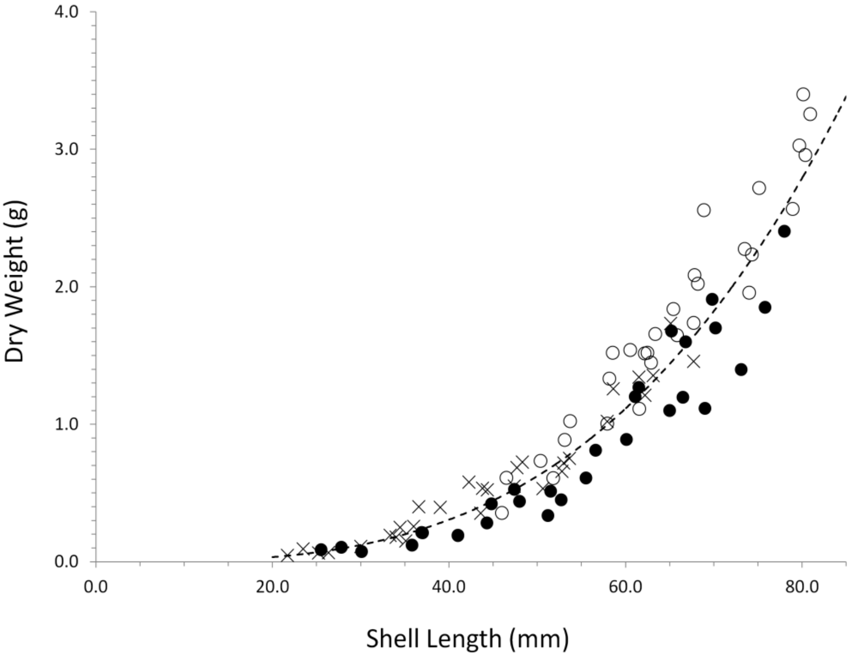


Figure XVI Relationship between tissue dry weight (g) and shell length (mm) of the Chilean oyster (*Ostrea chilensis*; filled circles), the blue mussel (*Mytilus edulis*; crosses) and the European oyster (*Ostrea edulis*; open circles). Regression model ($Y = a.X^b$) fitted to data pooled between species, where a = 2.37×10^{-6} and b = 3.19 ($R^2 = 0.963$).

Table II ANOVA table with shell length as a covariate, comparing the allometric relationships between dry flesh weight (g) and shell length (mm) of *Ostrea chilensis*, *Mytilus edulis* and *Ostrea edulis*.

Source	df	MS	F	p
Shell Length	1	8.586	1052.45	<0.001
Species	2	0.006	0.76	0.470
Shell Length x Species	2	0.003	0.33	0.717
Residual	84	0.008		
Total	89			

Chapter 6

General Discussion

Ever since the recognition that biological invasions pose one of the most prevalent threats to biodiversity and ecosystem functioning on a global scale, attempts have been made to identify predisposed characteristics that may assist in the detection of those non-native species that are most likely to show a high degree of invasiveness following their introduction (e.g. Rejmánek and Richardson 1996; Williamson and Fitter 1996; Goodwin *et al.* 2001; Kolar and Lodge 2001; Lockwood *et al.* 2005). 'Propagule pressure' (i.e. the product of the number of introduction events and the number of propagules released during each introduction event) is widely-acknowledged as being the primary determinant of establishment success (see Lockwood *et al.* 2005). Similarly, the likelihood of 'range expansions' or 'secondary spread' is highly-dependent on the extent of propagule release by the newly-established population (Rouget and Richardson 2003). Species with a large dispersal capacity and extended breeding season are thus more likely to become successful invaders.

Based on its reproductive dynamics and life history characteristics alone, the Chilean oyster is not what one would consider typical of a successful invader. A significant energetic investment in the production of large, yolk-rich ova results in the production of relatively few larvae compared with other oyster species (Cranfield and Allen 1977). *Ostrea chilensis* has the longest brooding period of any oyster species (6-8 weeks), with the release of larvae as pediveligers (Millar and Hollis 1963) meaning that propagule dispersal is generally limited to <1 km away from the adult stock population (Broekhuizen *et al.* 2011; Chapter 3). The breeding season of *O. chilensis* is also restricted to the warmer summer months within high latitude populations across both its native (Cranfield and Allen 1977; Westerskov 1980; Jeffs and Hickman 2000) and non-native geographic extent (Chapter 3). However, the strong stock-recruitment relationship consistently observed in the Menai Strait during all three years of study (Chapter 3) is contradictory to its relatively large range extension (>30 km) during the last 20 years (Chapter 2). This suggests that other important transport vectors (e.g. rafting, bivalve culture, periwinkle collection) are currently in operation (see Chapters 2 and 4). Despite its low reproductive output, a high degree of spat recruitment was observed within several established oyster patches along the Menai Strait (Chapter 3). In agreement with the 'Enemy Release Hypothesis' (Elton 1958), preliminary data suggests that, in the absence of natural predators, newly-settled *O. chilensis* may likely thrive due to their limited subjection to predation pressure

(Appendices III and IV). The occurrence of *O. chilensis* in locally high densities may also buffer against periods of physiological stress, even in the face of global climate change (Chapter 5).

The *O. chilensis* population in the Menai Strait offered a unique opportunity to study a biological invasion of known provenance. This study highlights what can happen when a seemingly innocuous and perhaps 'uncharismatic' non-native species is left unregulated (see Chapter 2). Following its deliberate initial introduction by the MAFF in 1962, a 50-year period has elapsed where any interest in the *O. chilensis* invasion in the Menai Strait has been, for the most part, non-existent. The remainder of this discussion chapter is intended to serve as a timely synopsis of three pertinent and topical points of interest emerging from this chapter, namely 1) the current uncoordinated strategies relating to the management of non-native species within the UK, 2) the potential of unregulated anthropogenic activities as facilitators of secondary spread, and 3) the potential impacts of extreme climatic events as regulators of the rate of biological invasions. I conclude with a discussion regarding several management options relevant to the *O. chilensis* population within the Menai Strait and Conwy Bay SAC, which will hopefully form a basis for numerous discussions in light of the information presented herein.

6.1 Bridging the gap – preventing new introductions and mitigating against secondary spread by improving coordination between decision makers

Equitable, efficient and sustainable management of natural resources has often been facilitated by the devolvement of specific conservation objectives to several distinct but collaborative entities (Dressler *et al.* 2006), but in the case of non-native species management, this may arguably be a case of 'too many cooks...'. Responsibilities regarding various aspects of biological invasions have traditionally been divided between several organisations within the conservation sector, including agriculture, fisheries, forestry, pest control and water resource management. As a result, management strategies and legislation concerning several adjacent habitats and ecosystems have often become uncoordinated, despite clear similarities in terms of their respective conservation objectives (Chapter 2). Whilst this predicament may seem trivial at first, it has critical implications for the regulation of marine non-native species which span both sides of the intertidal-subtidal boundary. It has meant that management strategies and legislation concerning one portion of a non-native population may not necessarily offer adequate provision for the remaining individuals which occupy what would strictly be termed a 'different' habitat. The successful management of non-native species whose vertical distribution spans both the intertidal and shallow subtidal thus brings with it a major discrepancy unless complete coordination and collaboration between all stakeholders are obtained. A sound understanding and empathy for the different environmental factors operating is also required if successful management strategies are to be implemented.

Whilst a considerable number of statutory authorities and non-governmental bodies are, to some degree, concerned with various aspects of non-native species management and legislation, there has been, until very recently at least, a lack of a central strategic approach adopted across all involved parties. In addition, the lack of cooperation and coordination between all those involved has occasionally resulted in periods where statutory and non-governmental bodies were completely unaware of the work carried out by one another (Fasham and Trumper 2001). The recent formation of the GB Non-Native Species Secretariat has facilitated some degree of coordination between all relevant parties. However, numerous fallacies have been identified in the current UK legislation framework which overlooks both transient and established non-native species that have yet fully demonstrated their invasive tendencies (see Chapter 2). The EC Regulation concerning the use of alien and locally-absent species in aquaculture 2007 provides a dedicated framework regarding *"the introduction of alien species and translocation of locally absent species for their use in aquaculture within the EC"*. Despite its rather unclear definition of the term *"introduction"*, this legislative instrument may encouragingly concern deliberate movements of a NNS to *"an environment outside its natural range for use in aquaculture"*, and may thus include the intentional movements of those species that have already established within the EU to areas beyond their natural dispersal capacity. It currently remains unclear how this Regulation will be transcribed to UK legislation, and I would therefore advocate further clarification for the inclusion of already established NNS within any new additions or amendments to the legislation framework at both UK- and EU levels.

6.2 Unregulated anthropogenic activities – inconspicuous facilitators of biological invasions

Coastal anthropogenic activities can act as transport vectors which facilitate the range expansion of many introduced species. Such activities which remain unregulated have the potential to obscure management efforts regarding non-native species, particularly when the species in question has a particularly short natural dispersal capacity (see Chapters 3 and 4). Whilst the spread of several species of algae, for example, is restricted to a distance of <100 m via natural means alone (Dudgeon *et al.* 2001), anthropogenic activities such as recreational boating can significantly augment their dispersal capacity (Johnson *et al.* 2001; Minchin *et al.* 2006; Mineur *et al.* 2008). Despite increasing vigilance and enforcement of legislation regarding commercial vessels and their ballast water activities in North America (see US Coast Guard – Implementation of Ballast Water Discharge Standard, 21 June 2012), there currently remains no monitoring of inter-state traffic of recreational boats within this region. The potential role of unregulated coastal anthropogenic activities as facilitators of both introductions and range expansions of non-native species is thus identified as a highly pressing issue in need of immediate further investigation.

6.3 Global climate change – more than just warming!

Evidence is gathering supporting the view that biological invasions and range expansions of introduced species are likely to increase in a warming climate. Native species are expected to struggle, whilst invasive species have been shown to become more persistent with warming sea temperatures (Stachowicz *et al.* 2002; Hellmann *et al.* 2008; Sorte *et al.* 2010). Whilst an increase in mean global surface temperature of 0.74±0.18°C is expected by the end of this century (IPCC 2007), the increasing *variability* in climatic parameters is also likely to affect both native and non-native species. Our generally warming climate is thus expected to be punctuated by numerous extreme climatic events (ECEs), including hurricanes, storms, flooding, heat waves, droughts and cold snaps (see Chapter 5) of increasing frequency and intensity (IPCC 2012). Whilst the ecological responses of marine communities to climatic means are becoming increasingly well-studied, the impacts of ECEs are relatively unknown (Smith 2011). It has been suggested that the failure to include the impacts of climatic extremities in modelled projections of future global biodiversity patterns may go some way in explaining some of the large variability observed (Pereira *et al.* 2010). This is of particular concern considering that maximum and minimum temperatures, as opposed to annual mean temperatures, are often of the greatest significance to the persistence and invasiveness of many non-native species (Stachowicz *et al.* 2002). Whilst not expected to prevent the poleward migration of non-native species indefinitely, future cold winter temperature aberrations have been touted as a critical 'reset' mechanism which may impede the rate of biological invasions in the near future (Canning-Clode *et al.* 2011; Firth *et al.* 2011).

Encouragingly, a 'Special Edition' on ECEs in the context of marine science was recently published by the Journal of Experimental Marine Biology and Ecology (see Firth and Hawkins 2011 and articles therein). However, many scientific endeavours concerning the potential effects of ECEs upon marine ecological systems forego what is clearly demonstrated in Chapter 5 to be critical parameters that significantly influence the overall outcome. For example, the metabolism of all ectothermic organisms is highly-influenced by both body size and environmental temperature. This relationship is most consistently observed in aquatic organisms, where the high specific heat capacity and heat of vaporisation of the surrounding water buffers against large fluctuations in temperature. However, populations occupying the intertidal zone are subjected to a distinct suite of environmental stressors compared to conspecifics inhabiting the shallow subtidal. The switch from fully submerged to fully emersed conditions is relatively rapid, making the intertidal zone one of the most stressful physical environments of all. The severity of intertidal life is further exacerbated by sessile organisms such as oysters, which cannot actively migrate to less stressful microhabitats and must instead rely on their shells as the only line of defence during times of stress. Surprisingly, several high-impact papers make no attempt to test for size-specific differences in the responses of ectothermic species to climate change (e.g. Canning-Clode *et al.* 2011). Likewise, many have restricted their analyses to observations of a single, specific density (e.g. Urian *et al.* 2010) despite clearly different responses between single specimens and those occurring in aggregations (see Denny *et al.* 2011; Chapperon and Seuront 2012; Chapter 5). Modelling and biomimetic studies are extremely useful tools in instances whereby the physiological responses of invaders within novel geographic localities are sought (e.g. Helmuth *et al.* 2006; Lima and Wethey 2009; Denny *et al.* 2011). However, such approaches do not incorporate the behavioural and/or phenotypic plasticity observed within species across their respective geographic distributions. For example, *C. virginica* at the northernmost limit of its geographic distribution has been shown to exhibit feeding activity at significantly lower temperatures than conspecifics from more southern populations (Comeau *et al.* 2012). Such traits need to be investigated and incorporated into models in order to provide a more accurate representation of the response of invaders to climate change. Additionally, community- and ecosystem-level approaches incorporating multiple complex interactions between the invader and other species which occupy the same or adjacent trophic levels are expected to reveal differential responses to climate change than those postulated from species-level studies. Over-generalistic extrapolations of individual species' responses to community- and ecosystem-level outcomes should thus be treated with caution (Walther 2010).

6.4 Management options for the non-native *O. chilensis* population in the Menai Strait and Conwy Bay SAC

The prevention of unwanted introductions is globally-acknowledged as the principal method for mitigating biological invasions (Leung *et al.* 2002), although this form of response is not always possible. Such is the case with deliberate introductions that occurred several years prior to the formation of any advice or legislation concerning non-native species introductions. In situations where prevention is not a viable option, a rapid response (i.e. early detection and eradication prior to its establishment) is recognised as the best management practice (see Wittenberg and Cock 2001). In an era of significant financial hardship, one can appreciate why policy, monitoring and management strategies have been required to focus their efforts on those species that are currently undergoing or have recently undergone a dramatic increase in their geographic distribution, overall density and threats to native ecosystems. However, bearing in mind the now well-established concept of a lag phase within the invasion process (see Crooks and Soulé 1999; Chapter 1), it is relatively straightforward to see why we may thus always struggle to overcome the threats of biological invasions.

Whilst beyond the scope of the present investigation, other studies have highlighted the potential influence of *O. chilensis* upon both the native biological community and one of the qualifying habitats of the SAC, namely 'reefs'. The provision of a hard substratum that gains increasing complexity with increasing numbers of *O. chilensis* is known to be linked with a significant increase in both epifaunal and mobile species richness (see Appendix I), although it currently remains unknown how this change translated into modifications in trophic level dynamics and ecosystem function. During my study period, an outbreak of the haplosporidian parasite (later identified as *Bonamia ostreae*) was detected in cultured native European oysters (*Ostrea edulis*) towards the south-western end of the Menai Strait. As well as the obvious implications for this locally endangered Biodiversity Action Plan-listed species, the presence of *B. ostreae* also enforced restrictions on the collection and movement of bivalves to, from and within the area, with significant impacts for the mussel industry in the region (currently the largest of its kind in Britain). Considering the likelihood that several natural and unregulated anthropogenically-mediated transport vectors are continuing to facilitate the spread of this species, the mere presence of *O. chilensis*, a known vector of *Bonamia* spp., within the Menai Strait and Conwy Bay SAC requires urgent attention. By coupling what has already been established from the current scientific literature regarding the Chilean oyster in Chile and New Zealand with the first-ever comprehensive study on the invasion dynamics of this species beyond its native geographic range presented in this thesis, a total of four management options are identified:

6.4.1 Do nothing – leave it to nature

A significant increase is expected in the frequency and intensity of acute cold winter temperature aberrations in Northern Europe (Wang *et al.* 2010; Liu *et al.* 2012). As demonstrated in Chapter 5, *O. chilensis* is poorly adapted to short periods (i.e. 2 hours) of exposure to freezing air temperatures. Future cold snaps may thus function as a critical 'reset' mechanism which could potentially eliminate the entire intertidal *O. chilensis* population within the Menai Strait. Financially, this is obviously the best option as it incurs no set-up costs whatsoever. However, Chapter 2 also documents the occurrence of significant numbers of *O. chilensis* within the shallow subtidal population which, given their survival in cold seawater temperatures (5°C; see Chapters 4 and 5), would most likely remain unaffected by forecasted acute cold temperature aberrations. Furthermore, the projected *mean* increase in seawater temperature due to global climate change (IPCC 2007) is also expected to extend the breeding season of *O. chilensis* within the Menai Strait (Chapter 3). Propagule rain is thus expected to increase, meaning that the Chilean oyster is likely to become more persistent and widespread. In addition, small intertidal oysters are expected to gain a spatial refuge away from the effects of future cold snaps by settling in and amongst high densities of adult conspecifics (Chapters 3 and 5). Intra-specific competition for food and space may thus be partly offset by the thermal benefits of aggregated behaviour.

6.4.2. Large-scale eradication effort – dredging or smothering

Eradication methods for non-native oysters can be economically and logistically feasible with rapid response to relatively novel invasions (Guy and Roberts 2010). However, eradication of this well-established species is likely to be a "high risk-high reward" approach in both ecological and economic terms. Coupling of eradication efforts with commercial aquaculture incentives may alleviate the financial burden. The eradication of the invasive gastropod, *Crepidula fornicata*, following its accidental introduction into the Menai Strait in 2007 was successfully accomplished by smothering them with several tonnes of the commercially-harvested mussel, *Mytilus edulis* (Morgan 2007b). Whilst no information is available regarding the ecological relationship between *O. chilensis* and *M. edulis*, the Pacific oyster, *Crassostrea gigas*, is known to out-compete the native mussel in the Wadden Sea due to its rapid growth and extremely high reproductive output (Diederich *et al.* 2005). Considering the much lower fecundity, slower growth rate and a highly-reduced spawning season for the Chilean oyster (Chapter 3), *M. edulis* is likely to show better competitive resilience against *O. chilensis* than that of the Wadden Sea population against *C. gigas*, although this would require empirical testing prior to its implementation. Dredging may offer a more robust approach to eradication and would also remove the allogenic engineering effects of *O. chilensis*. However, bottom-fishing gear is known to have multiple negative effects on several benthic habitats, particularly the response of biogenic habitats to scallop dredging (Kaiser *et al.* 2006). Given the current extent and dominance of the *O. chilensis* population (Chapter 2) and the expectedly high associated economic and ecological impacts upon native biodiversity, ecosystem function and health of the qualifying habitats of the SAC, this option is unlikely to be feasible and must serve as a timely reminder of the importance of early detection and a rapid response to biological invasions.

6.4.3 Mitigation – target transport vectors and trial (small-scale) eradication

The paradoxical relationship between the restricted natural dispersal capacity (Chapter 3) and relatively long-range range expansion of the *O. chilensis* population during the last 20 years (Chapter 2) highlights an urgent need to investigate both natural and anthropogenic transport vectors of non-native species within the Menai Strait and Conwy Bay SAC (see Chapters 2 and 4). The economic costs of a full, large-scale eradication effort would arguably be better partitioned into several small-scale investigations into various non-native transport vectors, coupled with trials to develop effective eradication techniques. Pilot studies, conducted following the detection of the invasive ascidian, *Didemnum vexillum* Kott 2002, upon floating pontoon structures within Holyhead marina (Anglesey, North Wales) (see Griffith *et al.* 2009), serve as good examples of a rapid response to halt the proliferation of a newly-detected invader. Plastic wrappings and bags were used to isolate, smother and kill *D. vexillum* colonies through stagnation. Further success was gained by accelerating the eradication process through the addition of calcium hypochlorite to the enclosed bags (see Holt and Cordingley 2011). However, despite their best efforts under bureaucratic and financial constraints, *D. vexillum* has since returned to the area. In this respect, consideration of all transport vectors (in this case, boats) and their respective significance throughout the invasion process is of prime importance (Chapter 4) in order to adequately reduce propagule pressure, thus eliminating the possibility of re-introductions following eradication efforts.

6.4.4 Commercial fishery – bespoke product for special occasions or comprehensive international exportation?

Walne (1974) highlighted several problems with regards to the culture of *O. chilensis* in the Menai Strait, leading to the termination of all growth trials involving this species and the abandonment of the Chilean oyster population *in situ*. Relative to the native European oyster, growth rates of *O. chilensis* were deemed to be inadequate. In addition, spat cultured from Chilean broodstock, in particular, showed high mortality during periods of "*quite moderate* frost". As mentioned previously, the Chilean oyster is also susceptible to infection by haplosporidian parasites within the genus, *Bonamia*. Another obstacle yet to be fully negotiated is the general lack of spat available for ongrowing. Traditional spat production methods involve the collection of pediveligers (i.e. larvae that are ready to settle) from sacrificed adult oysters. However, as highlighted in Chapter 3, the proportion of brooding females within a population can be extremely low (≤4.6% of oysters ≥60 mm shell length) and thus this method is extremely inefficient. In this respect, Jeffs *et al.* (2008) suggested the possibility of culturing premature veliger larvae, also obtained during the traditional method, thus increasing the supply of available spat for ongrowing. However, no follow-up experiments relating to this method was found in the scientific literature, hence comparisons of subsequent growth and vigour of cultured premature and mature larvae are currently lacking.

An alternative option, and one which would no doubt stimulate fervent discussion among potential stakeholders, would be to start a fishery for this species within the Menai Strait and Conwy Bay SAC. In New Zealand, *O. chilensis* (famously known locally as the 'Bluff' oyster) is a commercially-important species, with annual landings of over 8 million oysters in 2009 equating to a market value of approximately US$14.5 million. The much-celebrated Bluff oyster season takes place between February and July, with the interim period giving ample time for adult oysters to spawn and recover. The occurrence of *O. chilensis* in the Northern Hemisphere could supplement the New Zealand market during the close season of the Bluff oyster fishery, making the availability of fresh oysters in New Zealand an all-year round prospect. However, the presence of *B. ostreae* in the Menai Strait, the strict biosecurity measures enforced in New Zealand and the sheer logistical difficulties make this proposition a highly-unlikely option. A more feasible option would be the harvesting of the *O. chilensis* population as a bespoke commodity, reserved for exclusive occasions held, for example, by the New Zealand High Commission in the UK or during the International Rugby Union Test Matches (e.g. the 'Autumn Series').

Preliminary taste trials have revealed *O. chilensis* from the Menai Strait to be *"very similar, if not quite as strong"* to those harvested each year from Foveaux Strait, New Zealand (N. Anderson, pers. comm.). Whilst the general size and meat content were thought not to be quite as substantial as those from New Zealand waters, it should be noted that the oysters in question were hand-collected from the intertidal zone and were thus not fully representative of the larger conspecifics which inhabit the shallow subtidal in the Menai Strait (see Chapter 2). Collected in June 2012, evidence of spent gonads within several oysters meant that the flesh of certain individuals was watery and not representative of an 'in season' Chilean oyster from the Foveaux Strait. One other possible issue may be the high infestation rates of the shells by boring species such as the polychaete, *Polydora ciliata* Johnston 1838, and the sponges, *Cliona celata* Grant 1826 and *Halichondria panicea* Pallas 1766. *Polydora* is also commonly observed within oyster populations in northern New Zealand, where its boring activities cause 'blisters' within the shells which are both foul-smelling and unsightly (O'Sullivan 1996). Extreme infestations may lead to an increase in an energetic investment into shell repair at the expense of somatic growth, although treatment in a hypersaline solution has shown much promise without compromising the quality of the oysters themselves (see Dunphy *et al.* 2005). Infestations of oyster shells by *P.*

ciliata in the Menai Strait appear to become more prevalent with increasing oyster size/age (pers. obs.).

The commercial harvesting of the highly invasive yet economically valuable Chinese mitten crab, *Eriocheir sinensis*, from the River Thames is currently a hotly-debated topic (see Clark *et al.* 2011). Advocates of the fishery consider this to be the only viable method of controlling the population. Others may argue that reports of the potentially high economic value of this fishery could lead to unauthorised deliberate transfers of *E. sinensis* to other river catchments within the UK, thus facilitating its range expansion. Illicit transfers of non-native species have previously been documented within UK waters. Following its introduction to England during the 1970s, the invasive signal crayfish, *Pacifasticus leniusculus* (Dana 1852), is thought to have been deliberately relocated to several other rivers (Maitland 1987). As with any new commercial fishery, all potential environmental impacts pertaining from the commercial harvesting of *O. chilensis* would need to be satisfied prior to its commencement. As noted above, the impacts of dredging upon the oyster reef community, for example, would need to be addressed. Strict regulations regarding import/export, fishing techniques, permits and enforcement would also need to be employed.

6.5 Concluding remarks

Despite continuous recognition of the adverse economic and ecological effects of many invasive species, several aspects of the dynamics of the invasion process remain poorly understood, partly due to its complex and context-dependent nature. As well as providing a better understanding of the proliferation of an otherwise unfamiliar invader within a designated marine Special Area of Conservation, research presented in this thesis has helped prioritise topical and poorly-studied areas within the field of invasion ecology that require urgent consideration. Whatever the raisons d'être, our current approach to management effectively means that we will always be playing 'catch up' in the quest to halt biotic homogenisation and the loss of native biodiversity. Despite the recent financial recession, a change in attitude in tackling biological invasions is required which incorporates complete collaborative and cooperative commitment by all those involved which should then feedback into a single, central repository and legislation framework for all non-native species, regardless of their invasion stage. Novel, practical ideas regarding early detection and rapid response measures are also encouraged to ensure the successful eradicate of non-native species prior to their future proliferation. This attitude is of particular relevance in the face of a rapidly-changing climate.

Chapter 7

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