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NRW Evidence Report No 049 - The reproduction and connectivity of *Sabellaria alveolata* reefs in Wales – MAR4REF Bangor University

L.E. Bush, S.J. Balestrini, P.E. Robins and A.J. Davies

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Crynodeb Gweithredol

Mae adeileddau biogenig a ffurfiwyd gan organebau morol rhynglanol yn elfennau allweddol mewn cynnal â gwella bioamrywiaeth o fewn systemau morol lleol, drwy ychwanegu at gymhlethdod topograffig y dirwedd gysylltiol. Mae'r llyngyr tiwb *Sabellaria alveolata*, yn beiriannydd ecosystem bwysig ar draethlin y DU, ac felly meant yn cael i'w amddiffynni oherwydd ddosbarthiad prin y riffiau yr ydyn yn medru ffurfio. Er mwyn rheoli'n effeithiol a lleihau effaith newid yr amgylchedd ar boblogaethau'r llyngyr, mae angen mwy o wybodaeth er mwyn deall dosbarthiad a datblygiad y riffiau *S. alveolata.* Cyflwynwyd yr adroddiad hwn ddarganfyddiadau brosiect a noddwyd gan y Gronfa Ecosystemau Gwydn Llywodraeth Cymru, a arweiniwyd gan Brifysgol Bangor, yn ymchwilio i atgenhedliad a chysylltedd rhwng riffiau *S. alveolata* ar hyd arfordir Cymru. Yr ydym yn canolbwyntio ar dri safle allweddol ar gyfer casglu data, Llanddulas (Gogledd Cymru), Aberarth (Canolbarth Cymru), a Dunraven (De Cymru), ond fe ehangwyd i'r raddfa ranbarthol ar gyfer modelu symudiadau larfal rhwng safleoedd y prif riffiau ar draws y Môr Iwerydd. Y prif feysydd ymchwil a grynheir gan y prosiect yma oedd: 1) arbrofion labordy i ddisgrifio ymddygiad y larfa mewn perthynas â ffototacsis a meintoliad o gyflymder nofio ar draws nifer o gamau larfa gwahanol. 2) Fe aseswyd ffrwythlondeb gydag oes a maint wyau llyngyr a gasglwyd o'r tri safle ymchwil rhwng Chwefror a Medi 2014. 3) Mesurwyd dwysedd larfa yn y golofn dŵr yn y tri safle yn ystod y cyfnod ffrwythloni. 4) Cynhyrchwyd amcangyfrifon o'r boblogaeth gysylltiedig ar gyfer safleoedd riffiau gwahanol ar draws Cymru a darganfuwyd y tarddleoedd ac suddfannau pennaf trwy ddefnyddio model bioffisegol er mwyn creu amcangyfrif o symudiadau'r larfa.

Bu ein harbrofion "ymddygiad ffototacteg" dangos bod, wrth i larfau datblygu, fe fihafiwyd yn ffototactig negyddol (symud i ffwrdd o'r olau). Roedd cyflymder nofio yn cydberthyn yn bositif â maint corff, ond wrth i larfau metamorffeddu, bu chyflymder nofio disgyn yn ddramatig wrth iddyn nhw ddechrau ymlusgo. Arsylwyd disgyniad graddol mewn maint wyau yn ystod y cyfnod samplu ar draws bob safle, ond roedd yna anghysonrwydd mewn amser a hyd big y crynodiadau gamet ar draws pob riff. Roedd hyn yn cefnogi'r patrwm pig y silio deufodd a ddisgwylir yn ôl damcaniaeth ar hyd ein safleoedd Deheuol, ond fe sylwir hefyd bod diferyn o silio parhaus i weld ym mhob safle trwy gyfnod llawn yr arsylwad. Sylwir hefyd bod gwasgariad y larfau yn

newid rhwng safleoedd a ddangoswyd y rhain wahaniaethau mewn pig-cyflenwad. Roedd nifer y larfau o fewn y golofn ddŵr hefyd i weld bron yn barhaus (h.y. roedd larfau bron i weld yn gyson ar ryw gyflenwad), gyda phigau yn yr haf a'r hydref. Ddarganfuwyd tystiolaeth cryf dros gysylltedd rhwng is-boblogaethau riffiau o fewn y Môr Iwerydd drwy ddefnyddio model Tracio Gronyn, ac fe ddarganfuwyd tystiolaeth bod yna bosibilrwydd o gyfnewid rhwng tri phrif is-boblogaethau (Gogledd Cymru/Gogledd Lloegr, Canolbarth Cymru a De Cymru/De Lloegr). Mae ein darganfyddiadau ni yn dangos patrymau clir mewn ymddygiad, gwasgariad a recriwtio larfau yn safleoedd ar draws Cymru. Mae ymddygiad yn newid gydag oedran, gyda'r stad olaf y larfau yn dangos mudiad fertigol yn ymatebol i grynodiad golau o fewn y golofn ddŵr, a chynnydd yn y cyflymder nofio. Mae pellter y gwasgariad o safleoedd genedigol yn ddibynnol ar safle, ac o ganlyniad mae rhai safleoedd yn dangos hunan recriwtio, tra bod eraill yn gweithredu'n bennaf fel tarddleoedd neu suddfannau larfau i fannau arall. Mae hyn yn amlygu'r pwysigrwydd o ystyrio gwasgariad larfau yn y maes reolaeth ecosystemau, a sut i amddiffyn riffiau hunan-recriwtio a tharddleoedd (dros suddfannau), oherwydd mae'r un blaenorol yn gyfrifol i gynnal o boblogaethau is a gwelir ar hyd arfordir Cymru.

Executive Summary

Biogenic structures formed by intertidal marine organisms are key components in sustaining and enhancing biodiversity in local marine systems by adding to the topographic complexity of the associated landscape. The reef building tube worm *Sabellaria alveolata* is an important ecosystem engineer on UK shorelines and is subsequently protected as a result of its limited spatial distribution in reef form. To effectively manage and reduce impacts on populations resulting from environmental change, more information is required to understand the distribution and development of *S. alveolata* reefs. This report presents the findings from a Welsh Government Resilient Ecosystems Fund funded project lead by Bangor University investigating the reproduction and connectivity of *S. alveolata* reefs along Welsh coastlines. We focussed on three key sites for data collation, Llanddulas (North Wales), Aberarth (Mid-Wales) and Dunraven (South Wales), but expanded to the regional scale for modelling larval movements between major reef sites throughout the Irish Sea. The main research areas covered by this project included: 1) Laboratory-based experiments to describe larval behaviour in relation to phototaxis and quantification of swimming speed throughout different larval development stages. 2) We assessed adult fecundity and egg sizes from worms collected from all three study sites every month from February-September 2014. 3) We measured larval densities in the water column at all three sites during the theorised reproductive period. 4) We produced estimates of population connectivity for different reef sites within Wales and determined major source and sink sites by using a biophysical model to create estimates of larval movement.

Our phototactic behaviour experiments revealed that as larvae developed, they became negatively phototactic (move away from light). Swimming speeds were positively correlated with body size, but as larvae metamorphosed, speeds reduced dramatically as they began crawling. We observed a gradual reduction in egg sizes during the sampling period at all sites, but there were inconsistent timing and duration of peak gamete concentrations across the study site reefs. This supported the theorised bimodal peak spawning pattern at Southern sites, but we also detected continual trickle spawning at each site throughout the observed period. Dispersal of larvae was found to vary between sites and showed differences in peak abundance.

Numbers of larvae in the water column also appeared to be almost continuous (i.e. larvae were almost always present at some abundance), with peaks in the summer and autumn. We found strong evidence for connectivity between sub-populations of reef within the Irish Sea using a particle tracking model, and we found evidence that there might be limited larval exchange between three major sub-populations (North Wales/North England, Mid-Wales and South-Wales/South England). Our findings show clear patterns in larval behaviour, dispersal and recruitment at sites throughout Wales. Behaviour varies with ages with latter stage larvae demonstrating vertical migration in response to light concentrations within the water column, and an increase in swimming speed. Dispersal distance from natal site is site dependent and consequently certain sites show high self-recruitment, whilst others act as predominantly source sites, and others as sink sites for larvae from elsewhere. This highlights the importance of considering larval dispersal in the ecosystem management, and protecting self-recruiting and source sites above sink sites as the former sites are largely responsible for maintenance of sub-populations found along the Welsh coastlines.

1. Introduction

The adult Sabellariid polychaete *Sabellaria alveolata* occurs on many rocky coastlines of the northeast Atlantic, occasionally forming large biogenic reefs in suitable habitats from Morocco to the south of Scotland (Cunningham et al., 1984). Among the known requirements for *S. alveolata* reef development and persistence are: (i) the presence of *S. alveolata* larvae in the water column in sufficient abundance to ensure adequate temporal and spatial recruitment; (ii) a sufficient supply of suspended sediment to construct the aggregated tubes, and consequently a supply of sediment available for suspension, and a degree of wave exposure to transport sediment and provide particulate organic matter for filter feeding and; (iii) a hard, stable substratum for physical attachment; and (iv) food (Gruet, 1971, 1972, 1986; Porras et al., 1996; Holt et al., 1998).

Their biogenic structures take many forms, ranging from the tube of individuals through to aggregated low lying veneers or even to raised platforms of up to 1.8m high (Kirtley & Tanner, 1968; Dubois et al., 2003, 2007; Ayata et al., 2009; Fournier et al., 2010). These biogenic structures add topographical complexity to the intertidal and occasionally subtidal zones (Dubois et al., 2006, 2007; De Grave & Whitaker, 1997; Firth et al., submitted). Individual *Sabellaria alveolata* can live for 8 to 10 years, although the documented mean lifespan is 5 years (Wilson, 1971; Gruet, 1982). The biogenic reef structures created by the colony may last considerably longer as the structures are highly attractive to metamorphosed larvae, which often settle in numbers on existing reefs (Wilson, 1968b; 1970; 1976; Pawlik, 1988) *Sabellaria alveolata* structures are protected under Annex 1 of the EU Habitats Directive (Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora) and are listed under the UK's Biodiversity Action Plan (1994). Despite their importance, these worms have been subject to a low amount of research effort. In particular, current understanding of larval dispersal, population connectivity and resilience to environmental change is limited.

1.1. *Sabellaria alveolata* life cycle

Like many coastal benthic invertebrates, *Sabellaria alveolata* feature a complex life cycle, with a planktonic larval stage, in addition to a sessile bottom dwelling juvenile and subsequent adult stage (Cazaux, 1964) (Figure 1). *S. alveolata* is a gonochoric broadcast spawning species, becoming reproductively active within its first year (Ayata et al., 2009). Gruet (1982) suggested that individuals reproduce only once in approximately 3 years and consequently that the average individual would only reproduce twice in its lifetime. This has not been directly investigated at the individual level, although it has been speculated that *S. alveolata* populations may display semi-continuous spawning throughout the year (Dubois et al., 2007). Fertilisation is external, with fertilised eggs hatching and developing into free swimming trochophore larvae rapidly. Within the laboratory Pawlik (1988) found they developed to young trochophore stage within 12-18 hours at 20 °C, or 18-24 hours at 15 °C, whilst Wilson (1929) and Newstead & Davies (Unpublished) found they had developed to an equivalent stage in 2 days at room temperature (around 17°C). The trochophore larvae develop into obligate metatrochophore larvae, and then facultative erpochete larvae that are ready to settle and metamorphosise into benthic juveniles (Figure 1; Dubois et al., 2007, Elkin & Marshall, 2007).

Sabellarian larvae were original described from plankton samples off the coast of France by Caullery (1914), and it has long been known that they are some of the most common components of the spring and summer plankton around British coasts (Wilson, 1929). Larval settlement has been reported from Duckpool, Cornwall in the autumn (prior to October) and the winter months (Wilson, 1976). Larval settlement occurred prior to August at Llanddulas in 2012 (Bush, pers. obs). There is conflicting information available regarding the pelagic larval lifetime of *S. alveolata* (Wilson, 1968b, 1970, 1971, Dubois et al., 2007). Wilson (1929; 1968b, 1970, 1971) reported a highly variable rate of larval development in the water column, from fertilization to metamorphosis into a benthic juvenile, of between 1.5 to 8.5 months from both fieldwork (Duckpool, Cornwall) and laboratory based studies. Cazaux (1970) estimated

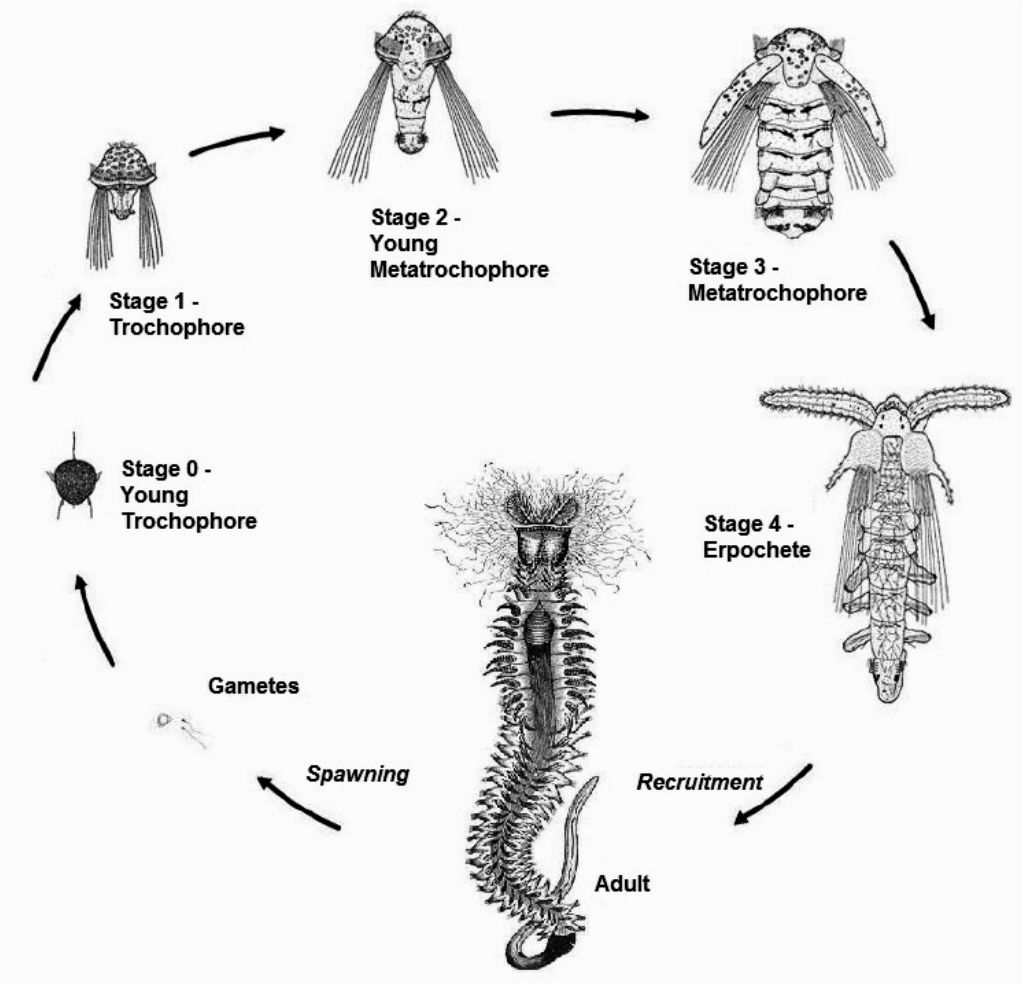


Figure 1: Life cycle of *Sabellaria alveolata.* Stages not drawn to scale. Adult males and females spawn gametes directly into the water column. External fertilization occurs and eggs hatch into a free swimming early trochophore larvae (Stage 0) within 2 days. These develop into trochophore larvae (Stage 1), reaching ~120 µm in length. The larvae continue to develop into the early metatrochophore (Stage 2) reaching 350 µm in length. The metatrochophore larvae (Stage 3) continues to develop with the initiating of tentacular palp formation, reaching ~500 µm before transforming into the erpochete larvae (Stage 4). The erpochete is the settlement stage larvae, which seek out a suitable settlement site to recruit to and begin tube construction (Wilson, 1929; Dubois et al., 2007; Slater, 2013).

a larval development time of 3 months from fieldwork in the Bassin d'Arcachon (France) during the colder months of October to March, whilst Dubois et al., (2007) reported a mean planktonic lifetime of 1 to 2.5 months during the warmer period of May to September in the Mont-Saint-Michel Bay (France). In this latter study Dubois et al., (2007) reported that larval development from fertilization to metamorphosis stage occurred in 2.5 months in May decreasing to just 1 month in September. This apparent variability in development time

may be attributed to temperature, food quality or quantity, factors that have been described as strongly influential in polychaete larval development and success (Reitzel et al., 2004; Qian & Chia, 1991 in Dubois et al., 2007). Dubois et al., (2007) reported rapid development in an autumnal bloom and slower development in a spring bloom of *S. alveolata* larvae in the Mont-Saint-Michel Bay. Both blooms were correlated with the presence of phytoplankton blooms, suggesting food is not limiting development at this site. With temperatures approximately 8 °C higher in autumn than spring, it is likely that, in this location, temperature is the dominant driving factor of the length of development time.

There is also conflicting information regarding the spawning season of *Sabellaria alveolata.* Wilson (1971) reported a short spawning period in July in Duckpool, North Cornwall, whilst studies from France suggest a longer, potentially bimodal, spawning pattern. Gruet & Lasse (1983) reported 2 long spawning periods in March- April and June - September from Noirmoutier Island whilst Dubois et al., (2007) reported an extended spawning period from April - October with peaks in abundance in early May and September from Mont-Saint-Michel Bay, supporting the hypothesis that *S. alveolata* display a poor seasonally synchronised semi-continuous spawning (Dubois et al., 2007). Studies on the reproductive biology of *S. alveolata* also support this, as mature ripe adults, capable of releasing eggs or sperm, have been reported to be present throughout the year (Gruet & Lasus 1983; Dubois et al., 2003; Dubois et al., 2007; Culloty et al., 2010; Bush and Balestrini, pers. obs.). Whilst ripe individuals are present in the population, they do not necessarily release gametes, but in some studies fertilisation has successful throughout the year. Although Gruet (1982) indicated no successful fertilizations occurred in laboratory studies in France from October to February, Davies & Newstead (2013) reported successful fertilization and metamorphosis into benthic juveniles throughout the winter in laboratory trials with UK *S. alveolata*. Within the field, Cazaux (1970) reported high densities of larvae in the water column during the winter months in the Bassin d-Arcachon, France. Stage was not specified but it is unlikely that high numbers of larvae would survive long-term in the water column due to high mortality (Dubois et al., 2007), despite their

ability to delay metamorphosis, indicating that successful fertilization does naturally occur later in the year.

1.2. Importance of *Sabellaria alveolata* larvae in population persistence and resilience

It is known that the Lusitanian polychaete *Sabellaria alveolata* is sensitive to extreme cold, with historic absences and extirpations in the far north of its range within the UK likely to have occurred in response to extremely cold air and sea water temperatures (Crisp, 1964; Gubbay, 1988; Frost et al., 2004; Mieszkowska et al., 2006; Firth et al., submitted). Active tube generation of the adult is suppressed by low temperatures, and absent at less than 5 °C (Bamber & Irvine, 1997), whilst early trochophore larvae had higher mortality rates with lower temperatures in conjunction with increased salinities (Slater, 2013).

Short term population survival is determined by the environmental tolerances of the adult, in addition to those of recruiting larvae and successfully settled juveniles. Ultimately species distributions are determined by the dispersal capacity of the mobile phase (Johnson et al., 2001). Historic population fragmentation through local extirpations, as has occurred in the recent past for *S. alveolata* within the United Kingdom (Crisp, 1964; Cunningham et al., 1984; Frost et al., 2004), can result in low connectivity between sub-populations of marine organisms and a loss of marine larval recruitment as discussed in a review paper by Levin (2006).

Mass extirpation of *S. alveolata* occurred on the North Wales coastline following the extremely cold winter of 1962-1963 (Crisp, 1964; Cunningham et al., 1984; Frost et al., 2004). Recolonisation of this area has been very slow. No reported sightings occurred prior to the 1990s. The first reported sightings of this species on the North Wales coastline, post 1962, was from the Menai Strait in the Marine Nature Conservation Review in 1992 (JNCC, record held by the National Biodiversity Network Gateway(NBN)). Several sightings of low abundance and encrusting populations were reported from the Isle of Anglesey and both the Little and Great Orme in the late 1990s by the Countryside Council of Wales (now Natural Resources Wales). However, the first record of a *S. alveolata* reef structure was from Llanddulas in 2008 (CCW reported an

Abundant population of *S. alveolata* at this site; record available from the North Wales Environmental Information Service).

It can be assumed that suitable habitat was available for the adult stage, as previously and subsequently inhabited substrate remained present throughout the extirpation period. Abundant sites were present in both Cardigan Bay, to the south, and Cumbria, to the north, throughout this time (records available on NBN), suggesting climatic conditions were also suitable. Despite this, successful settlement did not occur for approximately 30 years (Cunningham et al., 1984; Frost et al., 2004; Firth et al., submitted). Although sources of larvae were potentially available from both Cumbria and Cardigan Bay, recolonisation would be dependent on larval dispersal distances. This emphasises the importance of understanding the dispersal capability of the pelagic larval stage in predicting the spatial and temporal distribution of *S. alveolata,* as long term persistence and resilience of *S. alveolata* reef locations is dependent on successful recruitment, in addition to continued suitability of the habitat.

1.3. Factors influencing larval recruitment

The site-specific recruitment success of *S. alveolata* is highly variable year-to-year (Wilson, 1971; Gruet, 1986; Bush and Balestrini, pers. obs.). Hydrodynamic and biological processes can influence larval success. Both vary at different spatial and temporal scales, influencing larval dispersal patterns and hence transport of larvae away from a population (Pineda et al., 2007; Ayata et al., 2009; Ayata et al., 2011). Hydrodynamic factors that affect pelagic marine invertebrate larvae include wind induced and tidal residual currents, coastal upwellings, river plumes, eddies and gyres (Bradbury & Snelgrove, 2001). Thiebaut et al., (1998) demonstrated how wind induced currents modified the transport direction of *Pectinaria koreni* larvae, as well as increasing residual current velocity in coastal zones resulting in larval export to offshore waters. Cunningham et al., (1984) suggested that the absence of *S. alveolata* populations from both southwest facing mainland peninsulas and the Isle of Man may be due to prevailing currents influencing larval dispersal. It is likely residual currents reduce *S. alveolata* recruitment to these suitable areas simply because they are transported offshore rather than onto shores. River

plume waters that exhibit higher temperatures and lower salinities have been reported to have higher *S. alveolata* abundances than surrounding waters (Ayata et al., 2011), suggesting that these plumes act as a physical barrier to dispersal offshore and have a significant role in population connectivity. Eddies generated by headland effects may also restrict larval transport and contribute to the entrapment of larvae near their source reef (Ayata et al., 2009). A large anticyclonic eddy studied by Dubois et al., (2007) had a significant role in retaining high abundances of *S. alveolata* larvae to within the Mont-Saint-Michel Bay, France, perhaps explaining the stability of reefs in this region.

Spawning time, period and location, larval development time and larval behaviour are the main biological properties that influence *S. alveolata* larval recruitment as these alter interactions with the hydrodynamic regime (Ayata et al., 2009; 2011). Ayata et al., (2011) also suggest spawning time and location to be potentially more important than swimming behaviour. Larval mortality may have a greater role than hydrodynamics in the success of larval recruitment due to significant retention near source with high mortality, as illustrated in studies of another tube building polychaete, *Pectinaria koreni*, in the English Channel (Ellien et al., 2004).

Whilst self-recruitment is clearly important in the persistence of an isolated population, in other instances, sites of high abundance may be well connected to other persistent sites. Dependent on local hydrodynamics, and subsequent dispersal patterns, certain sites may act as sources of larval recruitment for others, supporting reef populations elsewhere. Larval dispersal patterns can be mono directional (Bode et al., 2006). Crowder et al., (2000) highlighted the importance of understanding source-sink population dynamics in the implementation of marine protective measures. As summarised previously, little is known about many of the determining biological parameters of *S. alveolata* larvae, and consequently very little is known with regards larval dispersal.

1.4. Study aims

Larval supply and continued habitat suitability are both essential requirements for the long term survival of individual reefs. This study aims to develop an understanding of how reefs interact, not just within their local environment but

also within regional hydrodynamics. Ultimately we aimed to understand their population connectivity that will establish the level of resilience that *S. alveolata* reefs may have to local changes in the natural environment. Current literature exposed the gaps in knowledge in this area, specifically concerning spawning, larval behaviour and the dispersal and vertical location of released larvae. Our objectives were to provide consistent long term gamete data from wild populations, investigate swimming speeds and phototactic responses of larvae at different development stages, measure larval densities in the water column and produce estimates of population connectivity of different reef sites within Wales, United Kingdom and identify major source and sink sites. These objectives provided the opportunity to test two hypotheses. Firstly, it was hypothesised that British *Sabellaria alveolata* will display semi-continuous spawning with a summer peak in larval abundance. Secondly it was hypothesised that larvae will be dispersed rapidly from their source reefs in regions of open coastline.

2. Methods

2.1. Study Area

Surveys were carried out at *Sabellaria alveolata* sites of high abundance on a latitudinal gradient: Dunraven Bay in South Wales (SS288731), Aberarth in mid Wales (SN475638) and Llanddulas in North Wales (SH914787) (Figure 2). The reef structures at Dunraven Bay, Glamorgan, dominate bedrock ledges to the west of the main sandy beach, whilst at both Aberarth and Llanddulas reef structures are mosaicked within cobble beaches. At all three sites, biogenic structures varied from encrusting veneers of a few centimetres in height, to reefs of more than 50 cm tall, with a spatial extent of > 200 m^2. Known *S. alveolata* colonies are present semi-continuously, on hard substrata, throughout the lower intertidal adjacent to the site of interest in both Dunraven and Aberarth unlike Llanddulas, which is a more contained site. The overall reef structures at Llanddulas and Dunraven were similar in size, with the Llanddulas reef showing signs of a successful recent recruitment and new growth throughout whilst in Dunraven new reef growth was restricted to the west of the site during the period of this study (February-September 2014). Aberarth is a predominantly encrusting site with the only reef formations taller than 50cm occurring approximately 500m to the south of the reef of interest. With these differences in reef size and quality and location, it is expected that there will be differences regarding larvae production, dispersal abundances and recruitment between these Welsh reefs.

Monthly sampling of *Sabellaria alveolata* larvae were carried out both on and offshore on a monthly basis from April to September 2014. Survey dates are listed in Tables 1 to 3. Offshore sampling was not possible at Aberarth or Dunraven in April as a result of vessel malfunction, or at Dunraven in August due to the unavailability of a qualified skipper at short notice. In Llanddulas, offshore sampling for June was delayed until July 1st due to poor weather conditions (high wind and waves) at each boat launch during this month leading to cancellation of survey for safety reasons.

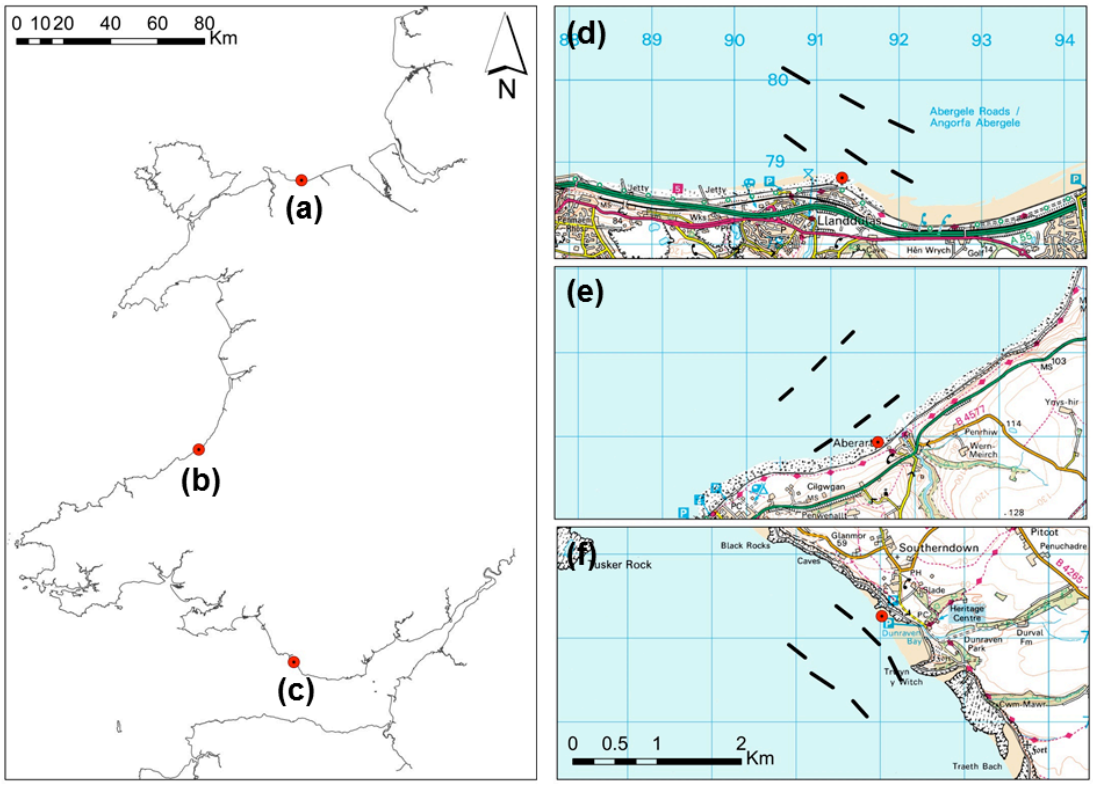


Figure 2: Sampled sites within Wales. (a-c) Location of sites within Wales showing latitudinal gradient from Llanddulas in the north to Dunraven in the south. (d-f) Location of sites on a 1:250000 Ordnance Survey map. Site location is pinpointed by the red circle, planned route of plankton tows by the black lines offshore. (a, d) Llanddulas, (b, e) Aberarth, (c, f) Dunraven.

Table 1: Dates of plankton tow surveys at all three sites.

Month	Llanddulas	Aberarth	Dunraven
April	30/04/2014	na	na
May	19/05/2014	28/05/2014	28/05/2014
June	01/07/2014	18/06/2014	26/06/2014
July	22/07/2014	11/07/2014	10/07/2014
August	07/08/2014	14/08/2014	na
September	16/09/2014	23/09/2014	22/09/2014

Table 2: Dates of trap surveys at all three sites

Month	Llanddulas	Aberarth	Dunraven
April	17/04/2014	na	na
May	13/05/2014	14/05/2014	22/05/2014
June	3/06/2014	12/06/2014	11/06/2014
July	28/07/2014	15/07/2014	17/07/2014
August	25/08/2014	26/08/2014	28/08/2014
September	25/09/2014	12/09/2014	12/09/2014

Table 3: Dates of fecundity surveys at all three sites

Month	Llanddulas	Aberarth	Dunraven
February	24/02/2014	17/02/2014	18/02/2014
March	27/03/2014	19/03/2014	20/03/2014
April	24/04/2014	22/04/2014	23/04/2014
May	12/05/2014	28/05/2014	29/05/2014
June	16/06/2014	11/06/2014	12/06/2014
July	28/07/2014	17/07/2014	15/07/2014
August	25/08/2014	27/08/2014	26/08/2014
September	13/09/2014	12/09/2014	12/09/2014

2.2. *Sabellaria alveolata* larval dispersal on shore

Larval presence onshore was monitored with low-lying larval traps (approximately 10cm off bottom) (Figure 3). The traps were designed so water would flow through the inflow mesh at the back, through a valve to prevent backflow and into the front section (Figure 3). Water would then flow out through the 50 µm mesh outflow, leaving potential larvae trapped inside. These traps were constructed from 33cm long, 110 mm diameter, plastic pipe sealed by a cap at one end. Two 50cm holes were drilled in the upper surface of the pipe, and two at opposite edges of the base of the cap with one above

that was sealed with a water tight removable rubber bung, whilst 50 µm meshes were fitted inside the other three holes and attached with Epoxy resin. A plastic funnel was secured within the pipe to prevent backflow of the retained sample. 100 µm mesh was secured over the inflow with Jubilee clips. The traps were attached to wooden base boards with plastic brackets using marine grade stainless steel screws and washers. When deployed onshore, the 50 µm outflows on the cap were always positioned at the base of the trap, facing down the shore, whilst the 100 µm inflow mesh was positioned up shore. The wooden base board was secured in position by (a) the weight of surrounding rocks on pebble areas, (b) cable tied to embedded steel poles on sandy areas or (c) cable tied to temporary marine grade stainless steel bolts on bedrock.

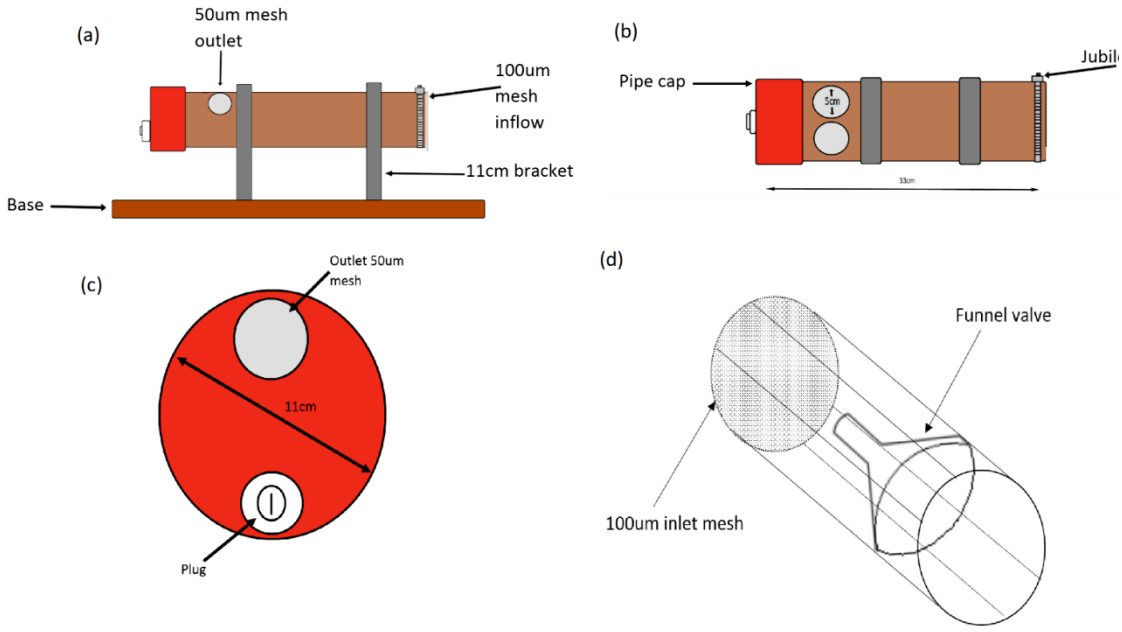


Figure 3: Design of larval traps manufactured in house by Bangor University. (a) Side profile showing longitudinal pipe with 5cm holes drilled in upper surface, sealed with 50um mesh. One end of pipe is sealed with a pipe cap and the other with a 100um mesh attached by a jubilee clip. Pipe in attached to a wooden base board by brackets. (b) Upper surface of trap showing position of the outflow holes. (c) End view of trap showing additional outflow and bung. (d) Internal schematic of trap showing funnel valve.

Eighteen traps were deployed for one tidal cycle, on a monthly basis (bimonthly at Llanddulas) (Table 2), at three tidal heights (low, mid and high), ~100m either side of the reef and within the reef, throughout the sampling

season (Figure 4). At each site, two traps were deployed simultaneously at the nine locations above. Following deployment, the water tight bung was removed and contents were washed through the opening with filtered seawater into a retaining bucket. Samples were partially filtered on a 50 µm sieve to reduce sample volume and collected in 500ml sample pots. Retained samples were preserved immediately in 10% formosaline solution, buffered with Borax.

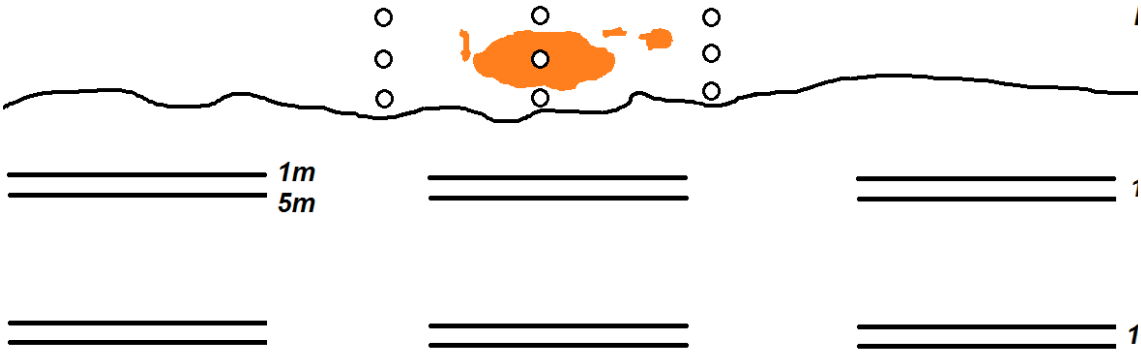


Figure 4: *Sabellaria alveolata* larvae monitoring design. Diagram not to scale. The hypothetical *S. alveolata* reef is shown in orange. Location of onshore sampling traps in relation to the reef is represented by circles, with each circle representing a pair of traps. Traps were located in 3 transects through the middle of the reef, above the reef and below the reef. Location of plankton tows is represented by black lines. Plankton tows were carried out monthly in 2 transects at 100m from the reef and 1km from the reef. In each transect 2 tows were completed to either side of the reef, and the middle of the reef, at both 1m and 5m depth.

2.3. Fecundity of *Sabellaria alveolata* adults

Fecundity of adults was also monitored monthly throughout the sampling period (Table 3). Adult worms were collected by removing a small hummock (~10cm^3) from within a 20 m^2 area of reef at each site. Sampling location was near the upper edge of the reef, adjacent to the access point and was consistent for each period. Adult worms were removed from their tube, agitated and placed in filtered seawater for 1 hour before being preserved on site in 200 ml of buffered formalin solution. All worms spawned under this treatment (Wilson, 1968b). Donor worms were sexed by gamete type (small white sperm or large pink eggs) and five representative adult worms of each sex were retained with their spawning liquid. These were weighed on a calibrated balance to 2 decimal places, and individual length was measured under a binocular microscope against a graticule. The spawning solution of

each worm was diluted to 20, 40 or 60 ml dependent on the concentration of gametes in solution. This solution was homogenised by repeated inversions of the tube and a 1ml subsample was placed on a 1 ml Neubauer Haemocytometer. This haemocytometer was divided into nine large grid squares, each divided into sixteen. The number of gametes in three randomly selected large grid squares were estimated for each subsample by counting the number of gametes in four diagonally connected small grid squares under a compound microscope. The number of eggs or sperm produced by each individual was estimated by:

$$n = 12d(c) \quad (1)$$

Where n is the number of gametes, c is the haemocytometer count and d is the original dilution factor.

Ten randomly selected eggs from each female were mounted under a Nikon inverted compound microscope, at x240 magnification. Images were captured with a microscope adapted 3.1 megapixel digital camera, and lengths were measured in calibrated ToupView (version 3.2) software that interfaced with the camera.

Despite being collected from a similar area of the reef each month, both male and female worms demonstrated variation in both weight and length both within site, as a result of inherent variation in population structure over a small spatial scale, and between sites (Appendix 1). Consequently mean egg and sperm counts were normalised using both the weight and length of the donors.

Statistical analysis was carried out in Minitab. If test for normality and homogeneity of variance were not satisfied, natural log transformation of the data was applied If the afore mentioned were satisfied in original data, or in transformed data, one-way between subject ANOVA was used to assess differences, in this case between sites and months. If these conditions were not satisfied in original or transformed data, a Kruskal-Wallis Test was utilised. Comparisons between egg and sperm count data and egg size were investigated through Poisson regression.

2.4. *Sabellaria alveolata* larval behaviour

2.4.1. Spawning and larval rearing

Sabellaria alveolata larvae were derived from the eggs and sperm of adult worms collected from an established reef in Llanddulas. After collection adults were maintained within their tubes in aquaria supplied with a continuous flow of 0.2 µm filtered seawater prior to laboratory-induced spawning. Spawning was induced by shock: adult worms were physically removed from their tubes, taking care not to damage the donor worms. Donors were placed in individual 60 ml vials with 5 ml of ambient temperature 0.2 µm filtered seawater. Within five minutes most individuals expelled the majority of their gametes (Wilson 1968b). Donor worms were sexed by gamete type (small white sperm or large pink eggs) and removed from the vial. Vials containing gametes of separate sexes were combined into two vials of known volume (200 ml). The concentrations of eggs and sperm were determined by manual count using a Neubauer Haemocytometer, using the same methodology for the fecundity study. Sperm at a ratio of 5:1 was added to the eggs, reducing the risk of polyspermy and left for 20 minutes to allow fertilisation.

Fertilised eggs were rinsed through a 50 µm mesh to remove redundant sperm and reduce risk of bacterial infection before being and transferred to 1 L of aerated ambient temperature seawater. Larvae remained in the flask for 18 hours, by which time most eggs were expected to have hatched into the free swimming trochophore stage. Initial larval concentration was determined by manual count using a Neubauer haemocytometer, using the same methodology as described previously. Larval solution was then transferred into aerated 12 L buckets, containing 0.2 µm filtered seawater at a concentration of 1 larvae ml^{-1}. Larvae were fed daily on the phytoplankton *Pavlova lutheri* and *Tahitian isochrysis* (initial concentration of 10^5 cells ml^{-1}) providing a 1:1 mixture of phytoplankton to larvae. The buckets remained in a thermostatically controlled room at a constant temperature of 18˚C under a florescent light setting at 13:11 hour light to dark cycle (mimicking summer sunlight exposure). Twice-daily partial water changes were made. Cleaning and removal of waste was conducted via reverse filtration through a 50 µm mesh until approximately

500 ml remained in the bucket. Larvae were transferred to a clean bucket with filtered seawater of the same temperature on alternating days.

During the rearing process, samples were taken at different stages post fertilisation for experimentation, and speed and length measurements: day 5 (trochophore), day 15 (young metatrochophore), day 20 and 25 (old metatrochophore) and day 30 (erpochete) (Table 4). Speed and length measurements were estimated by video recording and imagery respectively, by the same methodology as egg size in the fecundity study.

Table 4: A summary of *Sabellaria alveolata* larval developmental stages with descriptions and size classes (Wilson, 1929; Cazaux, 1964; Dubois et al., 2007; Newstead and Davies, Unpublished).

Larval stage	Description	Size
Young trochophore (Stage 0 = S^0)	Within days of hatching, very little elongation with no segmentation. Chaetae evident.	<90um
Trochophore (Stage 1 = S^1)	Some elongation, no segmentation, chaetae prominent	90-120um
Young metatrochophore (Stage 2 = S^2)	Elongation, segmentation has begun formation of first abdominal segments, ocellus present	120-350um
Old metatrochophore (Stage 3 = S^3)	Further elongation, segmentation has continued with an increase in abdominal segments, darker pigmentation, formation of 2 tentacular buds	350-500um
Erpochete (Stage 4 = S^4)	Ready for settlement, larger abdominal segments, intensification of dark pigment, elongation of tentacular palps, formation of future palae of the adult worm.	500um

2.4.2. Horizontal phototactic behaviour

Horizontal phototaxis experiments were carried out in acrylic chambers (20 cm x 2 cm x 2 cm) following a protocol similar to Miller and Hadfield (1986). One half of each chamber was coated in black paint to prevent light penetration to this half of the chamber, whilst the opposite side remained uncoated and exposed to light (Figure 5). At days 5, 15, 20, 25 and 30 post-fertilisation, chambers were filled to 1.5 cm depth with 0.2 µm filtered seawater containing approximately 20-70 larvae, taken from a well-mixed culture. A black screen was placed over the painted half of the chamber, shading it further from any light source. Following transfer from culture to chamber, the larvae were left

undisturbed in a dark room for 10 minutes to acclimate (Marsden 1984) (Figure 5a). Following acclimation, light from a single illuminator KL 1500 LCD halogen cold light source at an intensity of 0.6 x 10^{16} quanta s^{-1} cm^{-2} (measured using QSL-100/101 Complete Laboratory Quantum Scalar Irradiance Meter (QSL-100/101).) was projected vertically towards the translucent end of the chamber, illuminating one half for 20 minutes (Figure 5b). Following this, a partition was inserted at the mid-point of the chamber to prevent subsequent larval migration post light exposure (Figure 5c). Larvae in 10 ml of solution were then removed from each half using a pipette simultaneously, in total darkness, and manually counted under a dissection microscope.

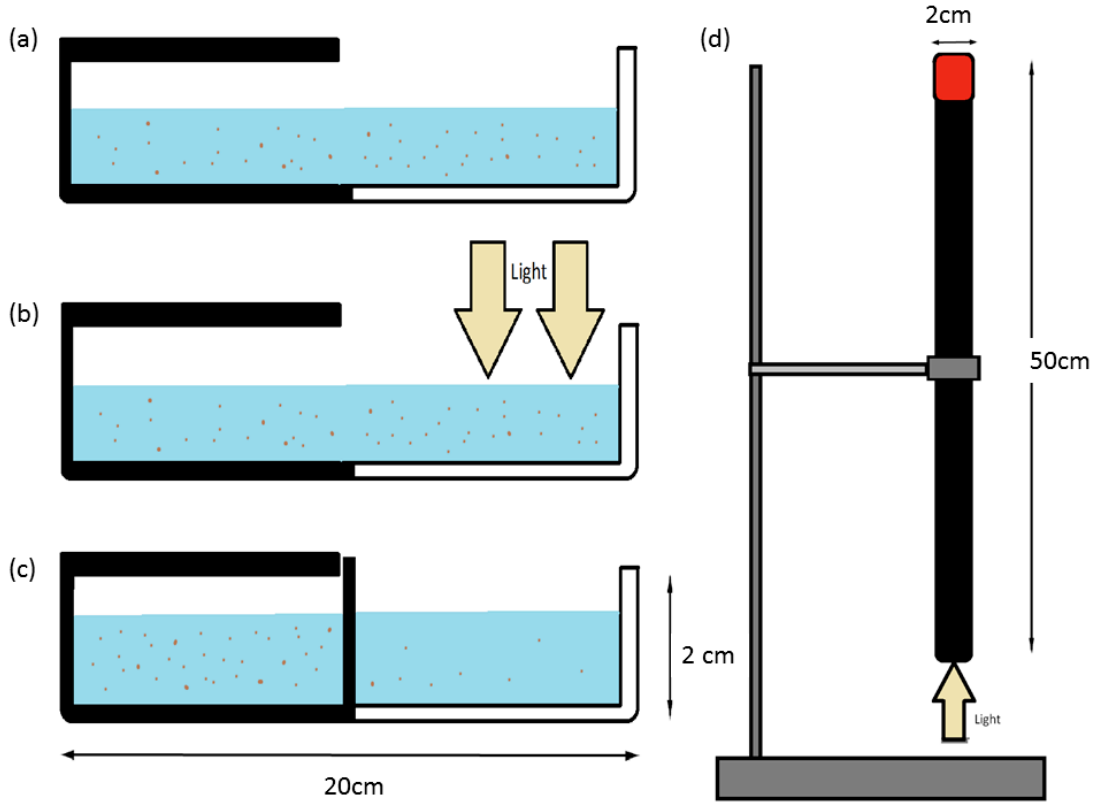


Figure 5: Larval migration experiment chambers (a-c) Horizontal experimental chambers. (a) Acclimation chamber. (b) Experimental procedure. (c) Chamber separation. (d) Vertical experimental chamber. Black plastic tube was filled with larvae in a seawater solution. Light was emitted from a light source through the base of the chamber.

2.4.3. Vertical phototactic behaviour

To mimic the vertical nature of the water column, vertical phototaxis trials were conducted in cylindrical tubes, coated externally in opaque black paint to shield from external horizontal light (Figure 5). The tubes (50 cm in length and 7mm cm in diameter) were filled with 20 ml of 0.2 µm filtered seawater. The tubes had graduations every 10 cm, with each graduation equating to a volume of 4 ml. The irradiance meter was held at the top of the tube as the light source was emitted from the base to determine the strength of light. To determine the intensity of light received at different graduations throughout the tube, 10cm sections of a replicate tube were removed sequentially from the top and irradiance measured. This removal and irradiance measurement was repeated every 10cm length down the tube. The light intensity at source (0 cm distance) was $0.6x10^{16}$ Quanta $s^{-1}.cm^{-2}$. For each 10 cm gradient light intensity reduced, which produced a vertical light gradient. These intensities were $0.22x10^{15}$ Quanta $s^{-1}.cm^{-2}$ (50cm from source), $0.6x10^{14}$ Quanta $s^{-1}.cm^{-2}$ (40cm from source), $0.4x10^{14}$ Quanta $s^{-1}.cm^{-2}$ (30cm from source), $0.3x10^{14}$ Quanta $s^{-1}.cm^{-2}$ (20cm from source) and $0.2x10^{14}$ Quanta $s^{-1}.cm^{-2}$ (10cm from source).

As with the horizontal experiments, approximately 20-70 larvae were taken from larval culture on equivalent days, and transferred to the vertical tube with 20ml filtered seawater. The larvae were again left undisturbed for 10 minutes in complete darkness to acclimate. Light was then projected vertically upwards through the tube from the base for 20 minutes. Separate controls were also conducted in completed darkness and with light projected vertically downwards. After the elapsed time, five sequential 4ml subsamples were released from the tube, by careful removal and replacement of the bung, and retained in separate labelled vials. The concentration of larvae in each subsample was enumerated separately under a dissection microscope. This protocol reduced vertical mixing of the larvae during the sampling period.

2.4.4. Larval measurements

Videos of actively swimming larvae were recorded on days 5, 15, 20, 25 and 30 post-fertilisation. Larvae were collected from a culture, filmed with the same methodology as for fecundity studies, and timed over a graduated microscope slide. The video was analysed and speed measured using ImageJ software by

measuring the distances moved by the larvae and the time of movement. Distances were measured over ~5mm in direct white light. At equivalent times, throughout the 30 day larval rearing process larvae lengths were measured and recorded in conjunction with light and swimming speed measurements. Single larvae were randomly selected from a well stirred culture. All subsequent image capture and analysis were undertaken using the same methodology as previously for egg size.

2.5. *Sabellaria alveolata* larval dispersal

2.5.1. Larvae distribution and dispersal

Larval concentrations offshore were monitored with plankton tows. At each site a 50 µm plankton net with an aperture of 50cm was towed through the water column at a speed of ~2knots, at two distances offshore from the reef of interest (~100m and ~1km), at three different directions from the reef (~500m either side of the reef in addition to directly in front of the reef) and at two different depths (1m and 5m from the surface). The volume of water passing through the net was measured with a calibrated Hydro-Bios digital flowmeter (model 438 110). Time, length and GPS position of each tow was noted to allow determination of tidal state. Retained samples were condensed on site by filtering through a 50 µm sieve and preserved in 10% borax-buffered formosaline solution.

2.5.2. Plankton and trap sample analysis

Larval abundance, life-history stage and size (length and width) in both net and trap samples were determined under a binocular microscope. Preserved larvae samples were filtered and cleaned in a fume cupboard over a 50 µm mesh immediately prior to analysis and resuspended in 500ml of filtered seawater. A 5ml subsample was transferred to a Bogorov Modified Counting Chamber with a Hensen-Stempel Pipette. Methylene blue dye was added to facilitate larval identification. Larval abundance was assessed in three replicate sub-samples of each trawl sample under a binocular microscope. Larvae were removed from the subsample with a glass dropper pipette and retained in an Eppendorf tube in 70% ethanol solution. If the initial count was greater than 250 larvae per 5ml, a 1ml subsample was counted for the remaining two subsamples as not only was significant time required to remove individual

larvae, but removal of larvae disturbed the sample and in dense samples more than the intended larvae could be removed. This methodology was validated and each subsample was consistent with the original count. All subsamples were rescanned under the binocular microscope until no further larvae were found. Ten percent of all subsamples analysed, selected at random across all sites and time periods, were quality controlled by another analyst.

Larval developmental stages of *Sabellaria alveolata* were characterised into size ranges following Dubois et al., (2007) (Table 4). An estimate of the concentration of each larval stage in each sample was generated by measuring the length of a random sample of retained larvae. All subsequent image capture and analysis were undertaken using the same methodology as egg size in the fecundity study. All larvae were measured in samples with less than 10 individuals, however in more abundant samples a maximum of 10 larvae were selected at random and measured.

Statistical analysis was carried out in Minitab in the same procedure as fecundity. In this instance a square root transformation was required. If tests for both normality and homogeneity of variance were satisfied in original data, or in transformed data, one-way between subject ANOVA was used to assess differences between sites and months. If these conditions were not satisfied in original or transformed data, a Kruskal-Wallis Test was utilised.

2.6. Biophysical modelling of *Sabellaria alveolata* larvae

This work is based on simulating the transport of virtual 'larvae' particles in the Irish Sea, which represent planktonic larval migration of *Sabellaria alveolata*, and predict the proportion of *Sabellaria* larvae that contribute to self-recruitment, in addition to connectivity within the metapopulation. The model domain is shown in Figure 6. The region extends northwards from the Celtic Sea (50° N) to beyond the North Channel (56° N), and from the UK coast in the east to the Atlantic in the west (-9° W). Larvae particles were released from known coastal reefs within the model domain, where they were exposed to shelf sea currents, and transported either locally within their natal habitat (self-recruitment) or to a similar habitat elsewhere (connectivity). The natal habitats investigated were 26 geographically distinct locations on the coasts of Wales, England, and Scotland (Table 5), which included the three sampled locations (Llanddulas, Aberarth and Dunraven) around the Welsh coast (Figure 2). These locations were identified from historical data sources (Cunningham et al., 1984; Frost et al., 2004) that were revisited in the summers of 2012-2014 and so known to be persistent through time (L. E. Bush, pers. obs)

Both the barotropic (gravity-driven; e.g., tides or wind) and baroclinic (density-driven; e.g., oceanic fronts) components of the circulation may play fundamental roles in larval dispersal (Robins et al., 2013). Tidal velocities are governed by tidal range and phase relationship, and by local bathymetry. Residual currents are controlled by atmospheric heating and mixing, or by wind stress. In the Irish Sea, tidal ranges on the eastern side are greater than in the west, with peak tidal ranges exceeding 12 m in the Bristol Channel and 8 m in Liverpool Bay, and degenerate amphidromes (regions of zero elevations) off the northeast coast of Northern Ireland and the southeast coast of Ireland. The differences in tidal ranges are due to Kelvin-type progressive waves which travel from the Atlantic and interact within the central Irish Sea (Robinson, 1979). As a result, the strongest tidal currents generally occur in the east, where tidal ranges are greatest, and where the flow is concentrated around headlands (e.g., Pembrokeshire, Llŷn Peninsula, and north Anglesey), or through channels in shallow water (e.g., the Bristol Channel). Regions of weaker currents occur in more sheltered bays, such as Cardigan Bay and

Liverpool Bay (including the North Wales coast). Water depths in the Irish Sea are generally shallow - less than 100 m - although greater and exceeding 250 m in the North Channel.

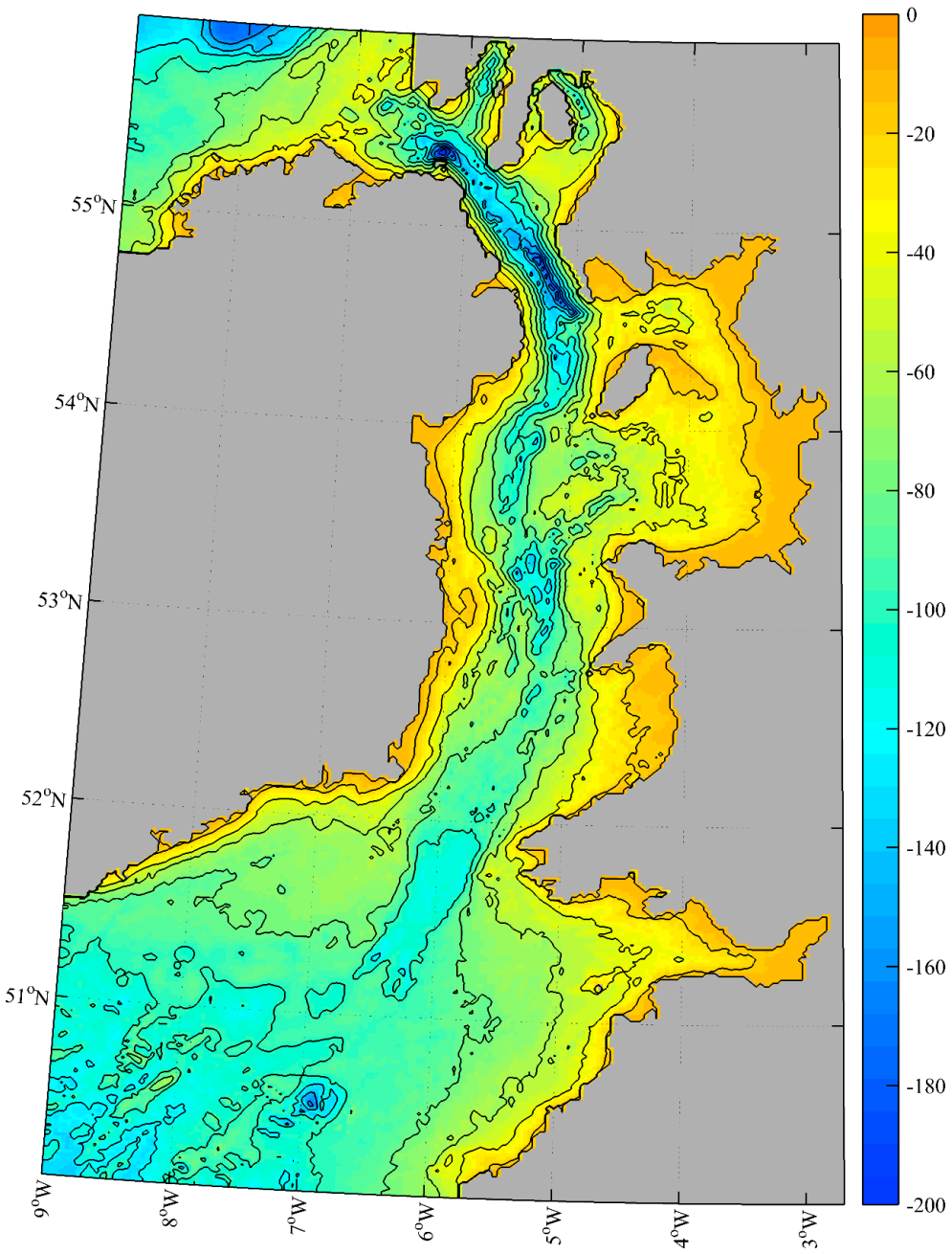


Figure 6: Map showing our bathymetric model domain (metres below mean sea level) of the Irish Sea. Land cells (UK and Ireland) are coloured grey (and the English Channel in the southeast is blanked out). Open model boundaries, forced by oceanic tidal elevations) are located at the southwest (Celtic Sea) and northwest (Atlantic Sea) corners of the domain.

Table 5: Particle Tracking Model release locations for *Sabellaria alveolata.*

Reef site	Name and location	Reef site	Name and location
1	Millook -4.575, 50.775	**14**	Barmouth -4.077, 52.677
2	Bude -4.558, 50.829	**15**	Shell Island -4.137, 52.824
3	Duckpool Coombe -4.561, 50.875	**16**	Criccieth -4.231, 52.916
4	Hartland Quay -4.533, 50.999	**17**	Llanddulas -3.650, 53.340
5	Downend Croyde -4.244, 51.126	**18**	Hilbre Island -3.230, 53.385
6	Clevedon -2.887, 51.430	**19**	Morecambe -2.917, 54.055
7	Dunraven -3.630, 51.390	**20**	Cross Dyke Scar -3.235, 54.067
8	Porthcawl -3.690, 51.4743	**21**	Annaside Bank -3.386, 54.245
9	Swansea Bay -3.944, 51.609	**22**	Drigg -3.477, 54.374
10	Limeslade Bay -3.984, 51.564	**23**	St. Bees Head -3.648, 54.539
11	Aberarth -4.290, 52.280	**24**	Maryport -3.474, 54.7408
12	Aberystwyth -4.158, 52.308	**25**	Dubmill Point -3.450, 54.795
13	Borth -4.057, 52.479	**26**	Auchenmalg Bay -4.746, 54.826

Residual currents are apparent in the Irish Sea, and have been shown to be important for larval transport (Robins et al., 2013). Although peak tidal flows are markedly stronger than residual currents (in most cases), tidal flows are oscillatory and, hence, over time scales of the order of weeks, residuals are responsible for advecting larvae along their conduit pathway. Most noticeably, the western Irish Sea gyre flows in a cyclonic direction, with residual currents reaching 0.3 m s 1, bounded to the east by northwest Wales and the Isle of Man. The gyre forms during spring and summer, when stratification in the west meets a tidal mixing front to the east (Simpson & Hunter, 1974; Horsburgh & Hill, 2003). Another significant residual current that is likely to be important for larval transport, flows southward along the eastern coast of Ireland, and towards the Celtic Sea. In addition, a westward residual current forms during

the summer, from South Wales towards Ireland, across St. George's Channel (Simpson & Hunter, 1974).

Our biophysical modelling is based on two components: a hydrodynamic model and a Lagrangian particle (larvae) tracking model, which are explained in the following two sections, followed by a description of how model outputs were used to calculate connectivity and self-recruitment.

2.6.1. Hydrodynamic model

A parallel version of the three-dimensional (3D), primitive equation, sigma-coordinate, hydrostatic, free-surface Princeton Ocean Model (POM) was used to simulate the hydrodynamics. The model (in its non-parallel form) is described in detail by Blumberg & Mellor (1987) and outlined for this application by Robins et al., (2013). The standard prognostic variables (e.g. 3D velocity, elevation, temperature, salinity, turbulence) are solved using finite-difference discretization on a staggered, orthogonal Arakawa-C grid in the horizontal plane and terrain-following sigma-layers in the vertical plane. The model grid covers the domain shown in Figure 6, where the horizontal cell size is 1/30˚ (longitude) by 1/60˚ (latitude), giving a resolution of approximately 1.85 km. In the vertical plane, 20 equally segmented sigma-layers gives minimum and average resolutions at mean sea level of 9.6 m and 4.3 m, respectively.

To validate the hydrodynamic model, a typical year (i.e., not an extreme weather year) was simulated; namely 1990, which was chosen based on an analysis of bed shear stress, significant wave heights and temperature records from a decadal simulation (1989-1998) (Neill et al., 2010). A six-month simulation was made for the period 01 April - 30 September 1990. The simulation was run for 1 month prior to the six-month harmonic analysis so that baroclinic currents were fully developed. The model was forced at the open boundaries with 6 tidal constituents including the dominant semi-diurnal M_2 (lunar) and S_2 (solar) constituents. Synoptic meteorological fields were sourced from the European Centre for Medium-Range Weather Forecasts (ECMWF)-Interim reanalysis (Simmons et al., 2006), available 3 hourly at a resolution of 1.5°. Validation was achieved by comparing amplitudes and phases of the modelled M_2 and S_2 elevations with records of known values from 20 coastal

stations within the domain; giving root-mean square errors of approximately 14 cm and 6 cm (M_2 and S_2, respectively) in elevation and 10˚ and 12˚ in phase. These values compare well with other models of this region (Xing & Davies, 2001; Horsburgh & Hill, 2003; Hartnett et al., 2007). In addition, simulated temperatures were compared to those produced by another model (Xing & Davies, 2001), and a temperature data record off the Isle of Mann (54.5° N, -4.5° W) provided by Port Erin Marine Laboratory (University of Liverpool). The two model simulations and data were in good agreement; importantly, strong stratification was simulated in the west, associated with the Western Irish Sea gyre and front. Strong baroclinic residual circulation along the western Irish Sea front, and along other strong fronts in the region, have implications on shelf-scale larval transport (Robins et al., 2013).

2.6.2. Particle tracking model

Lagrangian Particle Tracking Models (PTMs) are used to simulate the individual movement of larvae in space and time, based on advection and sub-grid-scale turbulent mixing. The dispersal probability distributions of the larvae, originating from distinct sites, was mapped, and the connectivity between geographically distinct populations estimated. Three-dimensional velocities and diffusivities were output from the hydrodynamic model for use in PTMs. This 'off-line' technique is sufficiently accurate, since the transport of larvae does not affect the hydrodynamics, and has the added advantage that a large number of PTMs can be performed from one hydrodynamic simulation, which is computationally more efficient. The velocities were tri-linearly interpolated from the hydrodynamic model to the position of each larva, and the interval of hydrodynamic model (30 minutes) linearly interpolated to 10 minutes for the PTM. Each larva was then iteratively advected in space and time.

The larvae are mixed locally through sub-grid-scale turbulence, based on random displacement models (random walks), where the eastwards change in position, Δx (m), over a PTM time step Δt (i.e., 600 s) is given by (Proctor et al., 1994):

$$\Delta x = \frac{R}{r}\cos(2\pi R)(2K_x\Delta t)^{1/2} \qquad (2a)$$

where R is a random number in the range [0,1] and r is the standard deviation of $R\cos(2\pi R)$ with a value of $1/\sqrt{6}$. The expression $(R/r)\cos(2\pi R)$ thus has the necessary properties of a mean of zero and a standard deviation of unity (Ross & Sharples, 2004). A similar expression for northwards diffusion is given by:

$$\Delta y = \frac{R}{r}\sin(2\pi R)\left(2K_y\Delta t\right)^{1/2} \quad (2b)$$

Spatially and temporally varying horizontal diffusivities, K_x and K_y ($m^2\ s^{-1}$), were output from the hydrodynamic model and linearly interpolated in the same way as the velocities. A random displacement model (Visser,1997; North et al., 2006) was used to simulate sub-grid-scale vertical turbulence:

$$\Delta z = K_z'\Delta t + \frac{R}{r}(2K_z\Delta t)^{1/2} \quad (3)$$

where K_z ($m^2\ s^{-1}$) is the vertical diffusivity, calculated by the hydrodynamic model and tri-linearly interpolated to the particle position, z, in the PTM algorithm, and $K_z' = \partial K_z/\partial z$ is evaluated at depth z.

Particles were neutrally buoyant and behaved passively within the water column, i.e., no habitual larval behaviour or vertical migration was simulated. Whilst the results of the larval behaviour in the laboratory show a response to light, the results of larval dispersal in the field were not conclusive. Over-dispersal of larvae is often predicted by including laboratory witnessed behaviour traits (Knights et al., 2006) so passive particles were modelled in this instance. Larval mortality was not considered, because a mortality function reduces the number of particles in the analysis. Mortality rates will affect overall connectivity but may not affect patterns of connectivity, unless mortality varies in space (Paris et al., 2007). Larvae that encounter a coastline were reflected back into the water column to their position at the previous iteration time step, rather than 'washed-up' on land, so that maximum dispersal can be investigated.

Cohorts of 10,000 larvae were released instantaneously from each of the 26 natal sites, which is sufficient so that the trajectories were not polluted by statistical outliers (Robins et al., 2013). Larvae were tracked for a pelagic larval duration (PLD) of 30 days, the minimum reported PLD of *S. alveolata* in the

wild (Dubois et al., 2007). The initial footprint of each reef (within which 10,000 particles were randomly positioned) was 1 km^2 at a point closest to the reef location. The above procedure was repeated at 4 different times throughout the summer; 01 May, 01 June, 01 July, and 01 August.

2.6.3. Larval dispersal, connectivity and self-recruitment

We analysed the sensitivity of larval dispersal distances, retention and connectivity, to the following variables: release location, release time (i.e., seasonal effects). We used oceanographic dispersal distances (i.e., the total distance travelled by each larvae) and release time as proxies for variations in tidal energy and residual currents, respectively.

Quantitative parameterizations of population connectivity have been produced using source distribution matrices p_{ij} (Ayata et al., 2010) which give the proportion of larvae that successfully settle in population *i* (i.e., a sink population), after the 30 day PLD, that came from population *j* (i.e., the source population). A value close to unity indicates high connectivity whereas a value close to zero indicates low connectivity. Retention at the source site is determined by p_{ii}. The overall success of larvae within a population *j* (i.e., larvae that manage to settle at any site) is given by:

$$T_j = \sum_{i=1}^{i=n} p_{ij} \quad (4)$$

where *n* is the number of sink populations. Larval wastage is denoted by $1 - T_j$. Upon completion of the PTM simulations, specific criteria were required to determine which larvae were successful. We used a similar approach to that taken by Cowen et al., (2006) for their larvae connectivity criteria; any larvae which were within 10 km of one of the 40 release sites at the end of the 30 days were deemed to settle at that site. The distance of 10 km was chosen because it is comparable to the tidal excursion of a passive particle in the Irish Sea (Xing & Davies, 2001). Larvae that did not meet these criteria (i.e., were located beyond 10 km from one of the 26 reefs) were considered unsuccessful. Evidently, using this approach, measures of connectivity are based only on a sub-set of possible environments. However, it is the comparative outputs, e.g., between release locations and seasonal effects, that are analysed here.

3. Results

3.1. *Sabellaria alveolata* larval dispersal on shore

Analysis of the solution retained by onshore traps revealed limited results due to a failure of the traps to retain larvae. When collected back off the shore some traps contained large grains of sand, and in some instances large crustaceans such as *Crangon crangon,* due to inadequate closure of the traps, ruptures in the flow mesh, or damage to silicon seals. It is assumed that despite routine maintenance these traps were unable to withstand the strong tidal and wind driven currents that occurred at all deployment sites. In other cases, traps contained many fine particles that may have blocked the mesh, reducing filtering efficiency. These traps were passive with the inflow mesh on the end facing away from the low water mark. Water movement as the tide came in may not have been enough to force larvae into the traps, and if it was, water may only have been forced through them for a short period of time on the ebb and flood tide. Additionally within the main frame of the traps, larvae had to be forced through a subsequent smaller elevated opening to be retained in the interior. Just 3 larvae were found from April to September at Llanddulas, and these were all from the July survey (1 in the mid shore and 2 from the low shore). Just 1 larva was found from both Dunraven and Aberarth in August (low shore in both instances). All larvae were less than 90 µm.

3.2. Fecundity of adult *Sabellaria alveolata*

3.2.1. Egg count

Egg count varied from 1.25 $x10^4$ to 1.5 $x10^6$ at Llanddulas, 2.5 $x10^4$ to 1.0 $x10^6$ at Aberarth and 1.25 $x10^4$ to 4.25 $x10^5$ at Dunraven. Mean egg count showed significant differences between Dunraven and both Llanddulas and Aberarth (One Way ANOVA, $p < 0.001$) when both ln(egg count) and ln(egg count normalised by length) were compared with site. When ln(egg count normalised by weight) was compared, Llanddulas and Aberarth also show significantly different mean egg counts (One Way ANOVA, $p < 0.001$).

Mean ln(egg count) and ln(normalised egg count) were significantly different between months, both between and within the 3 sites (pooled data, $p = 0.001$; site specific $p < 0.001$) (Figure 7). Mean egg count showed least variation at

Dunraven where mean egg count in March and July was significantly higher than April, June, August and September (One Way ANOVA, $p < 0.001$) (Figure 7a). Mean egg count decreased from a maximum of 1.4 $x10^5$ ±4980 eggs.individual^{-1} in July to 15626 ±1711.6 eggs.individual^{-1} in August, showing a decrease from one month to the next. At Aberarth mean egg count in February and April were significantly lower than in both March, and May to September (One Way ANOVA, $p < 0.001$), increasing from 4.75 $x10^4$ ±12870 eggs.individual^{-1} in April to 5.188 $x10^5$± 1.001 $x10^5$ eggs.individual^{-1} in July. Variation in mean egg count was greatest at Llanddulas where mean egg count in May was significantly different to February, August and September (One Way ANOVA, $p < 0.001$). Mean egg count at Llanddulas increased from 42813 ±5921 eggs.individual^{-1} in February to 7.588 $x10^5$ ± 1.462 $x10^5$ eggs.individual^{-1} in May.

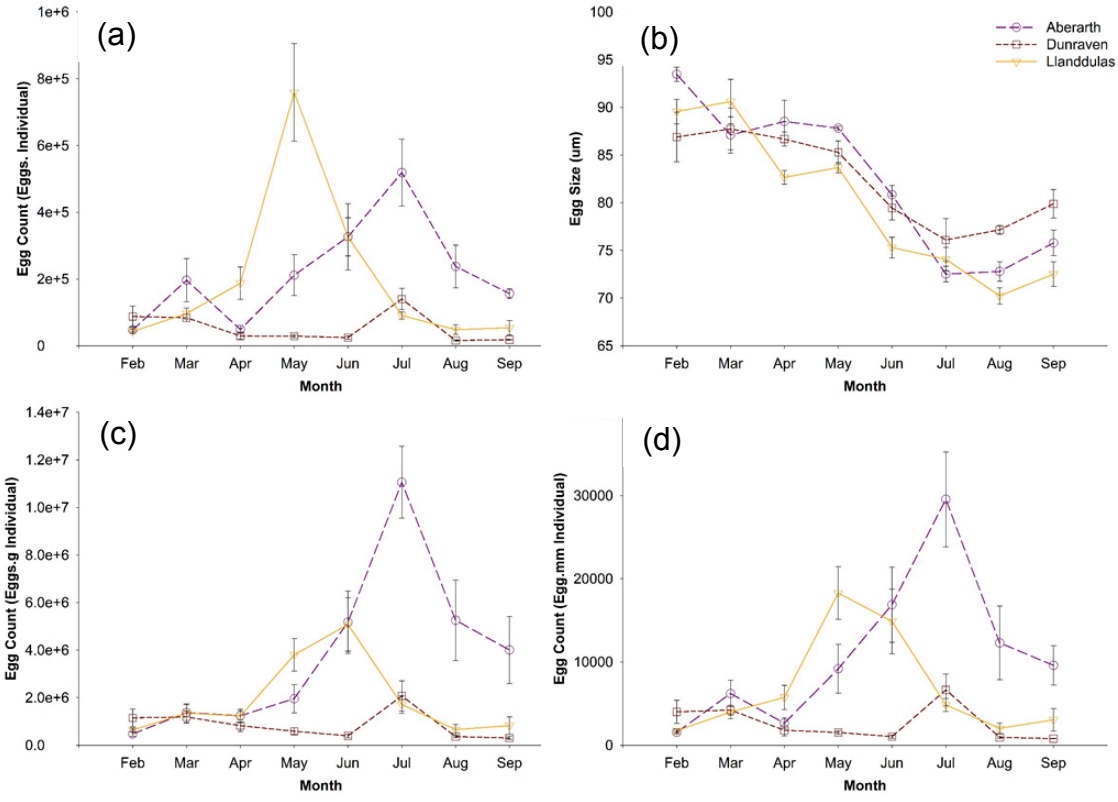


Figure 7: Monthly mean egg size and egg count from 5 individuals collected at 3 different sites, from February to September 2014. Llanddulas (yellow, solid line), Aberarth (purple, large dash) and Dunraven (maroon, small dash). Error bar represents standard error. (a) Mean monthly egg count. (b) Mean monthly egg size. (c) Mean monthly egg count normalised by mean monthly weight of donor females. (d) Mean monthly egg count normalised by mean monthly length of donor females.

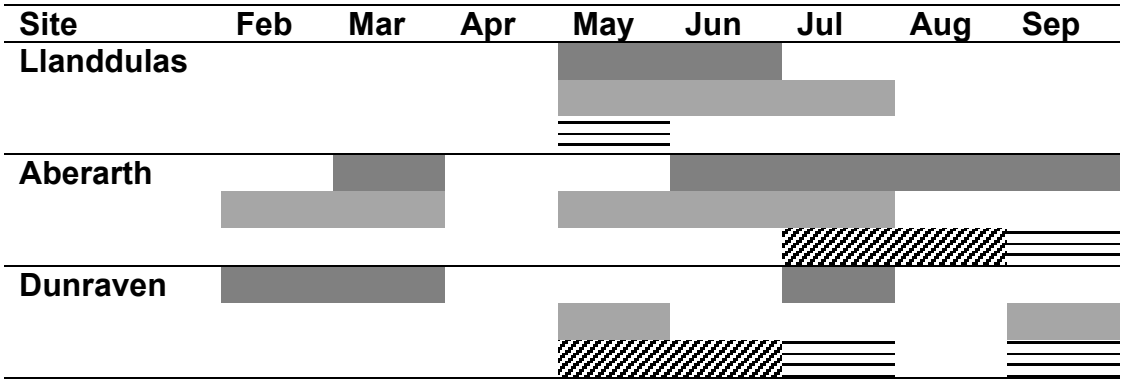
In normalisations by both weight and length of the donor female, a similar temporal pattern occurred to straight mean egg count (Figure 7b and c). At Llanddulas ln(mean egg count) in both normalisations was significantly greater in May and June than February, August and September, and in June than March (One Way ANOVA, $p < 0.001$). ln(mean egg count normalsed by weight) also showed June to be significantly greater than April (One Way ANOVA, $p < 0.001$). At Aberarth mean egg count normalised by weight increased exponentially from February to July, decreasing again in August and September but remaining at elevated levels in comparisons with both Llanddulas and Dunraven. ln(mean egg count normalised by weight) and ln(mean egg count normalised by length) in July were significantly greater than February to May, and significantly lower in February to June to September (One Way ANOVA, $p < 0.001$). Normalisation by length also shows February to be significantly lower than March (One Way ANOVA, $p < 0.001$). At Dunraven variation in mean egg count was suppressed in comparison to both other sites throughout the sampling period, in both normalisations. Despite the small range in data, a significant decrease occurred from March to September in ln(mean egg count normalised by weight). Overlaid on this was a very gradual decrease from March to June, followed by a significant increase to peak levels in July, and a subsequent significant decrease to low levels in August and September (One Way ANOVA, $p < 0.001$). ln(mean egg count normalised by length) concurred with the above but also showed a significant decrease in ln(mean egg count) from March to both June and August (One Way ANOVA, $p < 0.001$).

Egg count is related to both the length and weight of the female donor. Larger worms were inherently more fecund, with a higher egg count. Inclusion of normalisation by weight or length showed a suppression of egg count at Llanddulas in May (female worms were substantially larger in May than any other month (Appendix 1), and a magnification of egg count at Aberarth in July (female worms were substantially smaller in July than preceding months (Appendix 1)).

In summary, mean egg count at all 3 sites was relatively suppressed in winter showing a distinct peak at all 3 sites during the summer months (Table 6,

Figure 7a). This pattern was most defined at Llanddulas, whilst Aberarth and Dunraven demonstrated a slight bimodal distribution of mean egg count, with both sites showing significantly higher egg counts in March than subsequent months. Mean egg count was significantly different between sites when all data was pooled throughout the sampling season. These differences were particularly prominent in the summer peak egg production season, from May to August, which was site dependent (Table 6). Mean egg count at Dunraven was relatively suppressed throughout. Llanddulas was substantially higher than at both other sites in April and May, and than Dunraven in June, whilst mean egg count at Aberarth was substantially higher in May and June than Dunraven, and in July to September than at both other sites. Dunraven demonstrated reduced egg counts throughout, showing a peak in mean egg numbers in July, but with a mean egg count a third less than that reported at Aberarth.

Table 6: Synchronisation of mean egg and sperm count maxima. Mean egg count on the top (dark grey), mean sperm count middle (light grey), and larval blooms depicted at the bottom. A larval bloom with more than 400 larvae.m-3 are depicted by diagonal texture, whilst larval concentrations of more than 20 larvae.m-3 were depicted by horizontal stripes for each site.



3.2.2. Egg size

Egg size showed the greatest variability at Llanddulas where eggs ranged in size by ~70um throughout the sampling season, from 56.84 µm to 124.29 µm. Eggs collected in Aberarth varied from 61.69 µm to 110.02 µm and those collected from Dunraven varied from 45.88 µm to 105.43 µm. Mean egg size showed least temporal variation in Dunraven fluctuating from 87.75 ±2.12 µm in March to 76.09 ±2.24 µm in July. At Aberarth mean egg size ranged from 93.47 ±0.76 µm in February to 72.51 ±0.81 µm in July, and at Llanddulas from 90.62 ±2.32 µm in March to 70.23 ±0.84 µm in August. Egg size was greatest

at all 3 sites in the winter decreasing into the summer and increasing again in September (Figure 7b). Straight or natural log transformed egg size data did not conform to normality or show equal variance in comparisons with site. Consequently a non-parametric Kruskal-Wallis Test was utilised to compare egg size with site throughout the sampling period. There was no significant difference in egg size in seasonally pooled data between sites (Kruskal-Wallis, $p > 0.05$).

Mean egg size from February was significantly larger than in May, and from February to May significantly larger than in June to September. June was also significantly larger than August (One Way ANOVA, ln(mean egg size) $p < 0.001$). This pattern was also visible in site-specific data. At Llanddulas mean egg size in April and May was significantly less than in February and March. This trend continued with mean egg size in June to September being significantly less than in May (One Way ANOVA, $p < 0.001$). At this site, the lowest mean egg size was recorded in August with a slight increase in September. Mean egg size from Aberarth was substantially greater than at both other sites in February and May decreasing substantially in March and June. Eggs from Aberarth decreased in size significantly from February to March. Egg size in March to May was significantly greater than in subsequent months, with June also significantly larger than in July and August (One Way ANOVA, $p < 0.001$). A substantial decrease in egg size occurred in June and in July. Egg size then increased in August and September, when egg size was substantially greater than both other sites. Eggs collected from Dunraven were substantially larger than those from Llanddulas in April and June, and smaller than those from Aberarth in May. Eggs from Dunraven decreased less in size than those collected from the other 2 sites and were substantially larger in both August and September than the other 2 sites. Egg size from Dunraven was significantly larger in March than in June to. Additionally egg size in February to May was significantly larger than July or August (One Way ANOVA, $p < 0.001$).

In summary mean egg size showed little between site variation, but significant within site variation decreasing in size from spring (March/April) to summer (July/August) at all three sites. This decrease in egg size temporally correlates

with the overall season of peak egg production across all sites, but was consistent between sites whereas the timing and duration of peak egg production was site specific.

3.2.3. Egg count related to egg size

Mean egg count did not show a clear linear relationship with egg size (Figure 8a). Larger mean egg sizes were all associated with low egg counts at all sites, but small egg sizes are not always associated with high counts. When normalised by both weight (Figure 8b) and length (Figure 8c) of the donor female, there was greater variability in mean egg count at smaller egg sizes. At Aberarth a negative relationship between mean egg size and both normalised egg count, with larger egg counts composed of smaller eggs. However, this was not clear at either Llanddulas or Dunraven. Regression analysis shows an insignificant relationship between ln(egg size) and both ln(egg count) and ln(normalised egg count) in pooled and site-specific data ($p > 0.094$ for pooled data, $p > 0.059$ at Llanddulas, $p > 0.152$ at Aberarth, $p > 0.804$ at Dunraven).

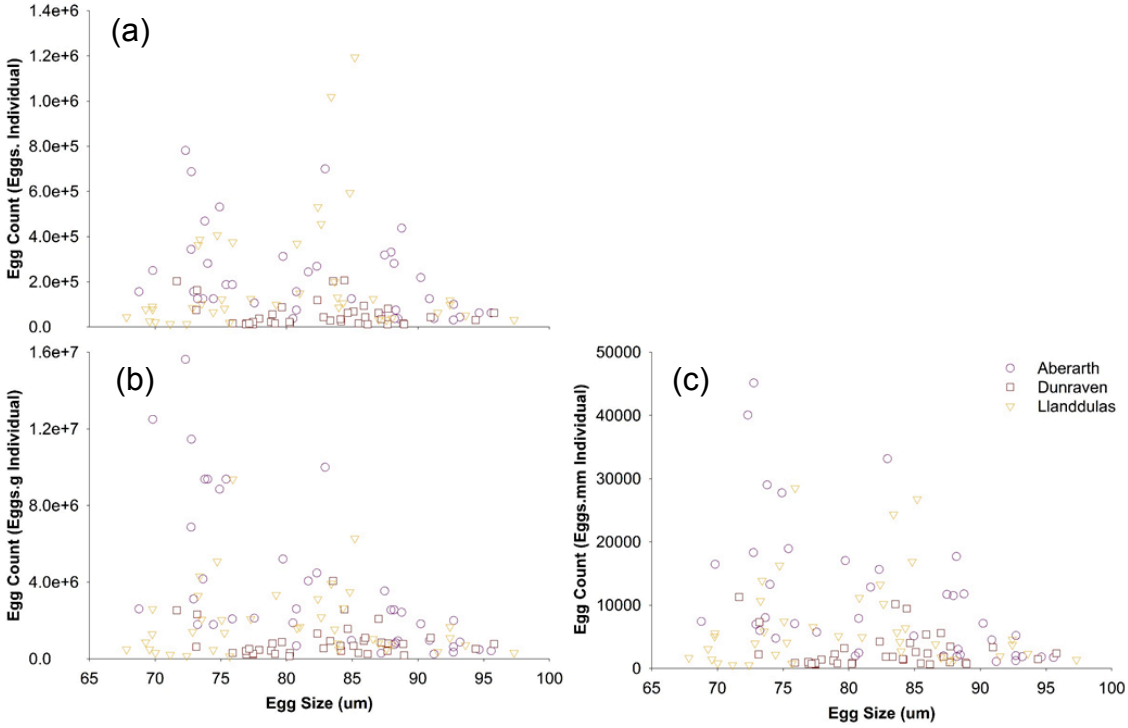


Figure 8: Egg count related to egg size at all 3 sites. Llanddulas is depicted orange (triangle), Aberarth in purple (circle) and Dunraven in maroon (square). (a) Mean egg count directly related to mean egg size. (b) Mean egg count normalised by weight related to mean egg size. (c) Mean egg count normalised by length related to mean egg size.

3.2.4. Sperm count

Sperm count varied from 2.46 $x10^7$ to 5.133 $x10^9$ at Llanddulas, 2.337 $x10^7$ to 2.523 $x10^9$ at Aberarth, and 3.03 $x10^7$ to 6.638 $x10^8$ at Dunraven. Raw or natural log transformed sperm count data did not conform to normality or demonstrate equal variance in comparisons of pooled sperm count data with site or month. Consequently a Kruskal-Wallis Test was used to compare sperm count with site, and subsequently month throughout the sampling period. Whilst there was no significant difference in mean sperm between sites throughout the sampling season (Kruskal-Wallis, $p = 0.186$), there was a significant difference in mean sperm between months in pooled data (Kruskal-Wallis, $p < 0.001$). In peak sperm production, in May, ln(mean sperm count) was significantly higher in Llanddulas than in Dunraven (One way ANOVA, $p = 0.011$).

When considered separately, the ln(sperm count) related to month demonstrates equal variance at each site. At Dunraven, mean sperm count in May was significantly greater than in February, June and July (One way ANOVA, $p < 0.001$). Of the 3 sites, Dunraven showed least variability in mean sperm count decreasing from a maximum of 5.125 $x10^8$ ± 5.417 $x10^7$ sperm.individual^{-1} in May to a minimum of 6.432 $x10^7$ ± 3.129 $x10^6$ sperm.individual^{-1} in June (Figure 9a). At Aberarth mean sperm count in May was significantly greater than in April or July to September (One way ANOVA, $p < 0.001$). Mean egg count varied from a maximum of 1.447 $x10^9$ ± 4.395 $x10^8$ sperm.individual^{-1} in May to a minimum of 1.968 $x10^7$ ± 6.340 $x10^6$ sperm.individual^{-1} in September. At Llanddulas, mean sperm count in May was significantly higher than in February to April, and July to September. Additionally, it was significantly higher in June than in February to April, August and September (One way ANOVA, $p < 0.001$). Maximum sperm counts of 2.846 $x10^9$ ± 6.523 $x10^8$ sperm.individual^{-1} were recorded in May and lowest mean sperm counts, of 6.504 $x10^7$ ± 6.361 $x10^6$ sperm.individual^{-1}, were recorded in September. Mean sperm count at all 3 sites was relatively stable throughout the sampling season with a peak in the summer.

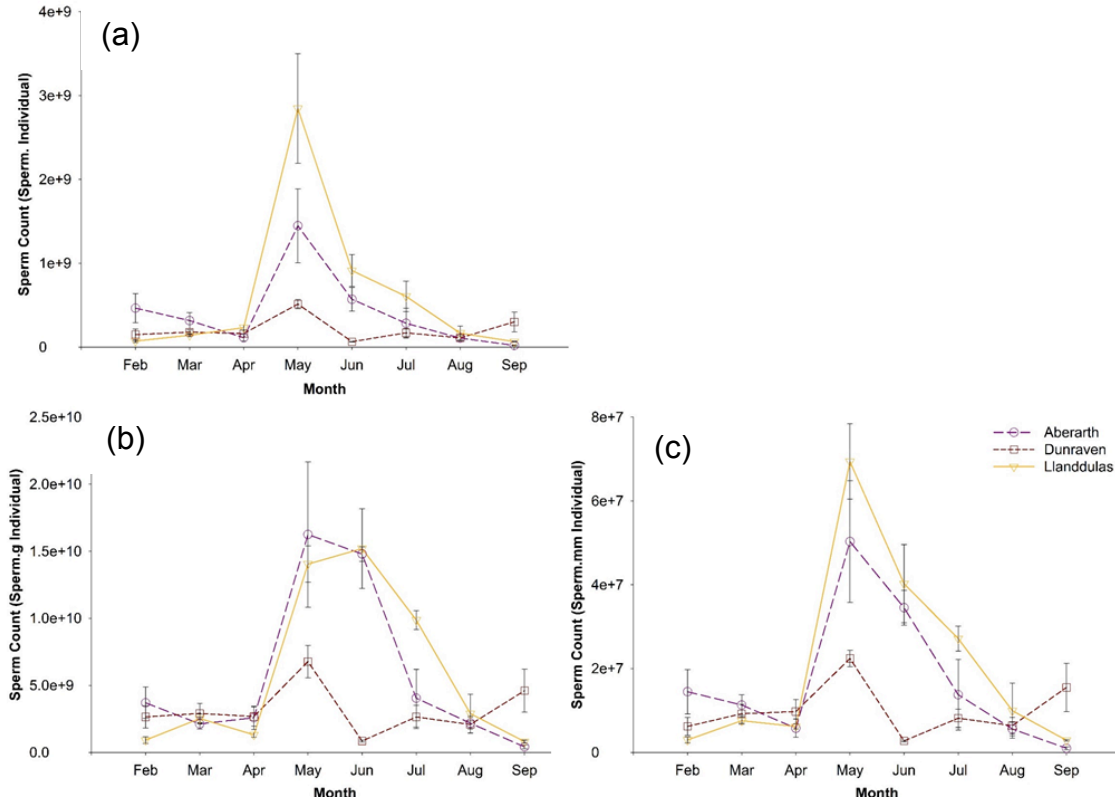


Figure 9: Monthly mean sperm counts from 5 individuals collected at 3 different sites, from February to September 2014. Llanddulas (yellow, solid line), Aberarth (purple, large dash) and Dunraven (maroon, small dash). Error bar represents standard error. (a) Mean monthly sperm count. (b) Mean monthly sperm count normalised by the mean monthly weight of the donor males. (c) Mean monthly sperm count normalised by the mean monthly length of the donor males.

Mean ln(sperm count) was also normalised by both weight (Figure 9b) and length (Figure 9c) of the donor males. In both normalisations a similar temporal pattern occurred as with raw sperm count, but the summer peak at both Llanddulas and Aberarth was extended at a result of worm size. At all sites sperm count was low from February to April, and returned to approximately equal levels in August, in both normalisations. In Llanddulas, both ln(sperm count normalised by weight) and ln(sperm count normalised by length) showed sperm count was significantly higher in May to July than any other sampled month. Additionally the ln(mean sperm count) in February was significantly lower than in any other month (One way ANOVA, $p < 0.001$). At Aberarth ln(sperm count normalised by weight) showed mean sperm count to be significantly higher in May to July than all other months. Additionally mean sperm count in September was significantly lower than all other months (One way ANOVA, $p < 0.001$). ln(sperm count normalised by length) concurs with

the above, and additionally shows sperm count to be significantly higher in February and March than April, August and September (One way ANOVA, $p < 0.001$). In contrast, the duration of peak sperm production at Dunraven was substantially shorter. At Dunraven ln(sperm count normalised by weight) May was significantly higher than in June alone (ANOVA, $p < 0.001$). Again, ln(sperm count normalised by length) showed the same pattern, but also mean sperm count in May was significantly higher than February and August. Although an elevated mean sperm count was recorded from Dunraven in May, this was a less than half the mean sperm count recorded at either of the other 2 sites and was a solitary event with the lowest mean sperm count recorded the following month. Unlike the other 2 sites, an increase in mean sperm count was also seen at Dunraven in September.

Sperm count is related to both the length and weight of the male donor. As with females, larger worms were inherently more fecund, with a higher sperm count. Inclusion of normalisation by weight or length showed a suppression of sperm count in Llanddulas in May, and a magnification in June (male worms were substantially larger in May than any subsequent month, and smaller in June (Appendix 1). Sperm count was also elevated at Aberarth in June (small male worms were collected in June (Appendix 1)).

In summary the mean sperm count showed a similar pattern between sites with low sperm count at the start and end of the sampling season and a peak in sperm production in the summer months. At both Aberarth and Llanddulas, mean sperm production was elevated from May to July, whilst at Dunraven, a short and significantly smaller peak occurred in May alone. Spring sperm counts at Aberarth and Dunraven were also significantly higher than subsequent months (Table 6).

3.3. Egg count related to sperm count

Mean egg count does not visually show a clear linear relationship with mean sperm count (Figure 10). However, Poisson regression did not show a positive correlation between ln(sperm count) and ln(egg count) when all data was pooled. Between site variation in this relationship also did not occur. At Llanddulas both sperm and egg count were high in May and June. However at both Aberarth and Dunraven, the peaks in sperm and egg count did not

correlate significantly. At Aberarth sperm count peaked in May to July whilst egg count peaked in June to August, whilst at Dunraven sperm count peaked in May and September, whilst egg count peaked in July.

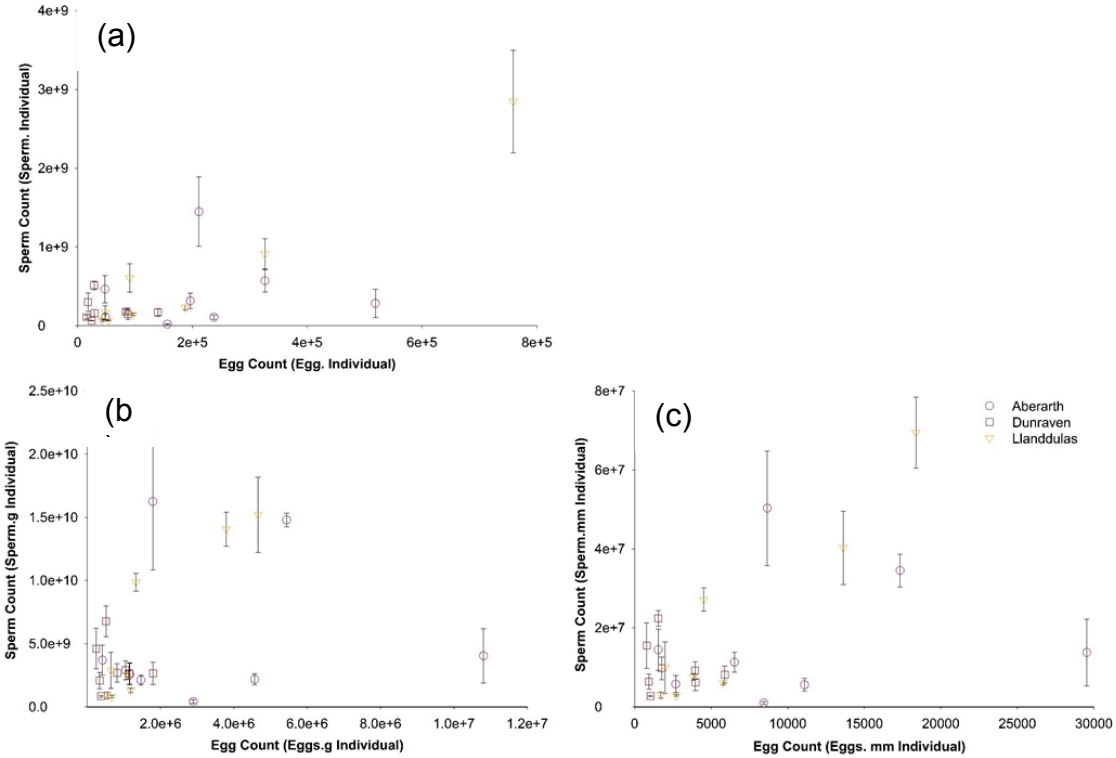


Figure 10: Mean sperm count related to mean egg count at all 3 sites. Llanddulas is depicted orange (triangle), Aberarth in purple (circle) and Dunraven in maroon (square). Error bars depict the standard error in the sperm count. (a) Mean sperm count related directly to mean egg count. (b) Mean sperm count normalised by the monthly mean weight of the donor males related to mean egg count normalised by the monthly mean weight of the donor females. (c) Mean sperm count normalised by monthly mean length of the donor males related to mean egg count normalised by the mean length of the donor females.

3.4. Sabellaria alveolata larval behaviour

3.4.1. Horizontal Phototaxis Response

Young larvae (five days post fertilisation) showed no positive or negative phototaxis (Figure 11). Negative phototaxis was significant for older larvae 15 days post fertilisation (One way ANOVA, $p < 0.05$) and greatest for larvae 30 days post fertilisation (One way ANOVA, $p < 0.001$), with larvae favouring the shaded half of the light chamber (Figure 11.). A 2 way ANOVA conducted showed a significant interaction between light exposure and the age of the

larvae on the larval phototaxis (Two way ANOVA, p <0.05), showing the greatest change on day 15.

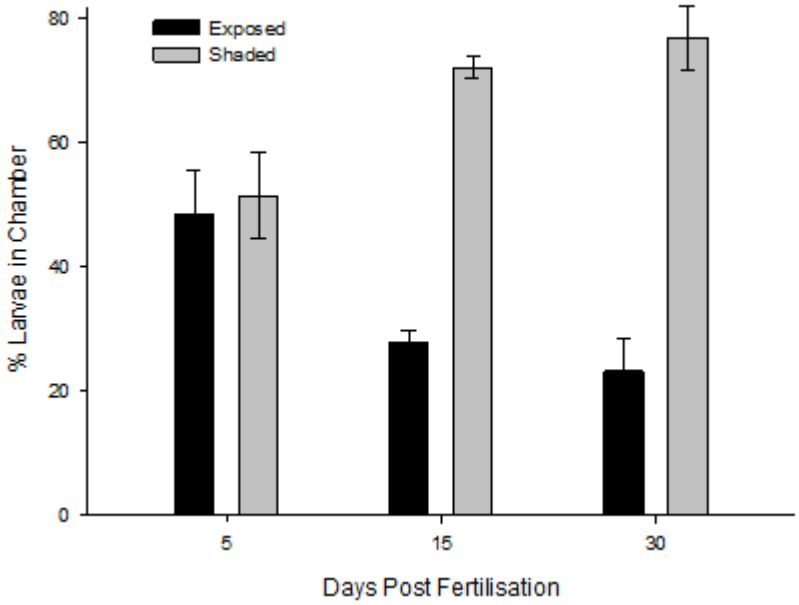


Figure 11: The phototactic response of *Sabellaria alveolata* larvae to horizontal light exposure. Graph represents the migration of larvae from light exposed to shaded half of an experimental light chamber over a 30 minute period at different stages of larval development. Error bars ±1 SE; P <0.05 (One-way ANOVA).

3.4.2. Vertical Phototaxis Response

The influence of vertical light exposure on phototaxis of larvae was more dependent on age of the larvae, with the only significant difference between negative and positive phototaxis occurring when larvae were 30 days post fertilisation (One way ANOVA, p <0.005). The larvae showed a steady increase in aversion to light, favouring the darker, upper end of the vertical cylindrical tubes, 50cm away from the light source (Figure 12.) There was a significant interaction between light exposure and age of larvae on phototaxis (One way ANOVA, p <0.005).

3.4.3. Swimming Speeds and Larval Size

Swimming speeds of larvae increased exponentially with larvae size until day 25, where the swimming speed declines to rates similar to day 15 (Figure 13).

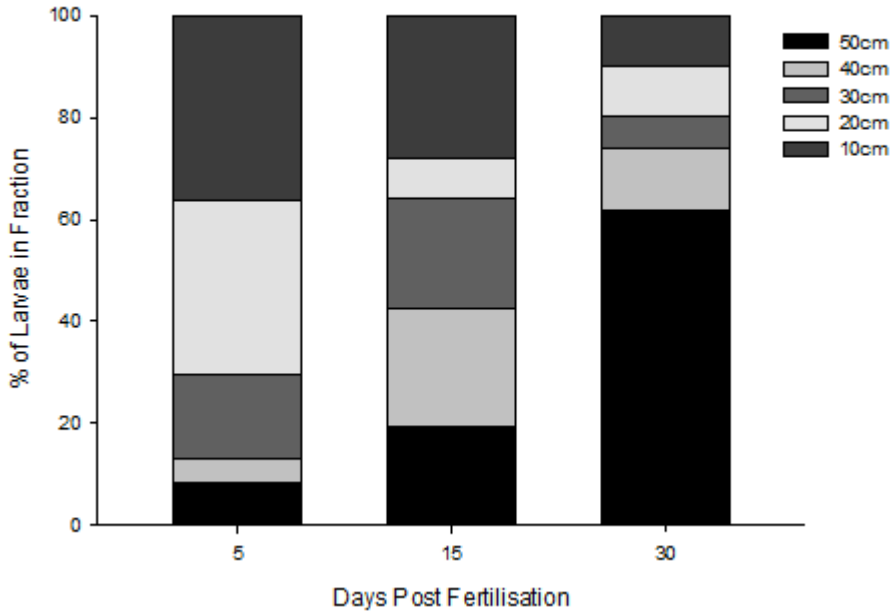


Figure 12: The phototactic response of *Sabellaria alveolata* larvae to vertical light gradient. Each fraction of each bar represents a 10cm vertical depth fraction (4ml) of a cylindrical tube exposed with a vertical light gradient from the base, with 10cm fraction exposed to the highest light intensity, and 50cm fraction exposed to the lowest intensity.

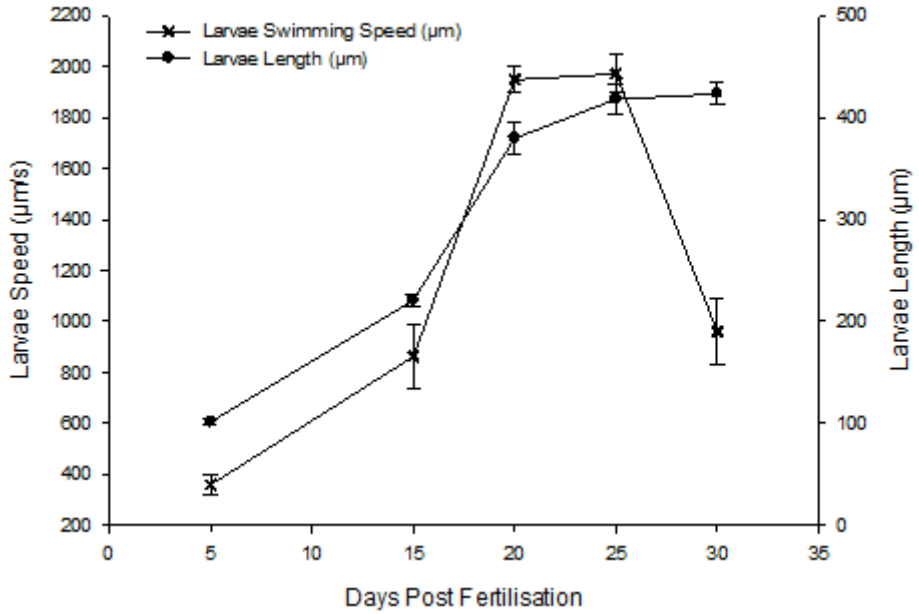


Figure 13: The change in *Sabellaria alveolata* larvae swimming speed and size with increasing age from day 5 to day 30 post fertilisation. Each point represents the average speed/lengths recorded from a range of replicates. Error bars ±1 SE; *P* <0.005 (One –way ANOVA).

One way ANOVA revealed a significant difference between the swimming speeds (One way ANOVA, p <0.001). A Tukey HSD revealed that each speed was significantly different (*P* <0.005) with the exception of speed measurements recorded between larvae aged day 15 and 30 (Tukey HSD , p = 0.936) and days 20 and 25 (Tukey HSD, p = 1). The lengths of *S. alveolata* larvae throughout the culture period showed significant growth (One way ANOVA, p <0.001) with growth being the most significant between days 5 and 15 and no significant difference between days 20, 25 and 30 (Tukey HSD; Figure 13.).

3.5. *Sabellaria alveolata* larval dispersal offshore

3.5.1. All sites and all months

Total mean larval abundance shows substantial variation both between and within sites (Figure 14). Significant difference in mean larval abundance occurred between sites in pooled data (Kruskal-Wallis, p = 0.002), and between months (Kruskal-Wallis, sqrt(larval abundance), p < 0.001). Maximum total mean larval abundance recorded at Llanddulas was 25 ±8 larvae.m^{-3}, 2 orders of magnitude less than at Aberarth where the maximum total mean abundance recorded was 11399 ±3094 larvae.m^{-3}. The maximum total mean abundance at Dunraven was intermediate but substantially different to both other sites at between 7129 ±934 larvae.m^{-3}.

No prominent peak in total mean larval abundance was seen at Llanddulas, were the total mean larval abundance recorded was greatest in May (Figure 14a). Despite the lack of a prominent peak in larval abundance, there was still a significant difference in mean larval abundance between months at this site (Kruskal-Wallis, P < 0.001). A larger abundance of Stage 0 (S^0) and Stage 1 (S^1) larvae were recorded in May than all other sampled months (15 ±7 S^0 larvae.m^{-3} and 5 ±2 S^1 larvae.m^{-3} respectively), but a lower abundance of Stage 2 (S^2) larvae than in subsequent months (4 ±2 S^2 larvae.m^{-3} in May compared to 14 ±7 S^2 larvae.m^{-3} in July, 5 ±2 larvae.m^{-3} in August and 11 ±7 larvae.m^{-3} in September). The only occurrence of Stage 3 (S^3) larvae at this site was in June.

At both southern sites a ≥2 month peak in total mean larval abundance was recorded. Both sites showed a significant difference in mean larval abundance with site (Kruskal-Wallis, p <0.001). This was initiated in May in Dunraven

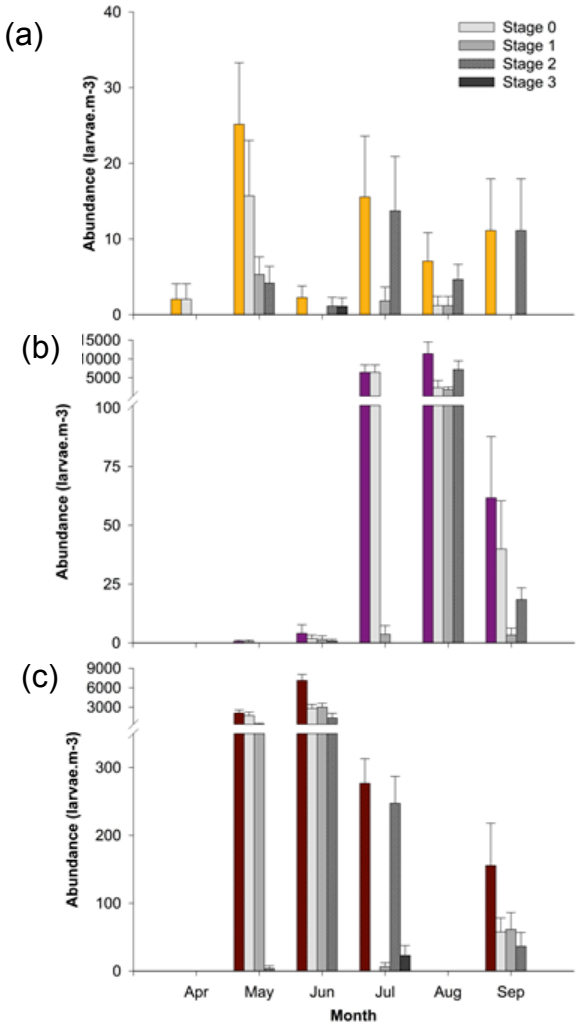


Figure 14: Total mean larval abundance of *Sabellaria alveolata* larvae collected from twelve plankton tows at 3 sites in a 6km 3-dimensional grid of the upper 5m of the water column grid monthly. In addition to total mean larval abundance, the overall proportion of larvae at each stage of development has also been depicted in grey scale. (a) Llanddulas; (b), Aberarth and (c) Dunraven. No sampling occurred in either Aberarth or Dunraven in April, or Dunraven in August.

when a total mean larval abundance of 2078 ±487 larvae.m^{-3} was recorded (Figure 14c). This increased 3 fold in June to 7129 ±934 larvae.m^{-3}. In contrast, by July, total mean larval abundance had decreased 4 fold to 277 ±36 larvae.m^{-3}. Total mean larval abundance decreased further in September but not substantially so (156 ±62 larvae.m^{-3}). In May the majority of recorded larvae at Dunraven were S^0 larvae, although a relatively large concentration of

S^1 larvae were also present (1701 ±546 S^0 larvae.m^{-3} and 374 ±142 S^1 larvae.m^{-3}). By June the concentration of S^0 larvae in the water column had increased but this developmental stage now made up less than half of the recorded larvae. S^1 larvae were now present in similar abundances, in addition to a smaller proportion of S^2 larvae (2787 ±642 S^0 larvae.m^{-3}, 2998 ±608 S^1 larvae.m^{-3} and 1344 ±696 S^2 larvae.m^{-3}). By July S^2 larvae dominated (247 ±40 S^2 larvae.m^{-3}). S^1 and S^3 larvae were also present (7 ±6 S^1 larvae.m^{-3} and 23 ±15 S^3 larvae.m^{-3} respectively) but no S^0 larvae showing a progression of the main larval bloom through time. In September, some S^0 larvae were still present suggesting secondary trickle spawning had occurred, and S^1 and S^2 larvae were also present in similar numbers (58 ±21 S^0 larvae.m^{-3}, 61 ±25 S^1 larvae.m^{-3} and 37 ±20 S^2 larvae.m^{-3} respectively).

In Aberarth larvae were present in all sampled months (Figure 14b). The larval bloom was not detected until July when a total mean larval abundance of 6247 ±1977 larvae.m^{-3} was recorded. The bloom increased 2 fold by August to maximum values (11399 ±3094 larvae.m^{-3}). By September a substantial decrease in total mean abundance had occurred (62 ±26 larvae.m^{-3}). In June the vast majority of larvae recorded from Aberarth were S^0 (6423 ±1978 S^0 larvae.m^{-3}). In August although S^0 were still present in high abundance, S^1 larvae were recorded in equivalent abundance and S^2 larvae now composed the majority of the recorded population with roughly two thirds of the total mean larval abundance (2351 ±1886 S^0 larvae.m^{-3}, 1869 ±664 S^1 larvae.m^{-3} and 7180 ±2316 S^2 larvae.m^{-3}). S^2 larvae were also present in September but the dominant developmental stage was again S^0 (18 ±5 S^2 larvae.m^{-3} and 40 ±21 S^0 larvae.m^{-3}).

In summary, total mean larval abundance demonstrated variability with significant differences recorded both between sites and months with Aberarth and Dunraven showing large summer blooms in larval abundance, and Llanddulas showing none. The duration of the larval bloom was consistent between Aberarth and Dunraven however the timing of initiation at Aberarth was at least 2 months after that at Dunraven (Table 6) and was substantially larger. Little consistent pattern in larval stage was seen between sites, although S^0 larvae dominated the initial bloom. They are present in most

sampled months at all sites showing continuous larval release through the sampling season from either the site of interest or an adjacent site. The lack of any S^0 larvae in July in Dunraven and the subsequent reappearance in September, suggests that at this site a secondary bloom has occurred. Older stage larvae increase in numbers as the blooms progress.

3.5.2. Along shore variability

At Llanddulas larvae were only recorded from the west of the reef in April (Figure 15a), from the west and middle in June, and middle and east in July and September. No significant difference was seen at any site between distance along shore in pooled data throughout the sampling season (Kruskal-Wallis, p = 1.000), however when months were considered separately the mean larval abundance to the west was significantly greater than in the middle or east (Kruskal-Wallis, p = 0.029), whilst in July the mean larval abundance was significantly greater in the middle of the reef than to either side (Kruskal-Wallis, p = 0.012). All larvae found in April to the west of the reef were S^0, and in May, the mean abundance of S^0 larvae was still substantially greater to the west of the reef than to the east of the reef (29 ±17 S^0 larvae.m^{-3} and 5 ±5 S^0 larvae.m^{-3} respectively). In June S^2 larvae were only found to the west of the reef and S^3 larvae in the middle of the reef. In July S^2 larvae were the majority stage recorded on both the middle and east of the reef (31 ±19 S^2 larvae.m^{-3} and 11 ±7 S^2 larvae.m^{-3} respectively) with S^1 larvae also present from the middle of the reef. In August S^0 and S^1 larvae were recorded from the west of the reef, but S^2 larvae remained the dominant stage recorded across the reef in low concentrations (maximum to the west of the reef at 7±3 S^2 larvae.m^{-3}). By September all recorded larvae were S^2 (maximum abundance of 31 ±18 S^2 larvae.m^{-3} in the middle of the reef).

At Aberarth larvae were only recorded in the middle of the reef in May, and from the middle and the south of the reef in June (Figure 15b). Total abundance was substantially greater to the north of the reef than both the mid or the south in July (14199 ±2836 larvae.m^{-3}, 3026 ±1925 larvae.m^{-3} and 2055 ±934 larvae.m^{-3} respectively) and the mid in August (16255 ±6844 larvae.m^{-3} in the north compared to 5267 ±1961 larvae.m^{-3} in the middle of the reef). No clear differences in total mean abundance with along shore distance were

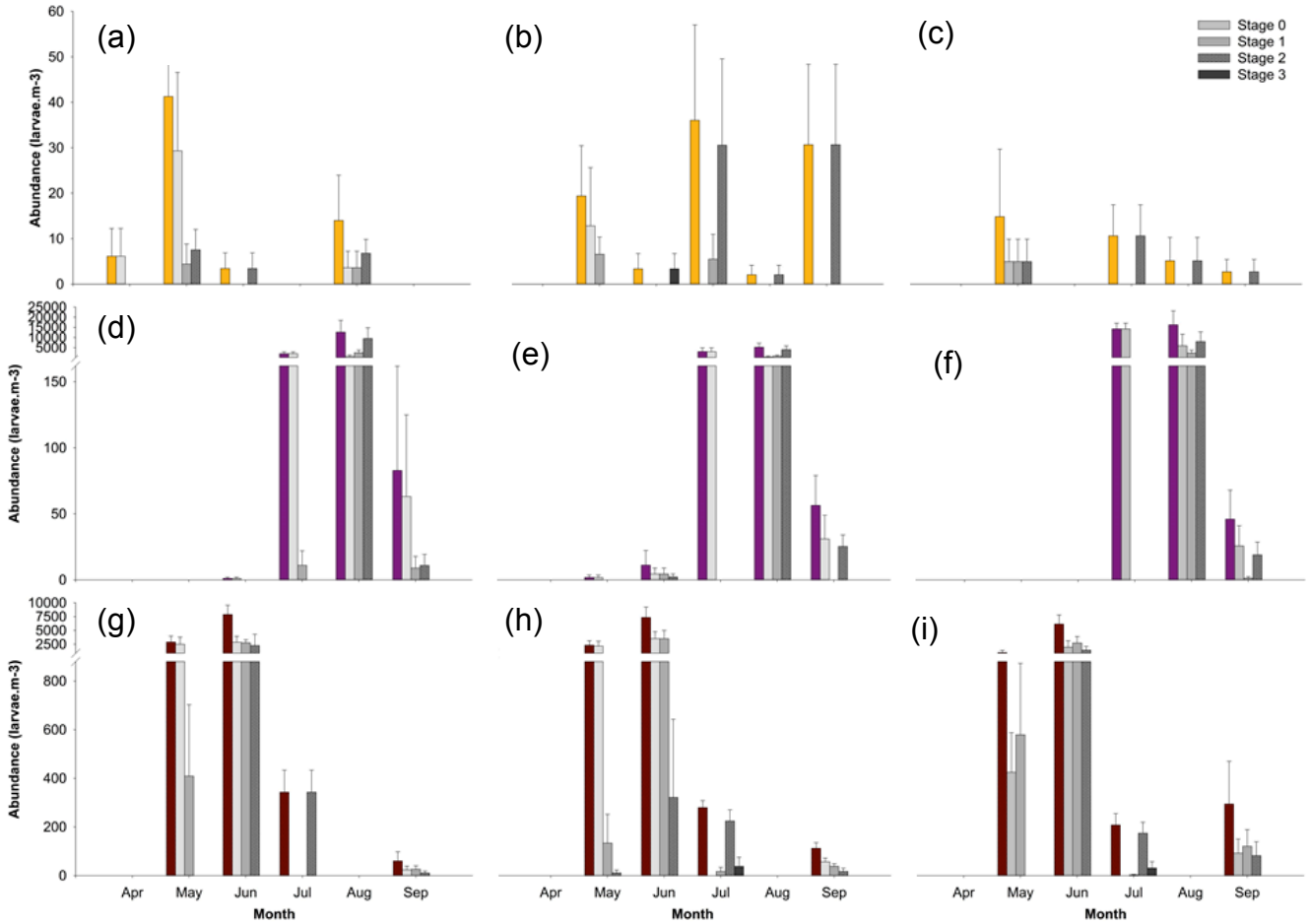


Figure 15: Mean larval abundance of *Sabellaria alveolata* larvae collected from four plankton tows 1km in each direction along shore from the reef and from the middle of the reef monthly at 3 sites. The overall proportion of larvae at each stage of development has also been depicted in grey scale. (a-c) Llanddulas where (a) represents west of the reef, (b) represents middle of the reef, and (c) represents east of the reef. (d-f) Aberarth and (g-i) Dunraven where (d, g) represent south of the reef, (b, h) represent middle of the reef, and (c, i) represent north of the reef. No sampling occurred in either Aberarth or Dunraven in April, or Dunraven in August.

recorded in September. No significant difference was seen between distance along shore in pooled data (Kruskal-Wallis, p = 0.482). However, when data was considered by month, mean larval abundance was significantly greater to the north of the reef than middle or south of the reef in July (ANOVA, p < 0.001) and to the north of the reef than middle in August (Kruskal-Wallis, p = 0.018). In May, S^0 larvae, and in June, S^1 and S^2 larvae, were only recorded from the middle of reef. S^0 larvae were recorded in both the middle and west of the reef in June. A substantially greater abundance of S^0 larvae was recorded to the north of the reef in July and August than either the middle or the south (14199 ±2836 S^0 larvae.m^{-3} compared to 3026 ±1925 S^0 larvae.m^{-3} and 11 ±11 S^0 larvae.m^{-3} respectively in July, and 5955 ±5657 S^0 larvae.m^{-3} compared to 418 ±418 S^0 larvae.m^{-3} and 679 ±679 S^0 larvae.m^{-3} respectively in August). S^1 larvae were only recorded from the south in July (11 ±11 S^1 larvae.m^{-3}), but were present in high abundance at all along shore locations in August (2498 ±1348 S^1 larvae.m^{-3} to the south, 812 ±487 S^1 larvae.m^{-3} in the middle and 2296 ±1493 S^1 larvae.m^{-3} to the north) as were S^2 larvae (9499 ±5257 S^2 larvae.m^{-3} to the south, 4036 ±1836 S^2 larvae.m^{-3} in the middle and 8005 ±4764 S^2 larvae.m^{-3} to the north). By September, relatively low numbers of all developmental stages were recorded at any along shore location, with highest abundance of S^0 and S^1 to the south (63 ±62 S^0 larvae.m^{-3} and 9 ±9 S^1 larvae.m^{-3} respectively), and of S^2 in the middle of the reef (25 ±9 S^2 larvae. m^{-3}).

At Dunraven larvae were recorded from on reef and at distance from the reef in both directions in all months (Figure 15c). In May the total abundance of larvae to the north of the reef (1004 ±413 larvae.m^{-3}) was substantially less than in the middle or south (2327 ±801 larvae.m^{-3} and 2904 ±1080 larvae.m^{-3} respectively). In June and July no difference in total mean larval abundance occurred with along shore distance, although an order of magnitude difference occurred throughout between months (In June 7887 ±1674 larvae.m^{-3} were recorded in the south, 7354 ±1877 larvae.m^{-3} in the middle and 6147 ±1654 larvae.m^{-3} in the north, whilst in July 343 ±91 larvae.m^{-3} were recorded in the south, 280 ±29 larvae.m^{-3} in the middle and 208 ±47 larvae.m^{-3} in the north). In contrast to May, in September the total abundance of larvae to the south of the

reef was substantially less than to the north (60 ±37 larvae.m^{-3} to the south, 294 ±176 larvae.m^{-3} to the north). When all months were considered, there was no significant difference was seen between distance along shore (ANOVA, p = 0.426). However, when considered by month, in both May and July, mean larval abundance to the north was significantly less than in the south (One way ANOVA, p = 0.012 and p = 0.019 respectively). In contrast, in September mean larval abundance to the north was significantly more than in the south (One way ANOVA, p = 0.008). In May, both S^0 and S^1 larvae were recorded at all along shore locations. A substantially lower abundance of S^0 larvae was recorded from the north of the reef (425 ±163 S^0 larvae.m^{-3}) than either the middle or south (2182 ±860 S^0 larvae.m^{-3} and 2495 ±1295 S^0 larvae.m^{-3}respectively), but a substantially higher abundance of S^1 larvae was recorded from the north than from the middle (579 ±294 S^1 larvae.m^{-3} and 134 ±119 S^1 larvae.m^{-3} respectively). S^2 larvae were also recorded in small numbers from the middle of the reef in May (11 ±11 S^2 larvae.m^{-3}). Similar numbers of S^0 and S^1 larvae were found at all alongshore locations in June (2864 ±1086 S^0 larvae.m^{-3} and 2759 ±556 S^1 larvae.m^{-3} to the south, 3523 ±1270 S^0 larvae.m^{-3} and 3510 ±1495 S^1 larvae.m^{-3} in the middle, and 1975 ±1161 S^0 larvae.m^{-3} and 2727 ±1177 S^1 larvae.m^{-3} to the north). A substantially higher abundance of S^2 larvae was recorded from the north than the middle of the reef in June (1446 ±664 S^2 larvae.m^{-3} and 322 ±322 S^2 larvae.m^{-3} respectively). In July the abundance of all previously observed developmental stages was reduced. No S^0 were recorded in Dunraven Bay at any along shore location in this month, S^1 were reported from in both the middle and north in low abundance (maximum in the middle of 17 ±17 S^1 larvae.m^{-3}), whilst S^2 was reported from all along shore locations (maximum in the south of 343 ±91 S^2 larvae.m^{-3}). S^2 larvae were recorded in both the middle and north of the reef in July (38 ±38 S^2 larvae.m^{-3} in the middle and 31 ±26 S^2 larvae.m^{-3} to the north). In September S^0 to S^2 larvae were present at all alongshore locations in reduced abundance, but with greatest abundance in the north (92 ±58 S^0 larvae.m^{-3}, 120 ±69 S^1 larvae.m^{-3} and 82 ±57 S^2 larvae.m^{-3}. Substantially less S^1 and S^2 larvae were recorded from both the middle and south of the reef than the north (37 ±11 S^1 larvae.m^{-3} and 17 ±13 S^2 larvae.m^{-3} and 24 ±15 S^1 larvae.m^{-3} and 10 ±8 S^2 larvae.m^{-3}respectively).

In summary, mean larval abundance demonstrated some variation with direction from sampling site in specific months, however this was not consistent throughout the sampling period. At Llanddulas, the greatest number of larvae were reported from either the middle of the reef or to the west (The highest number of S^0 larvae were reported in May from the west of the reef). At Aberarth, the greatest number of larvae were reported from the north of the reef in both July and August. However, at Dunraven the greatest number of larvae were recorded from the south of the reef in May and July, and the north of the reef in September.

3.5.3. Offshore variability

At Llanddulas, larvae were only found 1km offshore in April, whilst in May there was a higher abundance of larvae 100m offshore (34 ±14 larvae.m^{-3} near shore and 16 ±8 larvae.m^{-3} 1km offshore respectively) (Figure 16a). In June there was no difference in the total mean abundance of larvae in comparisons near and offshore, whilst in July all recorded larvae were from 100m of the reef. In contrast, in August there was a substantially greater abundance of larvae 1km offshore (12 ±7 larvae.m^{-3} offshore and 2 ±1 larvae.m^{-3} near shore respectively). At this site, a significant difference was recorded between on and offshore when all data was pooled (Kruskal-Wallis, $p = 0.046$). When considered by month, significantly more larvae were found near shore than offshore in July (Kruskal- Wallis, sqrt(larval abundance), $p = 0.002$). All larvae recorded in April were S^0 larvae, and in May a substantially greater abundance of these were found 100m from the reef than 1km offshore (27 ±13 S^0 larvae.m^{-3} near shore and 4 ±3 S^0 larvae.m^{-3} off shore respectively). In June only S^2 larvae were recorded offshore, whilst only S^3 larvae were recorded 100m from the reef. In July both S^1 and S^2 larvae were recorded 100m from the reef of which S^2 composed the majority (27 ±12 S^2 larvae.m^{-3} and 4 ±4 S^1 larvae.m^{-3}). In both August and September S^2 larvae composed the majority of larval abundance in both near and offshore samples (2 ±1 S^2 larvae.m^{-3} near shore and 7 ±4 S^2 larvae.m^{-3} off shore, and 12 ±10 S^2 larvae.m^{-3} near shore and 10 ±10 S^2 larvae.m^{-3} off shore respectively). S^0 and S^1 larvae were also recorded 1km offshore in August in lower abundance.

At Aberarth larvae were only found 1km offshore in May (Figure 16b). No substantial difference was found in mean larval abundance between 100m and 1km offshore in June, July, or September, but in August a substantially higher

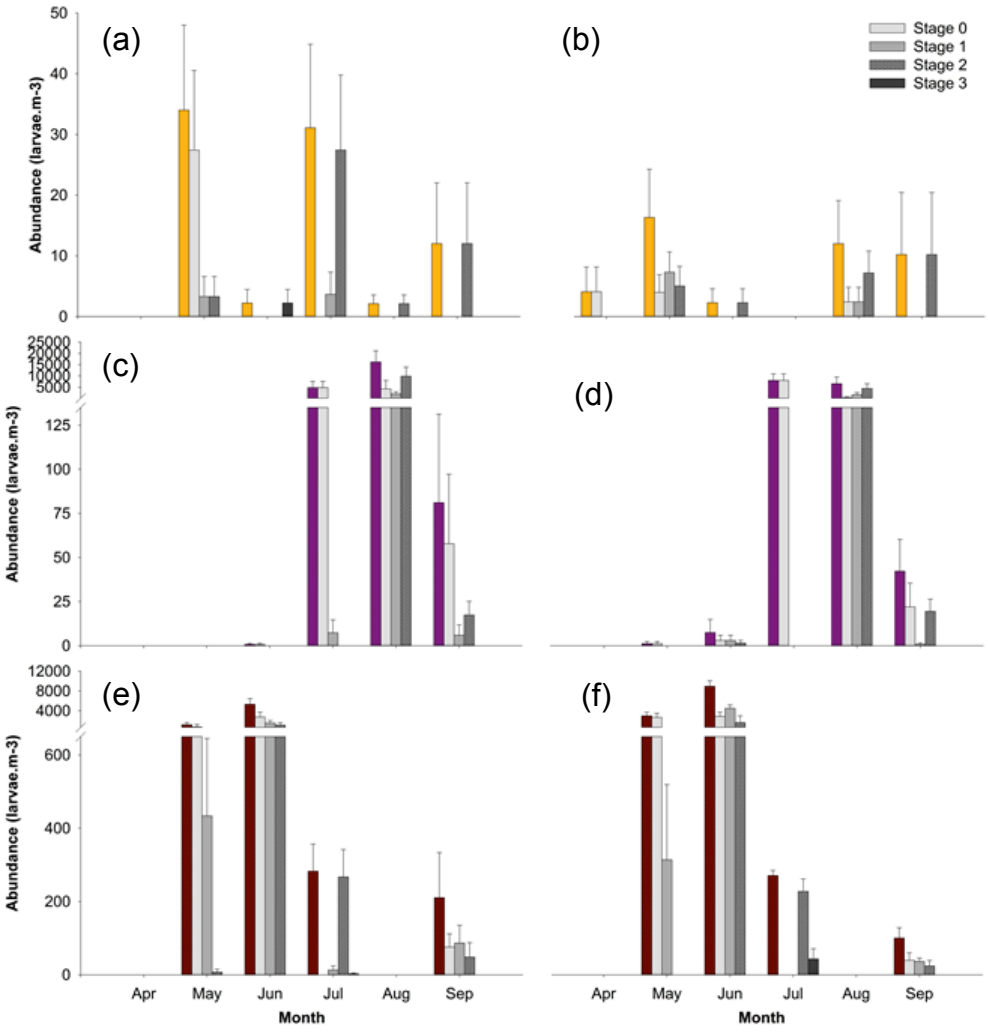


Figure 16: Mean larval abundance of *Sabellaria alveolata* larvae collected from six plankton tows at both 100m and 1km offshore from the reef monthly at 3 sites. The overall proportion of larvae at each stage of development has also been depicted in grey scale. (a-b) Llanddulas, (c-d) Aberarth and (d-e) Dunraven where (a, c, e) represent 100m offshore and (b, d, f) represent 1km offshore. No sampling occurred in either Aberarth or Dunraven in April, or Dunraven in August.

abundance of larvae was found 100m from the shore than 1km offshore (16231 ±4896 larvae.m^{-3} near shore and 6567 ±2968 larvae.m^{-3} offshore respectively). No significant difference was recorded between on and offshore when all data was pooled (ANOVA, $p = 0.2$). However, when considered by

month, significantly more larvae occurred near shore than offshore in August (Kruskal-Wallis, sqrt(larval abundance), p = 0.014). All larvae recorded in May were S^0, whilst in June both S^1 and S^2 were also recorded 1km offshore. In contrast, in July S^1 larvae were only recorded 100m from the shore. In August there were substantially more S^0 larvae 100m from the reef than 1km offshore (4271 ±3755 S^0 larvae.m^{-3} near shore and 430 ±289 S^0 larvae.m^{-3} offshore respectively), but in both locations the majority of larvae were S^0 (9914 ±3991 S^0 larvae.m^{-3} near shore and 4445 ±2166 S^0 larvae.m^{-3} offshore respectively). In September no clear difference in the proportion of developmental stages was detected between on or offshore locations.

In Dunraven, mean larval abundance was substantially greater offshore than near shore throughout the larval bloom (2977 ±725 larvae.m^{-3} offshore and 1190 ±443 larvae.m^{-3} near shore in May, and 8948 ±1096 larvae.m^{-3} offshore and 5311 ±1146 larvae.m^{-3} near shore in June) (Figure 16c). No clear differences in mean larval abundance were recorded in latter months. Again, no significant difference was recorded between on and offshore when all data was pooled (Kruskal-Wallis, p = 0.155). However, when considered by month, in both May and June, mean larval abundance was significantly greater offshore than onshore (One way ANOVA, p <0.001 in both cases). The offshore and near shore differences in the larval bloom in Dunraven are due to the proportion of early stage larvae. A substantially higher abundance of S^0 larvae was recorded from 1km offshore than 100m offshore in May (2663 ±840 S^0 larvae.m^{-3} offshore and 739 ±485 S^0 larvae.m^{-3} near shore), whilst in June, S^1 larvae were substantially more abundant 1km offshore than 100m offshore (4484 ±690 S^1 larvae.m^{-3} offshore and 1513 ±516 S^1 larvae.m^{-3} near shore). S^2 larvae were only recorded 100m from the reef in May, and S^1 from in 100m from the reef in July. In July, a greater mean abundance of S^3 larvae was recorded offshore (43 ±28 S^3 larvae.m^{-3} offshore and 3 ±3 S^3 larvae.m^{-3} near shore). No substantial differences between near and offshore larval abundance were seen at Dunraven in September with S^0 majority stage at each location (76 ±36 S^0 larvae.m^{-3} offshore and 40 ±20 S^0 larvae.m^{-3} near shore).

In summary, as with direction, some significant changes in mean larval abundance occurred with distance offshore from the reef, however these are not consistent with site or sampling month. At Llanddulas more larvae were found near shore than offshore in July, whilst at Aberarth, more larvae were found near shore in August. In both instance, S^2 larvae made up the largest proportion of the total mean larval abundance. In contrast, at Dunraven, significantly more larvae were found offshore than onshore in May and June, and in both cases the largest proportion of the total mean larval abundance was of S^0 larvae.

3.5.4. Depth variability

At Llanddulas larvae were only found at 5m depth in April and June (Figure 17a). There was no substantial difference recorded in total mean larval abundance between 1m and 5m depth in May, July, August or September. At this site, when all data was pooled, there was no significant difference in mean larval abundance with depth (One way ANOVA, $p = 0.322$). There was also no significant difference with depth at Llanddulas when data was considered by month (One way ANOVA, sqrt(larval abundance), $p > 0.151$). In April S^0 larvae, in June S^2 and S^3, in July S^1, and in August S^0 and S^1 were only recorded at 5m depth. All larvae recorded from 1 m depth, in all sampled months, were S^1, with exception of May when both S^0 and S^1 were also recorded. S^0 dominated abundance at both depths in May (15 ±8 S^0 larvae.m^{-3} at 1 m and 16 ±13 S^0 larvae.m^{-3} at 5 m respectively), whilst S^2 dominated abundance at both depths in July to September (15 ±13 S^2 larvae.m^{-3} in July and the only developmental stage present in August and September at 1 m, and 12 ±18 S^2 larvae.m^{-3} in July, 6 ±4 S^2 larvae.m^{-3} in August and also the only developmental stage in September at 5 m). No stage showed a significant difference with depth in any month (One way ANOVA, $p > 0.05$).

At Aberarth, when all data was pooled, there was no significant difference in mean larval abundance with depth (One way ANOVA, $p = 0.067$). Larvae were only recorded from 1m depth in May and from 5m depth in June (Figure 17b). When considered by month, there was significantly more larvae at 5m depth than 1m depth in August (One way ANOVA, sqrt(larval abundance), $p = 0.011$). In May, S^0 larvae were recorded from 1m depth only. In contrast, in

June no developmental stages recorded at 1m depth whilst S^0 to S^2 were all recorded at 5m depth. In July S^0 dominated abundance at both depths with no substantial difference recorded. However, in August, S^0 larvae were again recorded from 1m depth only and in high abundance (4701 ±3667 S^0 larvae.m^{-3}). Conversely a substantially higher abundance of S^2 larvae was recorded at 5m depth than 1m depth (12546 ±3426 S^2 larvae.m^{-3} at 5m depth and 1813 ±610 S^2 larvae.m^{-3} at 1m depth). In September, despite being present in relatively low abundances, S^0 larvae again composed the larval majority at both depths (55 ±40 S^0 larvae.m^{-3} at 1m depth and 24 ±13 S^0 larvae.m^{-3} at 1m depth). S^2 larvae were present at both depths, whilst S^1 larvae were only

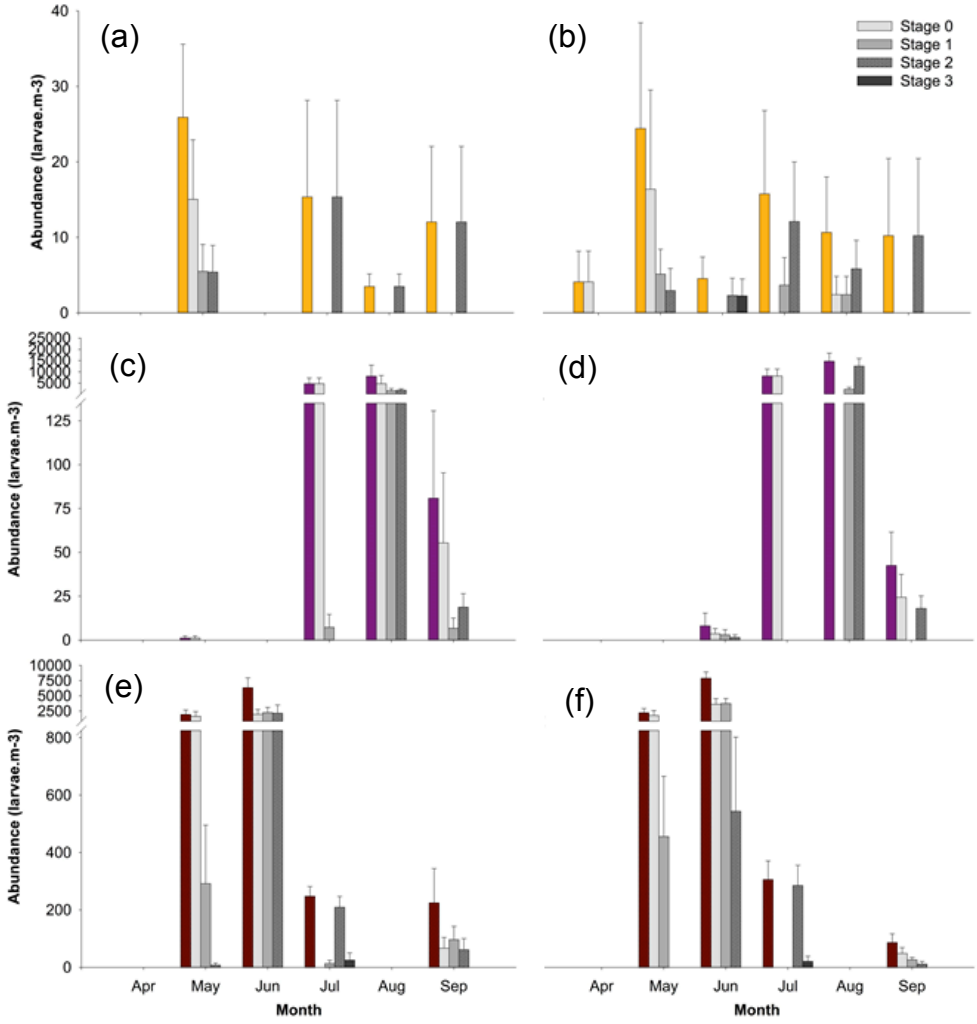


Figure 17: Mean larval abundance of *Sabellaria alveolata* larvae collected from six plankton tows at both 1m and 5m depth monthly at 3 sites. The overall proportion of larvae at each stage of development has also been depicted in grey scale.(a-b) Llanddulas, (c-d) Aberarth and (d-e) Dunraven where (a, c, e) represent 1m depth and (b, d, f) represent 5m offshore. No sampling occurred in either Aberarth or Dunraven in April, or Dunraven in August.

recorded at 1m. No stage showed a significant difference with depth in any month (One way ANOVA, p >0.05)

At Dunraven, mean larval abundance was substantially greater at 1m depth than 5m depth in both July and September (Figure 17c). No substantial differences in total larval abundance between 1m and 5m were recorded during the larval bloom in May or June (i.e. the water column is saturated with larvae). When all data was pooled, there was no significant difference in mean larval abundance with depth (One way ANOVA, p = 0.444). However, when considered by month, mean larval abundance was significantly less at 5m depth than 1m depth in September (One way ANOVA, sqrt(larval abundance), p = 0.027). At both depths S^0 larvae dominated in May (1636 ±789 S^0 larvae.m^{-3} at 1m depth and 1766 ±828 S^0 larvae.m^{-3} at 5m depth), and approximately equal abundance of S^0 and S^1 occurred in June (1957 ±813 S^0 larvae.m^{-3} and 2250 ±855 S^1 larvae.m^{-3} at 1m depth, and 3618 ±938 S^0 larvae.m^{-3} and 3746 ±919 S^1 larvae.m^{-3} at 5m depth). S^2 larvae were only reported in low abundance from 1m depth in May (13 ±12 S^2 larvae.$m^{-3)}$, whilst in July they dominated at both depths (210 ±37 S^2 larvae.m^{-3} at 1m depth and 285 ±71 S^2 larvae.m^{-3} at 5m depth). S^3 larvae were also present at both depths in July, although S^1 larvae were only recorded from 1m depth. There was a substantially higher abundance of both S^1 and S^2 larvae at 1m than 5m depth in September (96 ±46 S^1 larvae.m^{-3} and 61 ±39 S^2 larvae.m^{-3} at 1m depth, and 26 ±8 S^1 larvae.m^{-3} and 12 ±9 S^2 larvae.m^{-3} at 5m depth). No stage showed a significant difference with depth in any month (One way ANOVA, p >0.05).

In summary, generally, there was little significant variation with depth in the water column either within or between sites. However, there were a few exceptions, at Aberarth, in August, there were significantly more larvae at 5m depth than at 1m depth, S^0 larvae were absent whilst S^2 larvae showed a substantial greater abundance at 5m depth. In contrast, in September, in Dunraven there were significantly more larvae at 1m depth, and a greater abundance of all stages of larvae overall.

3.6. Biophysical modelling of *Sabellaria alveolata* larvae

Output from the particle tracking model for *Sabellaria alveolata* larvae show potential larval dispersal patterns for a population spawned from a known reef within the Irish Sea. Dispersal at the end of each 30 day pelagic larval duration (PLD) are presented, when the larvae are deemed to have developed to settlement stage. In addition, the cumulative oceanographic dispersal distances over the 30-day PLD are presented, which provides a proxy for the characterisation of the energy available to advect the particles from source reef to sink reef. Based on our settling criteria of being within 10 km of a source reef at the end of each 30-day PLD, metrics for particle retention and connectivity have been calculated and population connectivity matrices presented.

Output from all 26 known *S. alveolata* reef locations in the eastern Irish Sea, which includes the three Welsh sample reef locations at Llanddulas, Aberarth, and Dunraven, are shown. Spatial variability in larval dispersal and connectivity, between the reefs, has been characterised, according to several likely oceanographic processes and geographic features. Based on our PTM dispersal and settlement criteria, sub-populations of the Irish Sea Metapopulation have then been identified. Detailed 30-day PLD dispersal maps at the three sample locations, Llanddulas, Aberarth, and Dunraven, have been presented and compared with the field data. Finally, sensitivity to seasonal variability has also been examined through comparisons with four different release dates throughout May to August.

Daily-output dispersal animations from each PTM simulation (release dates: 01 May, 01 June, 01 July, and 01 August) have been created, and the animations are available in the digital appendix (Appendix 2-5).

3.6.1. Simulations of larval dispersal

Our particle tracking model predicts the dispersal of *S. alveolata* larvae populations from 26 known reefs within the Irish Sea, over a 30-day PLD, during May, June, July and August (see digital appendix for daily–output PTM animations). The PTMs show larvae being advected back and forth with the flood and ebb tidal oscillations and, in general, not travelling far from their natal reef, i.e., populations are generally located within 100 km of their natal reef

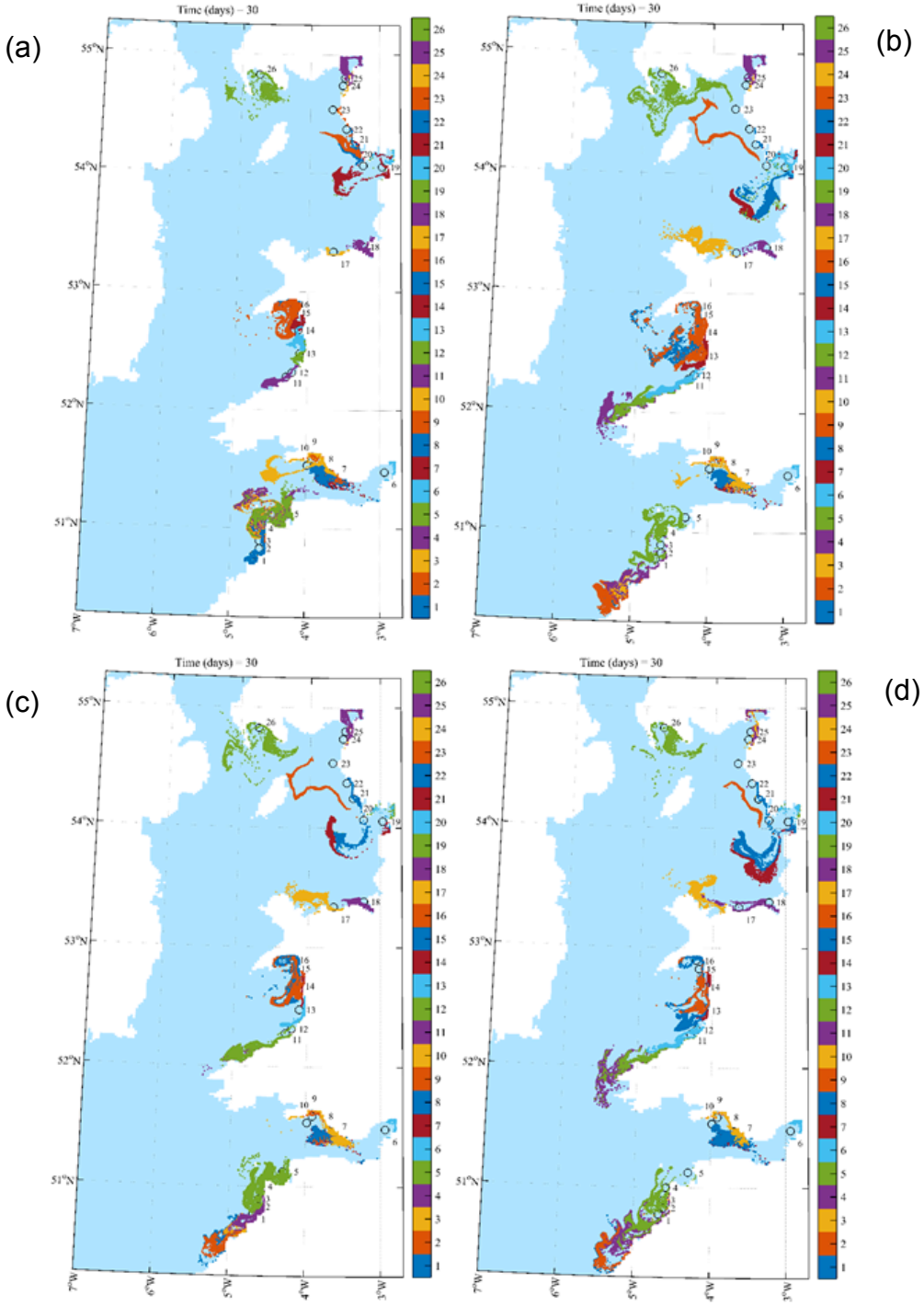


Figure 18: Particle Tracking Model (PTM) simulations of larval dispersal for *Sabellaria alveolata*, representing the potential dispersal of cohorts of larvae released ('spawned') from 26 selected reefs around the eastern Irish Sea (See Table 5 for reef names). Each cohort of 10,000 particles are coloured according to the colour bars which correspond to their release reef location, which are marked on the maps by black circles and corresponding numbers. Site 17 is Llanddulas (yellow), Site 11 is Aberarth (purple) and Site 7 is Dunraven (maroon). Each map shows dispersal after a typical 30-day pelagic larval duration, for particle releases on: (a) 01 May, (b) 01June, (c) 01 July, and 01 August.

after 30 days simulation (Figure 18). Our results suggest that far-field and across-shelf population connectivities (i.e., >100 km) are unlikely given a 30-day PLD.

Our larval dispersal model simulations predict that several neighbouring populations could be connected to form a sub-population, whereby larvae may travel from one source reef and settle at any other reef in the sub-population, depending on controls such as atmospheric conditions and larval release date. For example, *S. alveolata* reefs in Cardigan Bay (Sites 11-16: Aberarth, Aberystwyth, Borth, Barmouth, Shell Island, and Criccieth) form a sub-population that are isolated from all other populations, such as those in South Wales and North Wales (Figure 18). Consequently, *S. alveolata* from Cardigan Bay may connect within the reefs within Cardigan Bay but are unlikely to travel farther north, south, or west to Ireland, given a 30-day PLD and passive transport.

Reefs located in South Wales (Sites 7-10: Dunraven, Porthcawl, Swansea Bay, and Limeslade Bay) appear to form another sub-population (Figure 18); although our simulations show larvae dispersing south, close to a sub-population of reefs along the north coasts of Cornwall and Devon, and to an isolated population at Avonmouth. This implies that, perhaps under different atmospheric conditions or given a slightly longer PLD than 30 days, the South Wales sub-population could travel to these reefs, and vice-versa, hence, connecting these sub-populations. Nevertheless, under the conditions in our simulations, the three sub-populations were always distinct. *S. alveolata* populations along the North Wales coast (Sites 17 and 18: Llanddulas and Hilbre reefs) appear to form another distinct sub-population. Again, due to the close proximity of simulated larvae from English source reefs in Liverpool Bay after 30 days PLD, there is a suggestion that these larvae from could travel further south to the North Wales sub-population under different conditions (e.g., a northerly wind which would be likely for earlier spawning in the spring), especially given a longer PLD.

Predicted larval dispersal from all 3 sites of interest, both total larval dispersal and dispersal from the source reef, was less for larvae were released in May

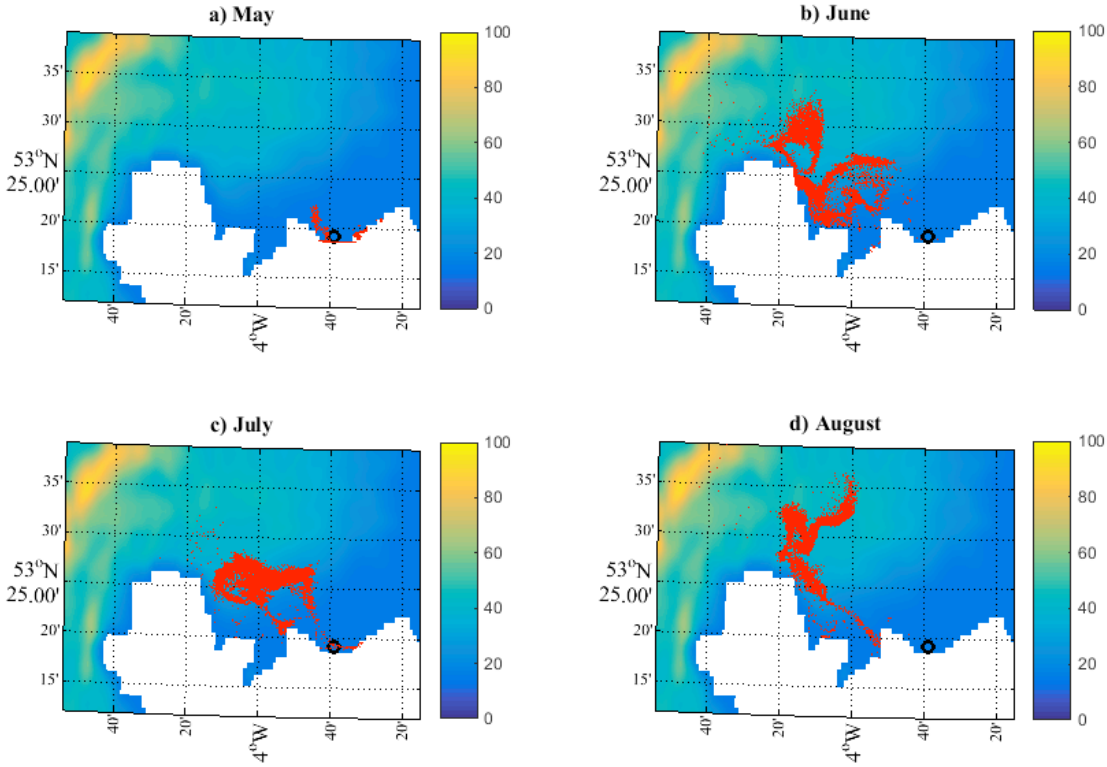


Figure 19: PTM simulations of larval dispersal for *Sabellaria alveolata*, representing the potential dispersal of cohorts of 10,000 larvae released from Llanddulas, North Wales. Each map shows bathymetry at mean sea level, overlain with larval dispersal (red particles) after a typical 30-day pelagic larval duration, for particle releases on: (a) 01 May, (b) 01 June, (c) 01 July, and (d) 01 August.

than in June, July or August 2014 (Figure 18). This difference was especially pronounced in Llanddulas (Figure 19). In May, at Llanddulas passive particle dispersal was minimal with a bimodal spread east and west washing over the site of origin multiple times in the first 30 days. Particles released in May, when maximum larval abundance was recorded at this site (Figure 14), were predicted to extend west to the Little Orme and east along the north coast of Wales with approximately sixty percent present at the site of origin after 30 days (Figure 19a, Figure 22a). Much higher dispersal occurred in all subsequent months, with net larval movement rapidly westwards away from the source. In June, dispersal was predicted to be greatest extending as far west as the north coast of Anglesey. By day 30 passive larvae released in June from Llanddulas could be present in the waters off the site of origin, the Great Orme and the west coast of Anglesey and as far west along the north coast as Cemlyn (Figure 19b). Dispersal of larvae released in July and August showed a similar westwards movement (Figure 19c-d), but larvae were

transported offshore from the west coast of Anglesey and the Great Orme. Whilst the majority of larvae released in latter months were predicted to be offshore on day 30 (Figure 19), approximately ten percent of larvae released in all months from June to August were expected to be retained at the source site at day 30 (Figure 22b-c). Some larvae were also predicted to be present along the north coast of Wales to the west of the source and up the west coast of Anglesey.

Hilbre Island (Site 18) is the only other abundant site on this coast. We simulated that particles released from this site, in May and July 2014, would not have reached Llanddulas (Figure 22 a,c). In contrast, in both June and August, larvae from Hilbre Island were predicted to disperse westwards along the coast, some as far west as Llanddulas (ten percent each month) (Figure 22 b, dc). Dependent on the timing of the larval bloom and the environmental conditions, larvae from Hilbre Island can connect with the Llanddulas reef at settlement stage. The greatest chance of settlement for larvae spawned from Llanddulas, is through retention at Llanddulas. Additionally Llanddulas may act as a source site providing larvae to many sites to the west along the coast of mainland north Wales and Anglesey.

Sabellaria alveolata larvae from our sampled reef at Aberarth in Cardigan Bay mainly travelled southwest and along the Welsh coast, except for a small proportion of larvae released in May that travelled a few kilometres (<10 km) north (Figure 20). A bimodal spread of larvae from Aberarth occurred in May with particles washing back over the site of origin many times. By day 30, particles are predicted to extend north to beyond Borth and south to Newquay (Figure 20a).Larvae travelled less than 30 km over 30 days. However, during June, July and August, larvae travelled markedly further. By day 30, particles were predicted to have dispersed; beyond St. Davids Head and towards Milford Haven and Skomer Island in Southwest Wales (Figure 20b-d). Thirty percent of larvae released from Aberarth in May (Figure 22a) are predicted to be retained at the site of origin, compared to ten percent in June and August (Figure 22b and d respectively), and none in July (Figure 22c). Twenty percent of the larvae released from Aberarth in May were predicted to be present in the waters near Aberystwyth after 30 days.

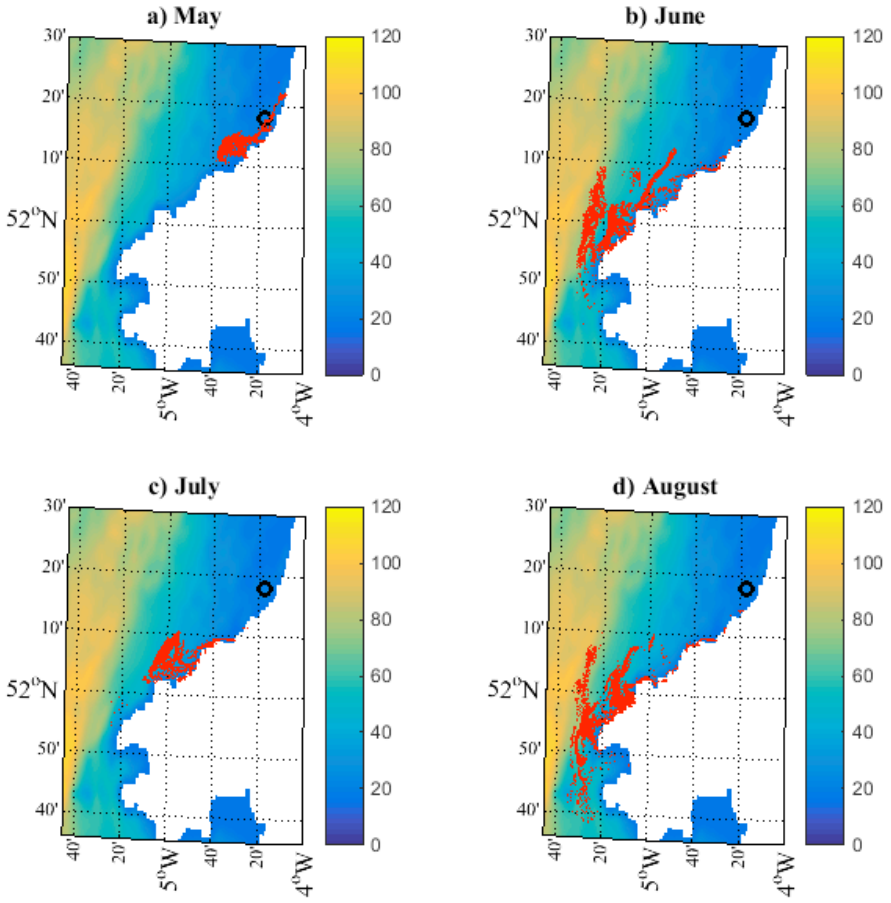


Figure 20: PTM simulations of larval dispersal for *Sabellaria alveolata*, representing the potential dispersal of cohorts of 10,000 larvae released from Aberarth, Cardigan Bay (black circles). Each map shows bathymetry at mean sea level, overlain with larval dispersal (red particles) after a typical 30-day pelagic larval duration, for particle releases on: (a) 01 May, (b) 01 June, (c) 01 July, and (d) 01 August.

This model output suggests that a southerly residual coastal current develops in Cardigan Bay during summer months. Tidal currents around Southwest Wales are known to be strong (>2 m s 1), rectilinear in a north-south direction, and with a net northerly residual (Robins et al., 2013). In addition, a distinct density-driven residual develops in summer, directed west from Southwest Wales towards southern Ireland (Lee et al., 2013). Therefore, larvae from Aberarth that reach Southwest Wales, are likely to either be advected northwards (offshore), and also westwards towards Ireland during summer months. However, a longer PLD than 30 days may be required for larvae to reach the Irish coast.

Our sampled reef in South Wales, Dunraven, was simulated in our PTMs (Site 7; Figure 18), and 30-day dispersal patterns for each release date are shown

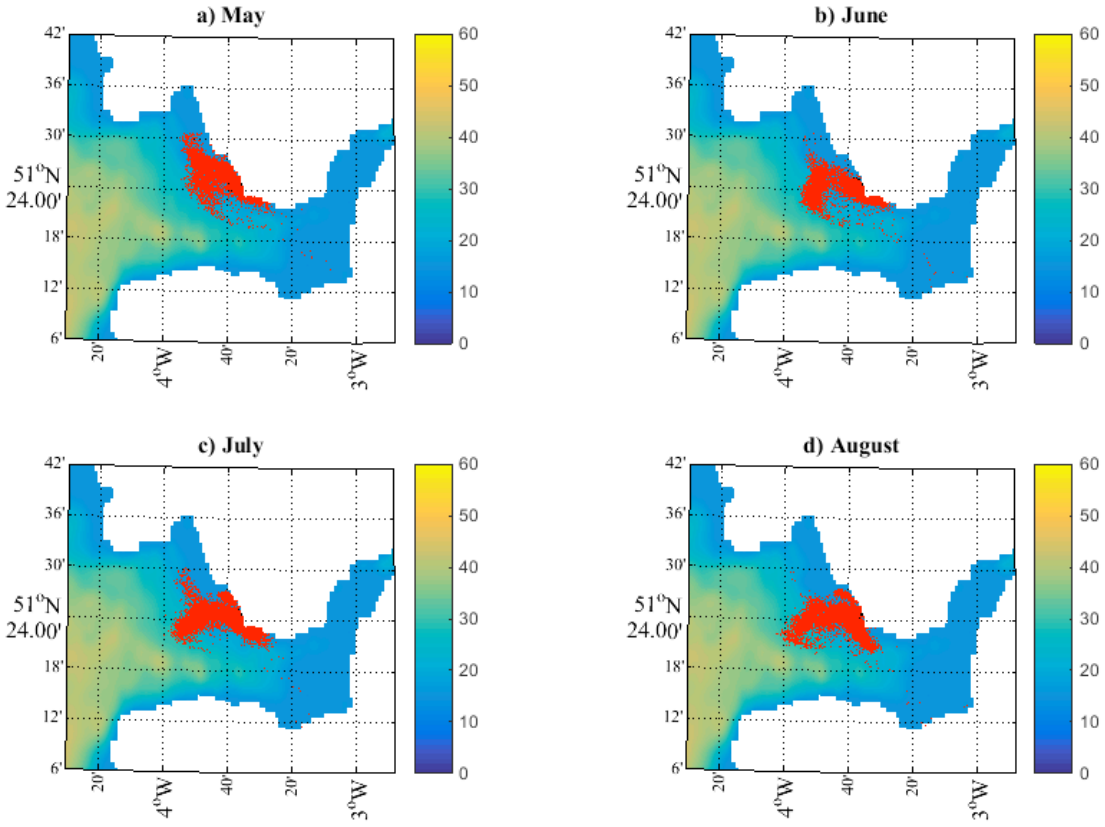


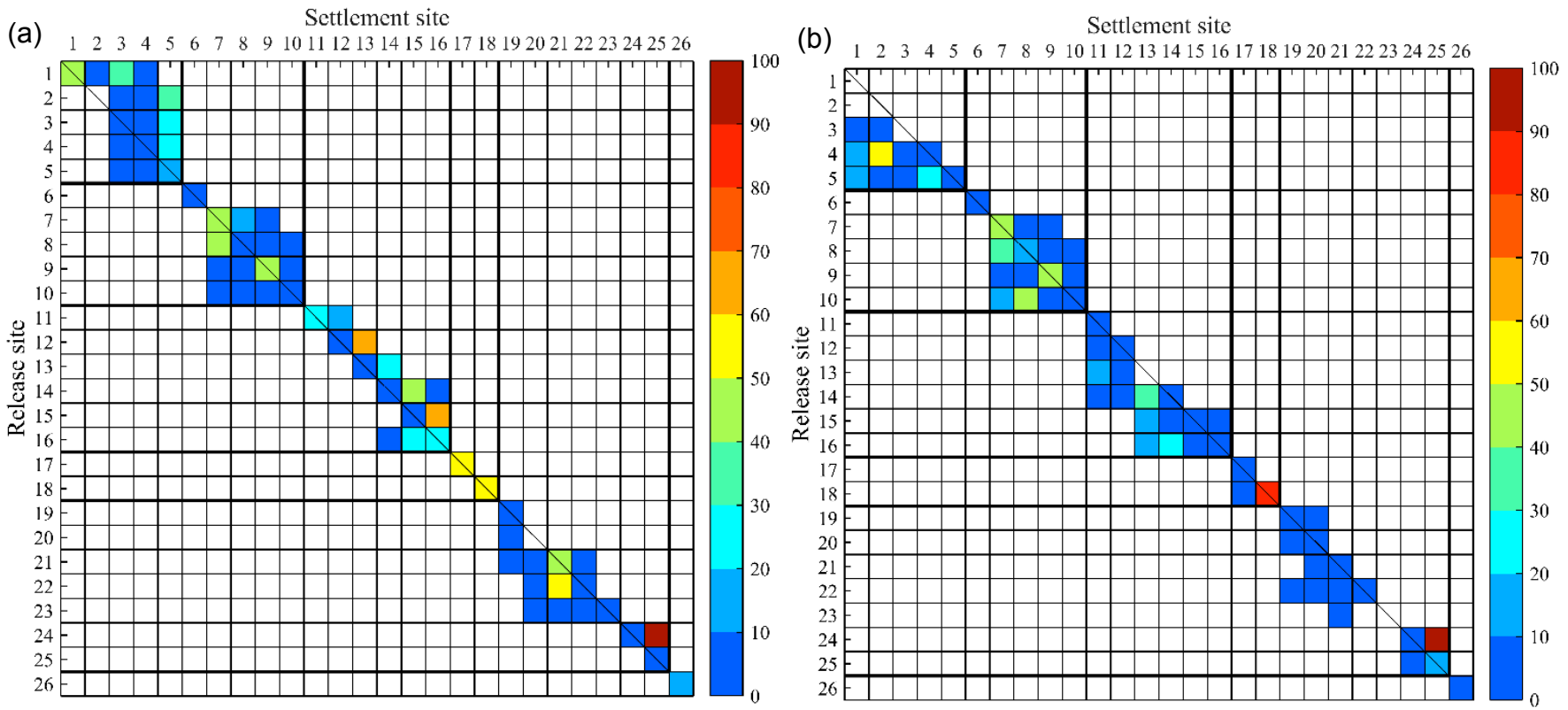
Figure 21: PTM simulations of larval dispersal for *Sabellaria alveolata*, representing the potential dispersal of cohorts of 10,000 larvae released from Dunraven, South Wales. Each map shows bathymetry at mean sea level, overlain with larval dispersal (red particles) after a typical 30-day pelagic larval duration, for particle releases on: (a) 01 May, (b) 01 June, (c) 01 July, and (d) 01 August.

in Figure 21. The Dunraven reef, along with Porthcawl to the north, were the most tidally energetic in our study, with Dunraven producing mean oceanographic larval dispersal distances of approximately 1,400 km (Figures 23-27), over the 30 day PLD, based on all simulated larvae (4×10,000 particles). However, due to the oscillatory nature of the tidal currents, the larvae did not disperse more than 60 km from Dunraven, with the majority dispersing less than 30 km from the reef, and mainly in a NW or SE direction (Figure 18). We simulated some seasonal variability between the four release dates, in terms of oceanographic dispersal distance, with the population-averaged mean dispersal increasing from ~1,200 km in May to over 1,450 km in July and August (Figures 23-29). Predicted dispersal patterns of passive particles released from Dunraven Bay were very similar in all months (Figure 21). Particles were predicted to move immediately offshore and to the west,

returning to shore at Porthcawl, travelling back over the site of origin and into the Severn. By day 30 some particles may potentially reach Hinckley Point, a known abundant site on the north coast of Devon (Figure 21 a to d). Whilst many particles released at Dunraven were predicted to migrate offshore, south and west, fifty percent of larvae released in May and June (Figure 22 a and b respectively) were predicted to be retained at source after 30 days, decreasing to forty percent when released in July (Figure 22 c) but peaking at seventy percent if released in August (Figure 22 d). Additionally passive particles released from known Abundant sites further north and west in the Bristol Channel are predicted to have been present in the waters off Dunraven after 30 days (more than ten percent of the larvae released from Porthcawl, Swansea Bay and Limeslade Bay in all four months are predicted to be present in the waters off Dunraven Bay 30 days after release) (Figure 22 a to d).

3.6.2. Oceanographic larval dispersal distance

The cumulative larval trajectory distance, from source to sink over the 30-day PLD, has been calculated for each larva. This 'oceanographic dispersal distance' may be hundreds or thousands of kilometres over the course of 30 days, even if the particle ends up near to where it was released from. If, however, the larva do not become exposed to offshore oscillatory tidal currents, and instead remain in weak coastal currents, the oceanographic dispersal distance will be small and the particle may also end up near to where it was released from. But characterisation of oceanographic dispersal is important, and can be used as a proxy for the ambient oceanographic conditions that each population is likely to experience. Histograms of oceanographic dispersal distances, for each population, and for each consecutive release month, are shown in Figures 23 to 27. A striking result from the PTM simulations is that most larvae cohorts remained close to one another throughout their 30-day larval stage, as a singular patch or splitting to form two patches (bi-modal distribution). Bi-model distribution usually resulted in one patch travelling a short distance, entrained in weak coastal currents, and another patch travelling offshore with large oceanographic dispersal.



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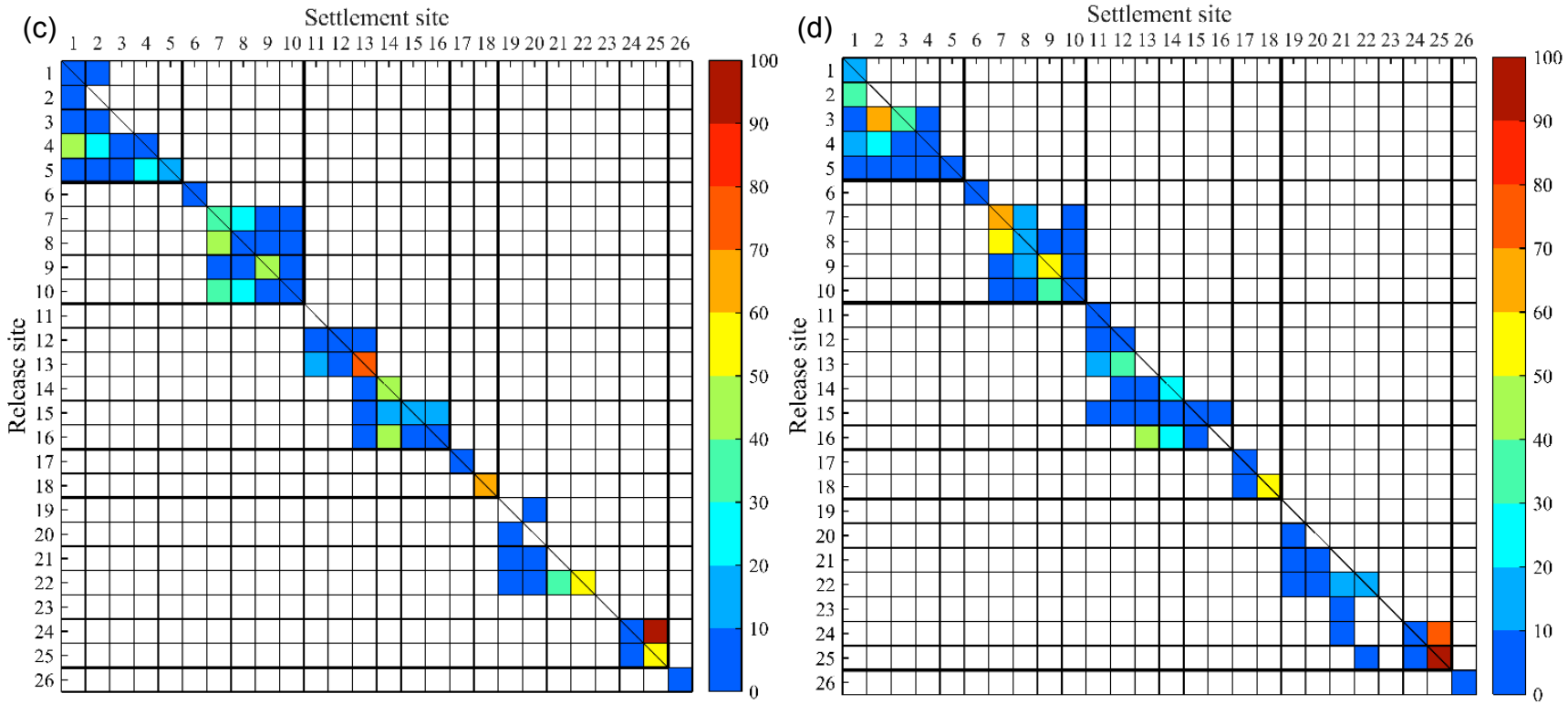

Figure 22: Connectivity matrices, showing our simulated predictions of larval retention and larval connectivity after a 30-day PLD, for release dates: (a) 01 May, (b) 01 June, (c) 01 July, and (d) 01 August. Larval retention (p_{ii}, Equation 4) is shown in the diagonal cells with a diagonal black line through. Larval connectivity (Equation 4) is shown by the other cells. The colour scale signifies the percentage of retention or connectivity of the population at each settlement site, where white cells indicate no connectivity. For example, during May, the Dunraven population (release Site 7, top left panel) has 50% retention, and connectivity with the adjacent settlement sites to the west (Site 8 at 20% and Site 9 at 10%). The remaining ~20% of larvae did not settle (i.e., all other cells in row 7 are white).

There are several examples of significant seasonal variability in oceanographic dispersal distances and distribution patterns. For example, we simulate spring vs. summer variability in oceanographic dispersal for larvae released from Llanddulas (Site 17), as mentioned above (i.e., we show less dispersal in May and more dispersal in June-August). In contrast, PTM simulations at the Limeslade Bay reef in South Wales (Site 10) show a reverse pattern; oceanographic dispersal was greatest during May (when the population mean was approximately 950 km) and lowest during August (when the population mean was approximately 550 km). Consistently throughout the four release scenarios, oceanographic dispersal from Dunraven in South Wales (Site 7) was greatest amongst the simulated populations (the population mean was above 1,200 km in all cases). However, no significant residual current was encountered by the Dunraven larvae, and so, the final stage larval positions were similarly close to the release location as in the other populations (i.e., within 50-100 km from the release reef). *S. alveolata* populations in Cardigan Bay (Sites 11-16), including our sampled Aberarth reef, were simulated to all travel similar distances, with patch-averaged dispersal between 200 and 500 km over their 30-day PLD.

There are several regions within the Irish Sea, which are known to be flood-dominant estuaries or bays that could potentially 'trap' larvae and lead to high levels of retention. Some such regions are Avonmouth, the Burry Inlet, Morecambe Bay, and the Solway Estuary (Robins et al., 2013), which are close to Sites 6, 9, 19, and 25, respectively. Hence, the flood tidal asymmetry could be attributed to their relatively short oceanographic dispersal distances (Figure 27). When we consider all simulated larvae, we predict increased oceanographic dispersal distances when larval were released later during the year (Figure 28). The mean dispersal distance increased by approximately 300 km over the four-month period, although variabilities from one population to another were large (Figure 28).

3.6.3. Recruitment and connectivity

Using our settlement criteria, a particle is deemed to settle successfully if it is located within 10 km of a reef after the 30-day PLD, and therefore contribute to the retention of the population or connectivity with another population.

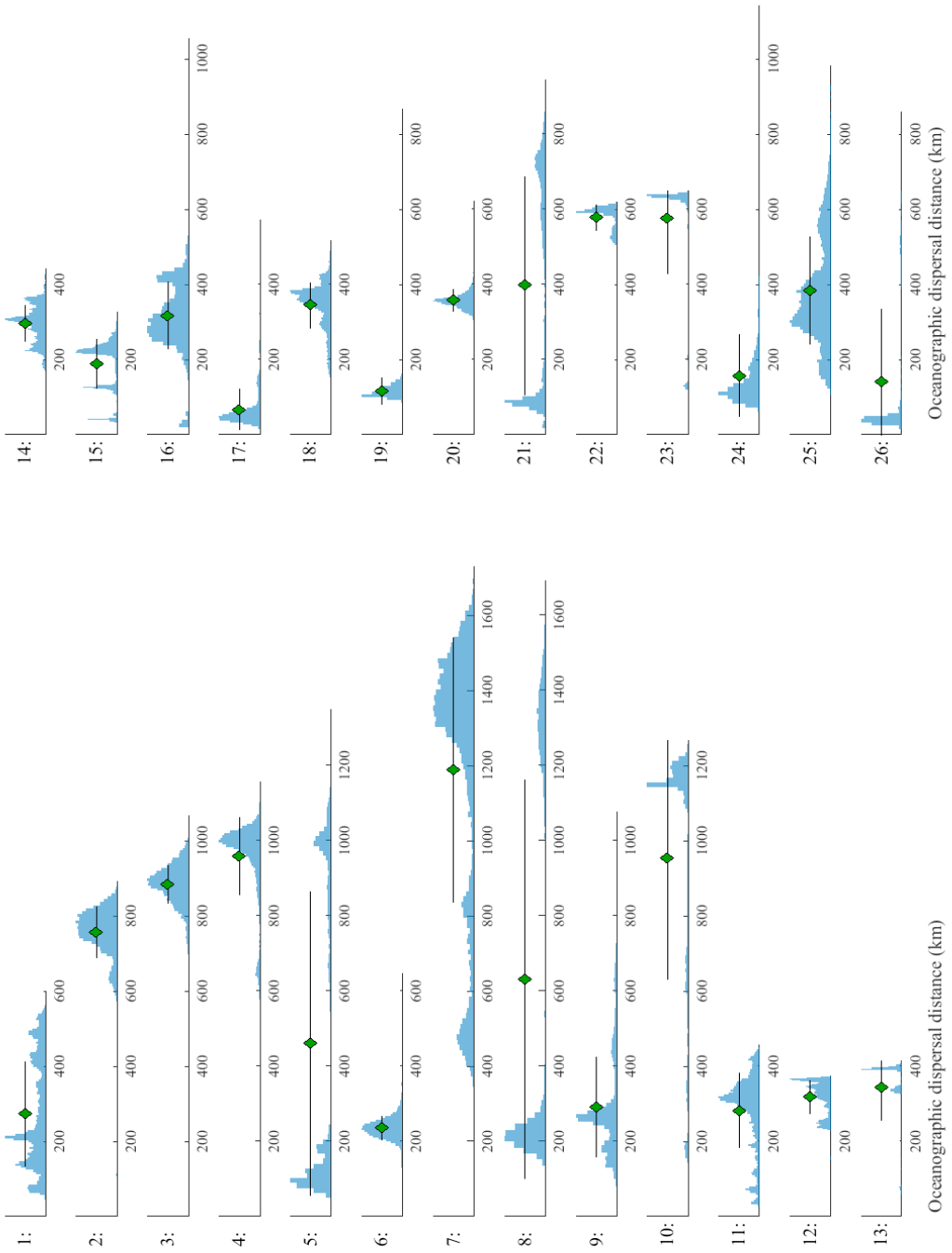

Figure 23: Histograms plots of oceanographic larval dispersal distance, for all particles released on 01 May. Each histogram shows the normalised distribution of oceanographic dispersal for 10,000 particles released from the numbered site. The patch-mean (green diamond) and standard deviation (black line) are also plotted.

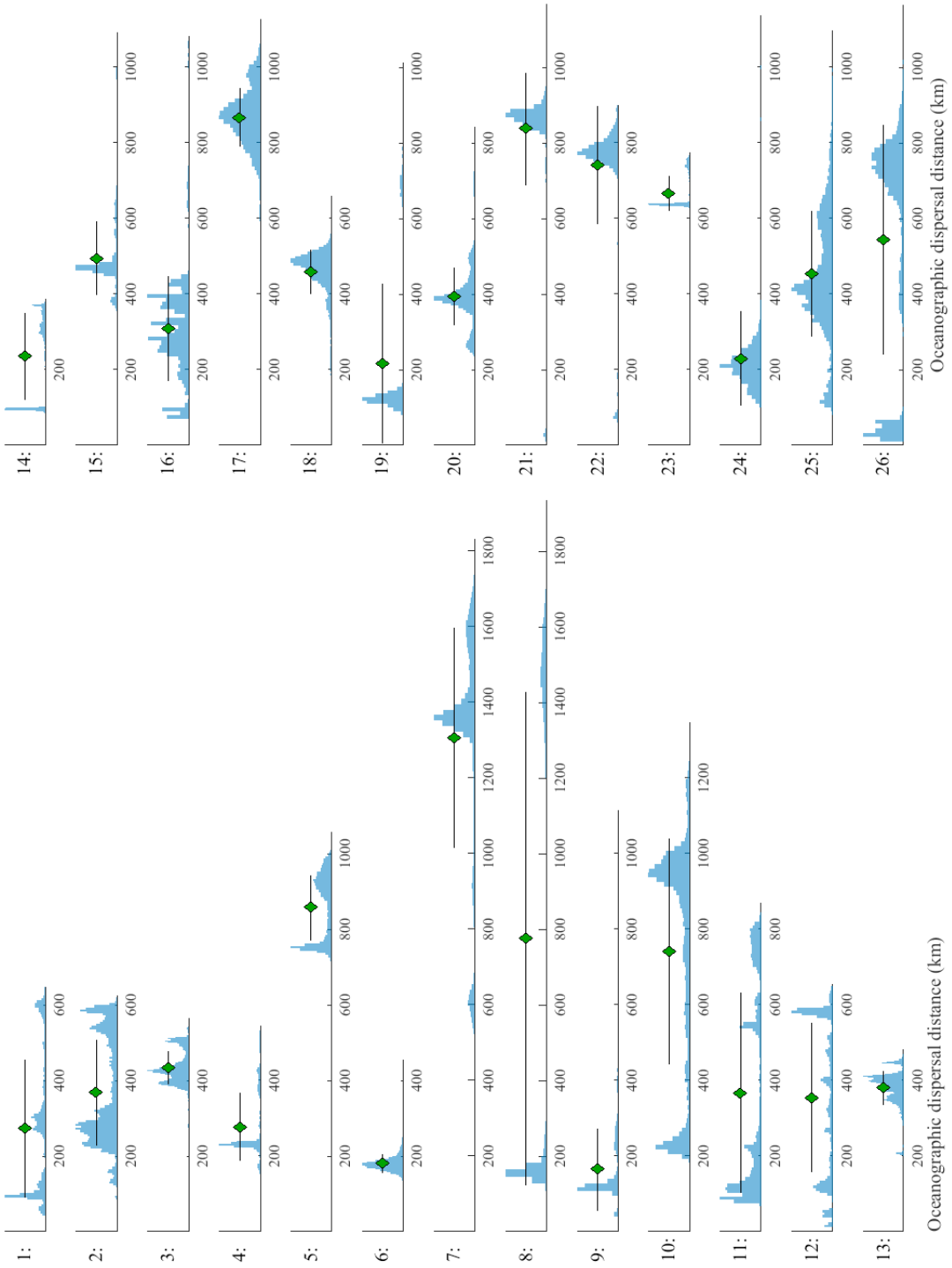

Figure 24: Histograms plots of oceanographic larval dispersal distance, for all particles released on 01 June. Each histogram shows the normalised distribution of oceanographic dispersal for 10,000 particles released from the numbered site. The patch-mean (green diamond) and standard deviation (black line) are also plotted.

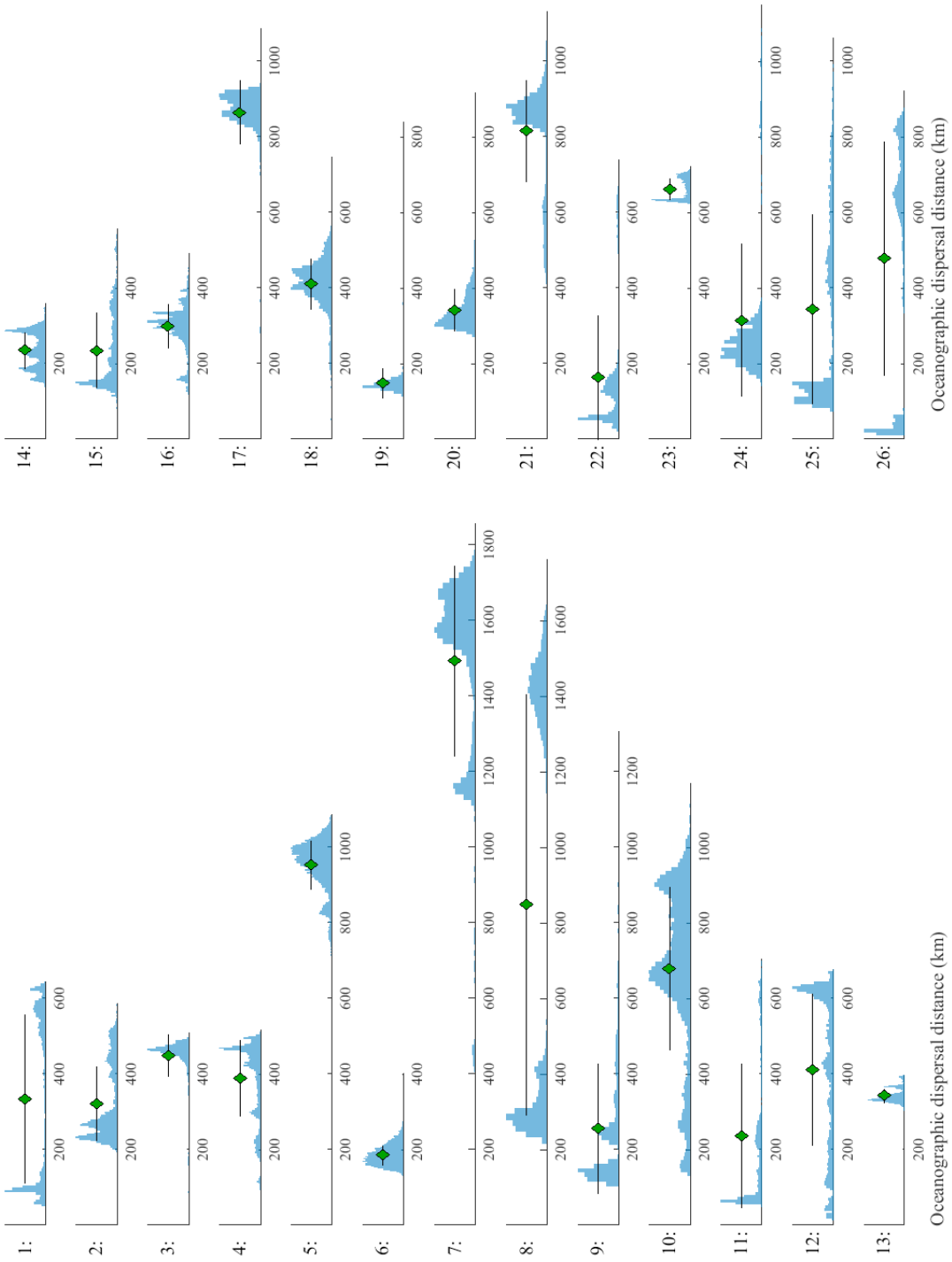

Figure 25: Histograms plots of oceanographic larval dispersal distance, for all particles released on 01 July. Each histogram shows the normalised distribution of oceanographic dispersal for 10,000 particles released from the numbered site. The patch-mean (green diamond) and standard deviation (black line) are also plotted.

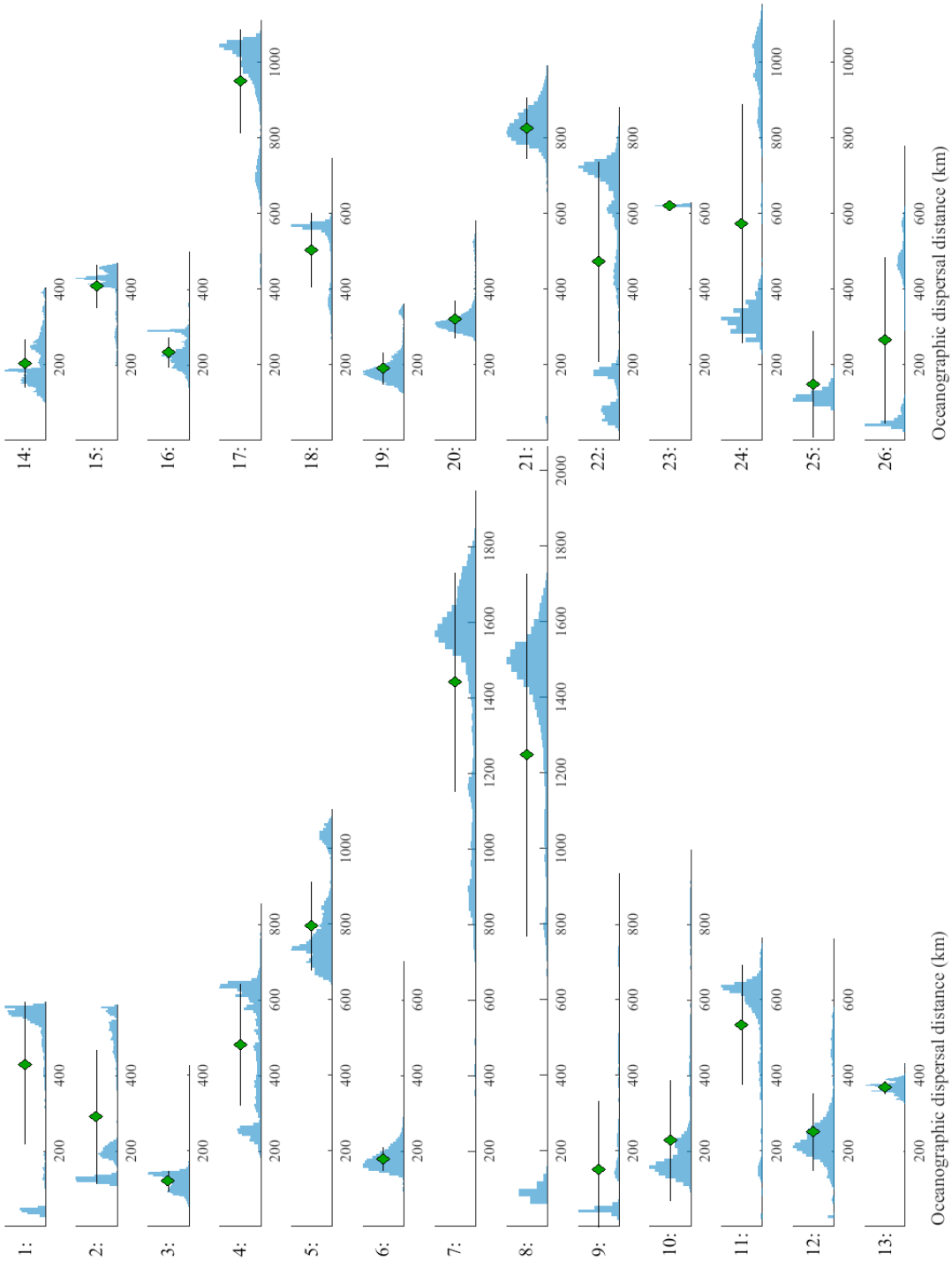

Figure 26: Histograms plots of oceanographic larval dispersal distance, for all particles released on 01 August. Each histogram shows the normalised distribution of oceanographic dispersal for 10,000 particles released from the numbered site. The patch-mean (green diamond) and standard deviation (black line) are also plotted.

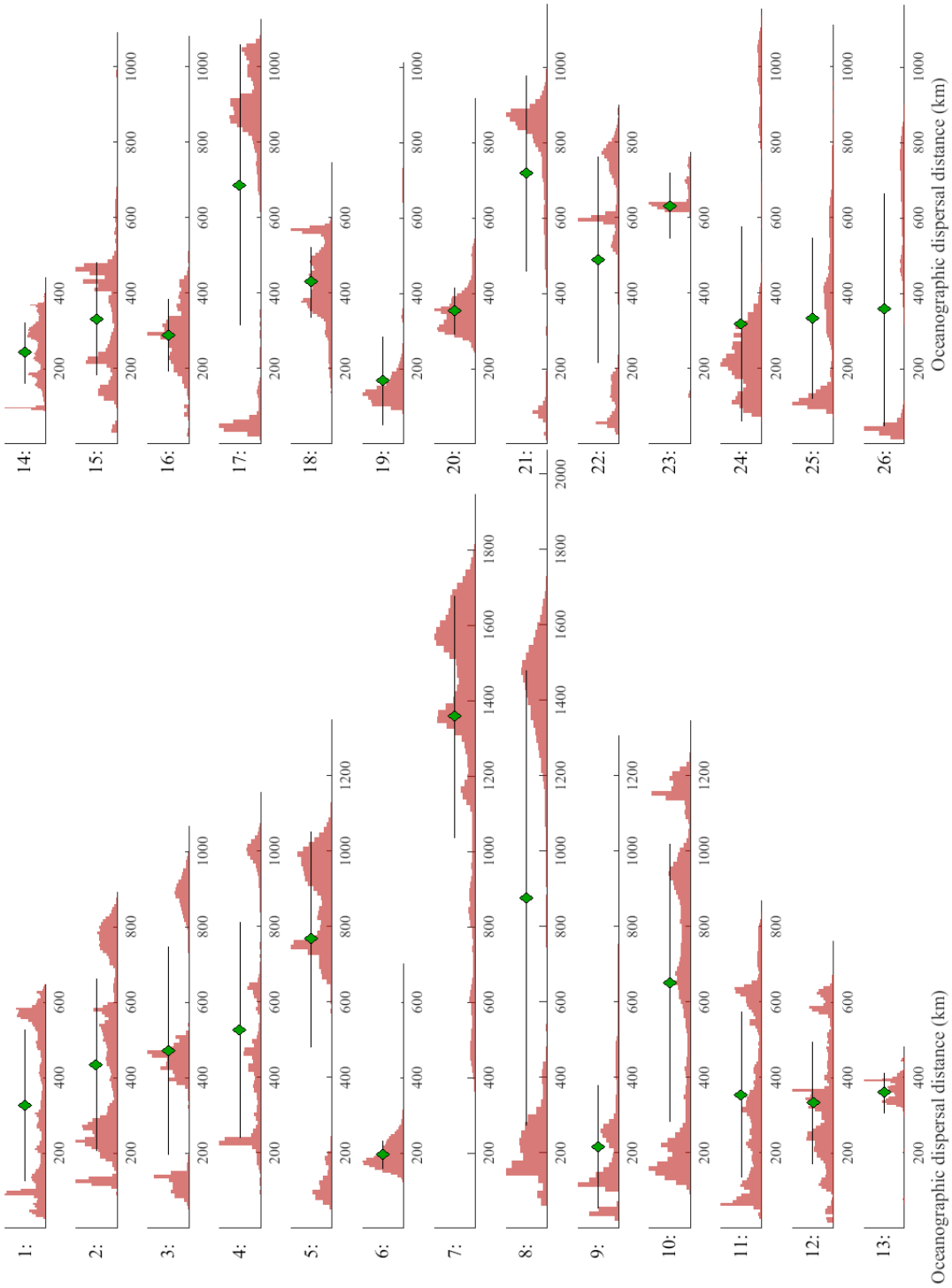

Figure 27: Histograms plots of oceanographic larval dispersal distance, for all particles released for all release dates. Each histogram shows the normalised distribution of oceanographic dispersal for 40,000 particles released from the numbered site. The patch-mean (green diamond) and standard deviation (black line) are also plotted.

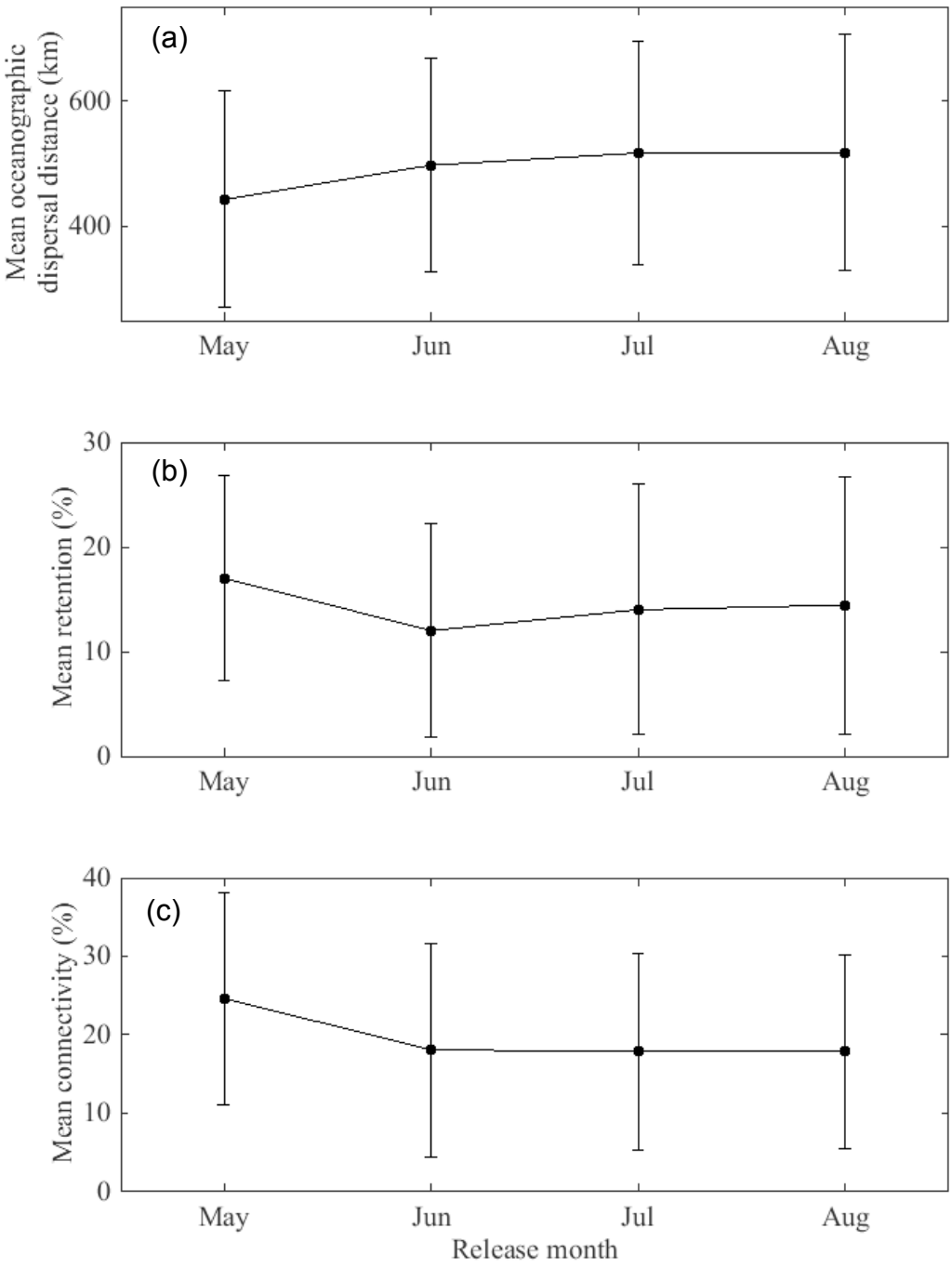

Figure 28: Predicted seasonal variability from our simulated PTMs, in terms of (a) mean oceanographic dispersal distance, (b) mean retention, and (c) mean connectivity. In all cases, each population cohort of 10,000 particles were averaged, then the 26 population averages were averaged to give the overall mean. The error bars signify one standard deviation from the mean.

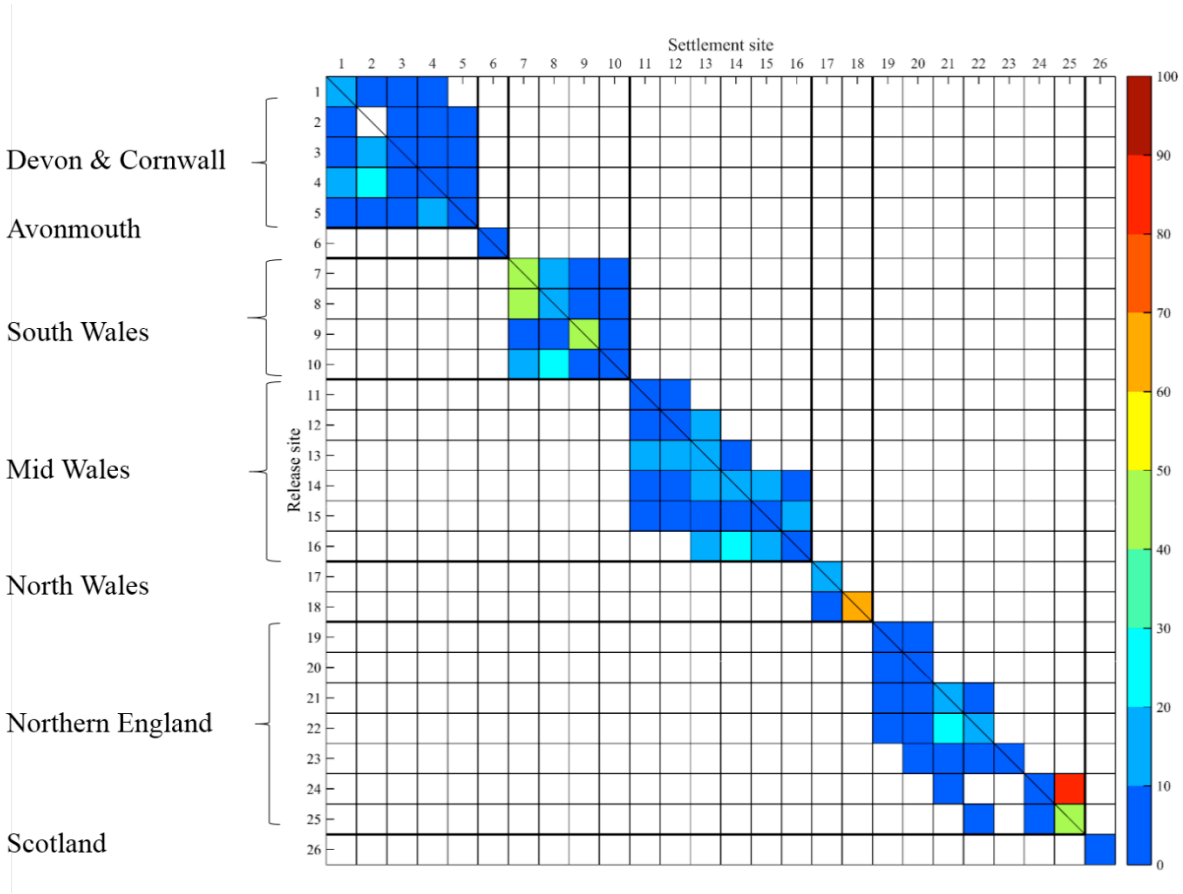

Figure 29: Seasonally-averaged connectivity matrix, showing our simulated predictions of larval retention and larval connectivity after a 30-day PLD, for all release dates. Larval retention (p_{ii}, Equation 4) is shown in the diagonal cells with a diagonal black line through. Larval connectivity (Equation 4) is shown by the other cells. The colour scale signifies the percentage of retention or connectivity of the population at each settlement site, where white cells indicate no connectivity.

Otherwise, the particle is deemed unsuccessful, e.g., stranded offshore in deeper water or washed up on a shore away from a reef colony. Connectivity matrices for each simulated particle release month, which illustrate such connectivity and retention described above, are shown in Figure 22. The seasonally-averaged connectivity matrix is shown in Figure 29. It is apparent from Figures 22 and 29 that, in most cases, there is at least some retention at each natal reef (i.e., the diagonal cells in each matrix are coloured). However, several populations were simulated where no retention was recorded (i.e., white diagonal cells in Figure 22, one population during May, five during June, six during July, and seven during August. This result suggests that, as oceanographic dispersal distances generally increase throughout the summer,

larval retention is reduced (Figure 28), meaning a correlation between dispersal distance and retention. Yet, Figure 28 shows a very weak decrease in retention through consecutive release months, when averaged over all populations. Indeed, when we plot patch-averaged oceanographic dispersal distance against larval retention, we do not see a strong correlation (Figure 30).

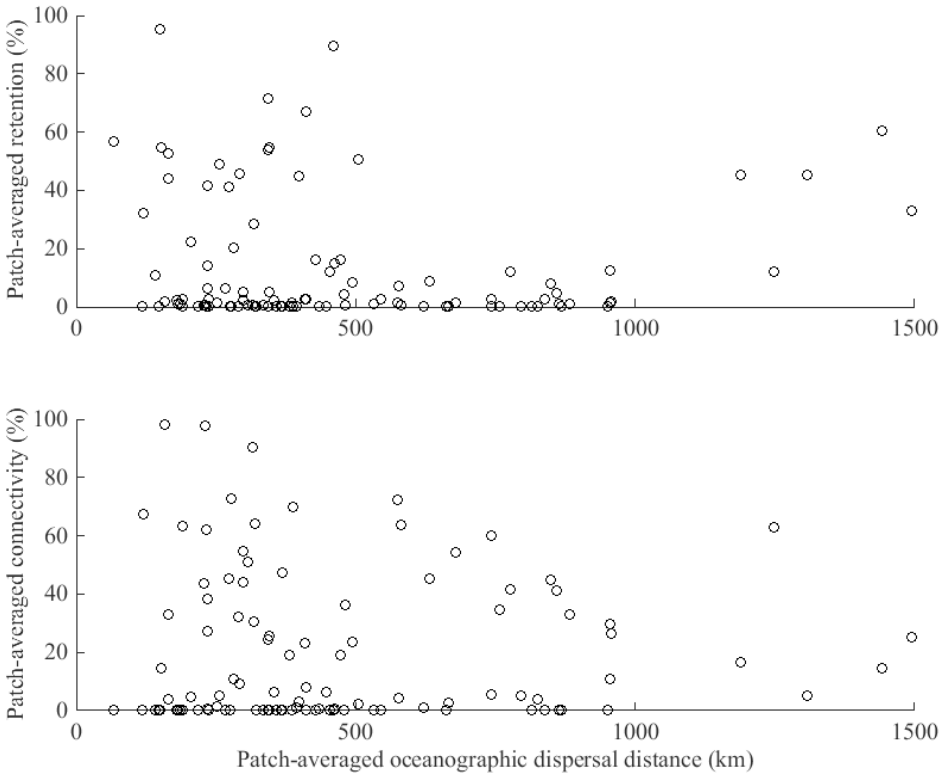

Figure 30: Patch-averaged oceanographic dispersal distances are plotted against (top panel) population retention and (bottom panel) population connectivity, for all 26 reef populations simulated and for all four release months.

Larval connectivity between populations was only possible between neighbouring reefs, less than 100 km apart. This result is illustrated in Figure 22 and 29 by coloured cells only being depicted adjacent to the diagonal retention lines (i.e., p_{ii}, Equation 4). The remaining cells of each matrix are white, signifying that there is no far-field connectivity (Figures 22 and 29). Again, there is a very weak, or non-existent, inverse correlation between

consecutive release dates and averaged connectivity (Figure 28), and between patch-averaged oceanographic dispersal distance and larval connectivity (Figure 30).

One of the most robust results of the connectivity analyses is that distinct sub-populations were isolated in the simulations, which have been highlighted by the bold black grid lines in Figures 22 and 29. These sub-populations are: South England (Sites 1-5), Avonmouth (Site 6), South Wales (Sites 7-10), Cardigan Bay (Sites 11-16), North Wales (Sites 17-18), North England (Sites 19-25), and Scotland (Site 26). These sub-populations are not sensitive to our settlement criteria. As a sensitivity test, we varied the settlement zone in the range 5 km to 20 km and, although the results did change, the sub-populations and connectivity patterns remained the same (results not shown). Of course, it is possible that larvae from other locations, that were not simulated here, could contribute to this system, such as *S. alveolata* populations in the English Channel, Scotland, or Ireland.

4. Discussion

This study conducted the first large scale observations of the reproduction and larval dynamics of *S. alveolata* in the UK. Adult worms that formed reefs were surveyed to assess the fecundity of adults in North, Mid and South Wales. These were supported by in and offshore larval capture using traps or nets. In the laboratory, we conducted trials to assess larval responses to light and swimming speeds at different life cycle stages. Finally, we successfully ran a high-resolution regional oceanographic model coupled with a Lagrange particle tracking module to release particles from known reef sites in the Irish Sea (UK side) to assess reef connectivity.

4.1. Fecundity of adult *Sabellaria alveolata*

In the current study, differences in counts between both male and female gametes of *Sabellaria alveolata* occurred both between and within sites from February to September 2014. All sites showed a summer peak in the production of gametes, but differences occurred in both the timing and the scale of this peak, in both sexes, between three geographically distinct populations. Female *Sabellaria alveolata* collected from Llanddulas showed elevated egg counts in the summer from May to June, whilst those from Aberarth showed bimodal peaks in mean egg counts with a small peak in March and a subsequent larger peak from June to August. Females from Dunraven showed a relatively suppressed mean egg count, remaining at relatively low fecundity in comparison to the other two sites throughout the sampling season. At Dunraven, as with Aberarth, egg count was slightly raised in the spring, in February and March, and in the summer, in July. This correlates precisely with the results of Culloty et al., (2010) who found female gametogenesis peaked in July. As with mean egg count, mean sperm count showed a single peak in Llanddulas but bimodal peaks in both other sites. All three sites showed highest mean sperm count in May. At both Llanddulas and Aberarth, this peak was maintained until July, again correlating with the results of Culloty et al., (2010) who found a distinct peak in male gametogenesis in the summer months from June to September. In contrast, at Dunraven, mean sperm counts returned to baseline levels by June. Mean sperm count was

substantially greater in both February and March than April at Aberarth, and in September than August at Dunraven.

Dubois et al., (2007) hypothesised that *Sabellaria alveolata* exhibited semi-continuous poorly seasonally synchronised spawning. Both Dubois et al., (2007) and Gruet and Lasse (1983) reported almost continuous spawning from April to September in France. Ripe male and female *S. alveolata* were present at all three sites throughout the sampling period. Gruet & Lassus (1983), Culloty et al., (2010) and Wilson (1971) also found some ripe individuals throughout the majority of the year. Wilson (1971) stated that after the sampling season a few ripe adults, particularly males, were present at Duckpool, whilst Culloty et al., (2010) found some ripe females throughout the year in Cork, Ireland, despite 100% of males being spent of gametes by November. Continuous spawning is also supported by the observations that settlement of larvae in the south-west of England occurs in most months from September to April (Wilson, 1968a), and in France from March to August (Gruet, 1986), in addition to other times of the year (Culloty et al., 2010).

Fecundity data from this study also supports the hypothesis of semi-continuous spawning. The gradual decrease in sperm and eggs to April at both Dunraven and Aberarth suggest a slow release of gametes was occurring throughout the spring. Mean sperm counts increased dramatically in all three sites from April to May. Although mean egg counts showed temporal variability in their peak, all three sites showed an increase in mean egg counts in the summer months. Whilst Dunraven showed a rapid decrease in egg count to baseline levels within one month, both Llanddulas and Aberarth showed a gradual release of eggs towards the end of the sampling period. In September at Aberarth, egg counts had not decreased to the levels recorded in the previous spring suggesting that this population would continue to release eggs throughout the following months, again indicative of year-round gamete release. Stored sperm was released slowly from May to September at both Llanddulas and Aberarth. In contrast, sperm from Dunraven was released rapidly between May and June. A month long, summer spawning period, as detected at Dunraven, has been reported previously at Duckpool, North Cornwall (Wilson, 1971). Culloty et al., (2010) suggested one main spawning

period in Cork, Ireland from June to September. Overlaying the pattern of semi-continuous spawning, both Gruet & Lasse (1983), and Dubois et al., (2007) reported a bimodal spawning pattern by *S. alveolata* in France. A bimodal pattern of spawning was also witnessed at both Dunraven and Aberarth with significantly elevated male and female gametogenesis in March in comparison with April. It is clear that temporal variation occurs in spawning on a small spatial scale with different sites following different patterns of gamete release. Within a small geographic region, we have detected short boom-bust spawning, and prolonged trickle spawning, with 2 main peaks in abundance, one in spring and one in summer.

Egg release correlated directly with sperm release at Llanddulas with mean egg count decreasing from May to August. Egg release from Aberarth also synchronised partially with sperm release with egg count decreasing from July to September. In contrast, eggs were predominantly released from Dunraven between July and August, at least one month after male spawning. At Dunraven, gametes were never stored within the adult at high abundance and gamete concentrations showed the least variability though time. The continued persistence of this reef, despite the low fecundity of the population could be indicative of an aged or unhealthy population. Equally it could be indicative that conditions are suitable for reproductive success throughout the year with fertile adults releasing gametes continuously. The persistence of the Dunraven population through time, despite this low fecundity, suggests high larval success rate or high larval connectivity with reliance on recruitment from other populations. In 2014, if Dunraven was a geographically isolated reef, this population would be relying on a high reproductive success from gradual spawning in the early spring.

Whilst an extended period of spawning, reproduction and subsequent larval abundance can increase the probability of some larvae correlating with successful environmental conditions and thus successfully settling (Dubois et al., 2007), Wilson (1968b) suggested that an advantage of rapid mass spawning was a release of fully matured eggs capable of producing successful larvae. In the current study egg sizes showed no statistical difference between sites throughout the sampling period with egg size decreasing throughout the

peak gamete production season, All sites showed a decrease in egg size from March to July and a subsequent increase in egg size from July and August to September in conjunction with the reduction in egg concentrations suggesting investment in individual eggs early in the spawning season is greater. Marine invertebrates exhibit trade-offs, whereby they either produce many small eggs with high mortality or fewer, larger eggs that develop quickly and experience reduced planktonic mortality and are fertilised at a higher rate (Levitan, 1996). This trade off pattern has been clearly shown for *S. alveolata* populations at each site, which suggest continuous gamete release to sustain the populations. What initiates changes in gamete trade-off could range from a variety of hydrodynamic and biological interactions and requires further analysis.

Peak larval abundance occurred in Dunraven from May to June, and in Aberarth from July to August. S^0 larvae were present in high abundance in all samples showing high reproductive success at both sites in both months. The larval bloom at Dunraven correlates with a period of gradual egg release and the most significant decrease of sperm at this site. Immediately following the initiation of the bloom, egg size decreases dramatically. The bloom at Aberarth correlates with highest mean egg counts followed by a period of rapid egg release. Whilst sperm was released in greatest abundance the previous month, continued decreases in sperm count occurred within this period. Egg size had reached a minimum for this site at this time.

The lowest mean sperm counts occurred between February and April at all sites in correlation with largest egg sizes in the same period. The high larval abundance in the following months highlights the species reproductive success throughout the summer at different sites. During the period of low sperm counts, this reproductive success can be attributed to large egg sizes. Levitan (1993) reported this relationship in *Strongylocentrotus droebachiensis* with high reproductive success at low sperm concentrations primarily because of increased egg size.

The differences in egg sizes and gamete concentrations between the three study sites provides an insight to the dispersal of larvae and the supply to the reefs. Egg sizes are highest at all sites between Feb and April. Large egg

sizes are associated with limited dispersal potential and enhance probability of self-recruitment (Levin, 2006). Recent research has shown larvae retention is more frequent in natural habitats than previously thought (Sponaugle et al., 2002; Levin, 2006). It is therefore possible larvae is retained in these colder months and potentially during the winter but dispersed in later months. The highest peaks in mean sperm and egg concentrations were recorded from worms collected in May in Llanddulas, when egg sizes had not yet dropped to their smallest recorded sizes. This excessive investment in gamete quantity and quality indicates attempts at high fertilisation rates showing that this site relies heavily on self-recruitment and is not connected to other populations and is therefore more susceptible to impacts from subsequent anthropogenic and natural impacts.

4.2. *Sabellaria alveolata* larval behaviour

This study has improved the understanding of basic larval behavioural traits of *Sabellaria alveolata* that have been previously overlooked. Although *S. alveolata* had not previously been shown to be clearly sensitive to light, vertical migration patterns have been observed in the field (Dubois et al., 2007), these horizontal and vertical light experiments show that *S. alveolata* larvae is negatively phototactic in the metatrochophore and erpochete stages, with light having a statistically significant effect on larval movement. In contrast early trochophore stages of larval development display no phototactic behaviour. The lack of eyespots in young larvae (Figure 31) partially explains the ambivalence of these larval stages to light, being neither negatively or positively phototactic. Swimming speed also increases significantly through the early stages of development but reduces when the larvae are ready to settle. Settlement stage larvae are instead actively seeking a suitable settlement site on the seabed.

This study found that *Sabellaria alveolata* larvae does not fit Thoron's generalised theory that intertidal larvae should remain photopositive throughout larval life (Thorson, 1964). It is also in contrast with the findings of Curtis (1973) in another Sabellariid polychaetes, *Sabellaria vulgaris*, who reported larvae to be photopositive. However, the findings of this study correspond with phototactic experiments by McCarthy et al., (2002) on the

Sabellariid polychaete *Phragmatopma lapidosa*, who found the larvae to switch from photopositive to photonegative response. Furthermore, findings compliment the vertical pattern reported by Dubois et al., (2007) who noted that early planktonic stages remain in surface waters, where light exposure is greatest, whilst older larvae migrate to the bottom waters towards the substrata where light intensity is lowest. Gravity may also be partly responsible for the sinking of older, larger larvae in the water column, however the continued significance of this negative phototactic response in response to under lighting in vertical tests, and the increase in swimming speed of these latter stage larvae, suggest descent in the water column with age is preferential in order to evade the light.

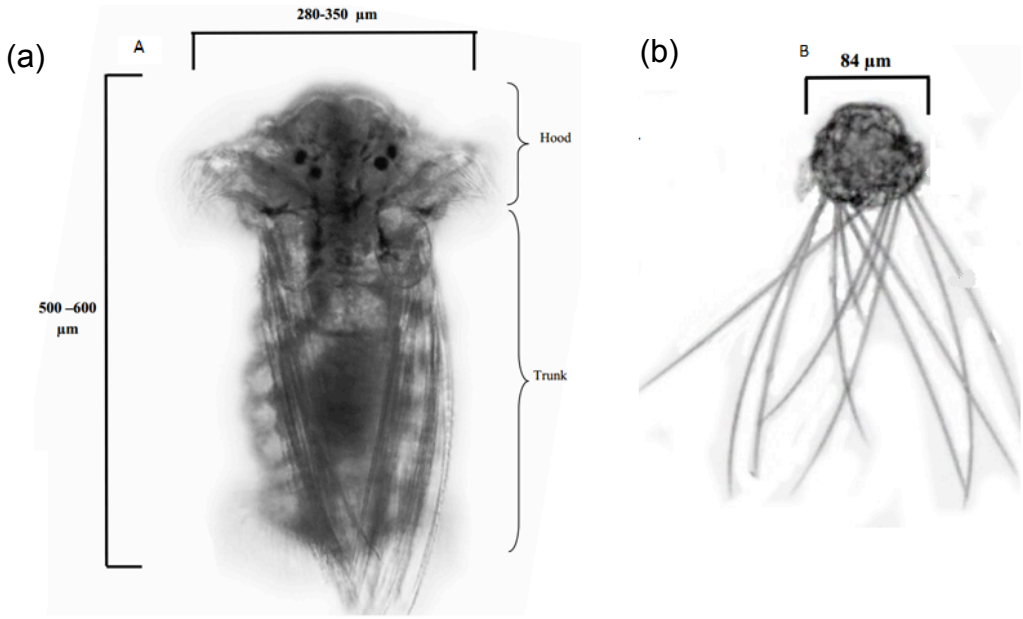

Figure 31: (A) Dorsal view of stationary larva on day 30 post fertilisation showing developed eyespots. (B) Dorsal view of stationary larva showing no developed eyespots. Images extracted from Newstead and Davies (Unpublished).

The differences in phototactic behaviour between older and younger S. alveolata larvae may contribute significantly to larval dispersal. In a similar study on larvae of the nudibranch *Phestilla sibogae*, Miller and Hadfield (1986) discuss that as the larvae age, the changes in phototactic response will cause further separation between larvae and parents and aid the spread and dispersal of the larvae. These nudibranch larvae, like *Sabellaria alveolata* larvae, moved away from the light source in a top-lighted vertical column. It is

possible that a similar separation occurs within *S. alveolata* communities where larvae separate and settle further from each other and the mother reef, leading to the spread, growth or shift in position and size of communities. Dubois et al., (2007) however, found no difference in vertical migration between day and night samples and found larvae to exhibit selective tidal stream transport migrating to near the surface during flood tides and descending to weaker currents near the bottom during ebb tides. This indicates that light may not be as influential in the water column as it is under laboratory conditions.

Although early stage trochophore larvae did not respond to visible light, there are similar studies investigating tube worms, whose early stage larvae were found to be positively phototactic (e.g. Thorson, 1964; Marsden, 1984). This suggests that *Sabellaria alveolata* is part of the minority of polychaetes that don't exhibit positive phototaxis at hatching. However, the early stage of development for this study was defined as day 5 post fertilisation. It is possible that larvae that have just hatched display positive phototaxis despite not having eyespots. Future studies should therefore consider phototactic experiments between day 1-5 post fertilisation. Further improvement to the methodology concerns using a range of light intensities. The purpose of this study was to see if *S. alveolata* larvae are phototactic, therefore 1 low level intensity was adequate. To test the effects a range of light intensities have on the larvae, a wider range of light intensities is necessary.

Through these behavioural experiments, *Sabellaria alveolata* larvae has been shown to be negatively phototactic in mid and latter stages of development and indifferent to visible light exposure in early stages. The horizontal swimming speeds have also been quantified and shown to increase exponentially until they levels off and decrease at the settlement stage. This study has improved working knowledge on the relatively understudied polychaete *S. alveolata*, which should prove useful in understanding the dispersal and connectivity of the biogenic reefs these polychaetes create.

4.3. *Sabellaria alveolata* larval dispersal offshore

Sabellaria alveolata larvae were detected in the water column in varying degrees of abundance in all sampled months at all sites. Abundant larval

blooms were detected in May and June at Dunraven, and in July and August in Aberath. However no peak in larval abundance was detected at Llanddulas where maximum abundance was two to three orders of magnitude less than other sites. Investment in egg size was equivalent at all sites, with Llanddulas actively producing more eggs and sperm than either Aberarth or Dunraven. Additionally, release of both eggs and sperm from Llanddulas was temporally correlated. Consequently it is highly unlikely that fertilization did not occur.

There are 2 possible explanations for the lack of larvae reported from Llanddulas: (i) larvae are rapidly dispersed away from source at this site. (ii) rapid mass mortality is occurring. Mussels are present in high abundance at Llanddulas, but not at the other 2 sites. They are known to be capable of significantly preying upon polychaete larvae (Davenport et al., 2000; Lehane & Davenport, 2006). Dubois et al., (2007) implicated this bivalve in the dramatic reduction of *S. alveolata* in a 24 hour time period. Additionally, residual currents are apparent in the Irish Sea (Robins et al., 2013).

The abundance of S^0 larvae was high throughout the bloom in both Dunraven and Aberarth suggesting at least 2 months of successful egg fertilization has occurred. S^1 larvae were initially present in high abundance in May in Dunraven suggesting the initiation of the larval bloom at this site may have occurred before sampling began. S^1 larvae were not recorded until August at Aberarth showing bloom progression. S^2 larvae were present in high abundance from June in Dunraven and August in Aberarth, again showing progression of the recorded larval blooms through time. The lack of S^2 larvae in May suggests bloom initiation was recently. Although no peak in abundance occurred at Llanddulas, S^0 larvae were present in highest abundance in May, and S^2 in July and September. At Dunraven S^0 larvae were not present in July, but were present again in September suggesting a secondary cohort. The same is true in Llanddulas in August.

Mean larval abundance showed some variation in the water column with direction from the reef. At Llanddulas mean larval abundance was greatest to the west and middle of the reef during May. S^0 larvae were only detected at this site in April to the west and in the middle, and in May and August to the west suggesting a movement of early stage larvae rapidly westwards. In

Aberarth mean larval abundance was greatest to the north of the reef during the larval bloom of July to August. In May, all larvae were S^0, whilst in June S^0 to S^2 larvae were present. However at Dunraven the greatest abundance of larvae were recorded to the south of the reef in May and July, but to the north of the reef in September. In May the majority of larvae to the south of the reef were S^0, however in July they were S^2. In September S^0 to S^2 larvae were all present in low numbers.

Mean larval abundance also shows some variation with distance offshore. At Dunraven more larvae were found 1km offshore than 100m offshore in during the larval bloom of May to June. In both months, S^0 larvae make up the greatest proportion of the larvae. Consequently larvae move rapidly offshore from Dunraven. At Llanddulas and Aberarth, more larvae were found near shore, in July and August respectively. This initially appears to be in direct contrast to the theory that early stage larvae are transported rapidly offshore from Llanddulas, however in both instances later stage S^2 larvae make up the greatest proportion of larval abundance.

From behavioural experiments, it was expected that a greater abundance of later stage larvae would be found at depth in the water column. This was not conclusive from field data. At Aberarth there were significantly more larvae at 5m depth than 1m depth in August, the majority of which were S2 larvae. In contrast, at Dunraven, in September, there were significantly more larvae near the surface at 1m depth, than at 5m depth, with a greater abundance of all stages of larvae present near the surface. Dubois et al., (2007) also found not relationship between larval stage and depth.

Settlement success is known to be highly variable year on year (Ayata et al., 2009, Bush and Balestrini, pers. obs). Additionally, it is clear that larval distributions in the water column are patchy (Dubois et al., 2007). The swimming speed of 5-15 $um.s^{-1}$ of early stage larvae, suggests movement off reef initially is likely to be the result of local hydrodynamics. Ataya et al., (2009) found that settlement success of *S. alveolata* larvae from some sites was predominantly dependent on tidal conditions at spawning with releases on neap sites retained close to source, whilst from other sites meteorological conditions were the dominant driver of success with onshore winds increasing

the likelihood of being retained near suitable habitat for settlement. The westerly prevailing wind is likely to keep larvae at Aberarth closer to the shore, perhaps explaining the retention of larvae near shore in this site. The importance of tide was highlighted further by Dubois et al., (2007) who reported that short term variations in larval abundance were dependent on tidal stage. Larvae moved up in the water column on the flood tide, and down on the ebb tide. Dubois et al., (2007) suggested larvae aggregated close to the surface on the high tide, and migrated deeper at low tide. This is in direct contrast to our study where the only significant difference in depth at Dunraven, where all sampling occurred on the ebb tide, was a greater abundance of larvae near the surface than at 5m depth in September. Additionally, there were significantly more larvae at 5m depth in Aberarth, where all sampling occurred in the flood tide, in August. On a site specific level, no difference should have been detected at either site due to tide. Tidal state showed some fluctuations at Llanddulas with sampling predominantly when the tide was coming in, but in July and August, sampling occurred as the tide was going out.

Dubois et al., (2007) and Ayata et al., (2011) both reported that larval densities were positively correlated with temperature and negatively with salinity with the highest larval densities in low-salinity, high temperature water. Consequently, dependent on the release site, *S. alveolata* larvae may be retained and transported within estuarine plumes (Ayata et al., 2011).

4.4. Biophysical modelling of *Sabellaria alveolata* larvae

Sabellaria alveolata larvae are not predicted to move far from their site of origin with populations generally within 100km of their natal reef after 30 days. This result of relatively low dispersal distances (compared with similar studies of species with programmed larval behavioural characteristics (Cosica et al., 2012; Robins et al., 2013)) stems from the *S. alveolata* populations being programmed as passive particles, and that fact that they are located in more sheltered bays and coasts, rather than near headlands which are exposed to stronger tidal currents and frontal circulation. Consequently, each reef population has the potential to settle at either their natal reef (larval retention) or at a close-by neighbouring reef (connectivity). Predicted larval dispersal

showed variation between and within sites in all months. From all 3 sites of interest, both total larval dispersal and dispersal from the source reef, was less for larvae released in May than in June, July or August 2014. However, at Limeslade Bay dispersal was greatest in May than in all subsequent months.

Llanddulas is the only abundant reef on the north coast of Wales. The reefs on Hilbre Island, on the Wirral, are the closest persistent abundant population. Reefs at Hilbre Island also suffered extirpation following the cold winter of 1962. They remained absent the early 1980s (Cunningham et al., 1984), with recolonisation reported by the early 2000s (Frost et al., 2004). Under certain hydrodynamics, meteorological conditions, and in conjunction with larval release, the westwards spread of larvae from Hilbre are predicted to potentially reach Llanddulas. It appears a stepwise progression of recolonisation occurred from persistent populations in Cumbria as a result of larval dispersal. Hilbre Island was recolonized first, Llanddulas later. The westwards spread of larvae from Hilbre Island may feed the low abundance populations that have recently reappeared on the north east coast of Wales, predominantly on sea defence structures (Firth et al., submitted). Additionally Llanddulas may act as a source site providing larvae to many sites to the west along the coast of mainland north Wales and Anglesey, dependent of the timing of larval release in relation to hydrodynamics and weather conditions. Transient low abundance populations have been reported from locations such as the Little Orme, the Great Orme, Puffin Island and Penmon which may be sinks for Llanddulas larvae (NBN website). However, it is suggested that Llanddulas is predominantly a self-recruiting reef, with larvae spawned from this Llanddulas having the greatest change of settlement at their natal reef.

It was predicted that Aberarth was not connected to either Llanddulas or Dunraven within 30 days of particle release, however dependent on the timing of the larval bloom and the environmental conditions Aberarth can act as a source site for many less abundant populations to the south, potentially within Pembrokeshire, and as a sink for larvae from the north. Whilst the majority of passive particles released from reefs at Aberarth were predicted to migrate away from the site of origin, passive particles released in the same time period from known abundant sites to the north in Cardigan Bay were predicted to be

present in the waters off Aberarth after 30 days. In Cardigan Bay the abundant and persistent reefs at both Criccieth and Shell Island may play a very important role in larval supply throughout the northern half of Cardigan Bay, north feeding larvae south to multiple in all months (Figure 22 a-d). The sink sites for Criccieth and Shell Island show connectivity with populations further south.

In South Wales, Porthcawl, Swansea Bay, Limeslade Bay and Dunraven Bay show interconnectivity between populations in all months. Both Dunraven Bay and Porthcawl retain high numbers of recruits near the origin after 30 days facilitating their own recruitment success, whilst Limeslade Bay is a particularly important source site for larvae to the Gower Peninsula, in addition to down the Glamorgan coastline. A limited number of larvae from Dunraven, and potentially from the other three sites, were predicted to disperse to the north coast of Devon, specifically at Hinckley Point, a site that has been reported as Abundant through time but was not included in this study as it was not visited by the authors previously.

The long-term persistence and resilience of *S. alveolata* reefs is dependent on successful recruitment by the larvae (either by retention of the natal population or by connectivity with another population), in addition to the continued suitability of the reef habitat. One of the most important results from this study is that distinct sub-populations within the Irish Sea were isolated by the simulations. These sub-populations are reefs along southern England, the Bristol Channel, South Wales (including Dunraven), Cardigan Bay (including Aberarth), North Wales (including Llanddulas), and northern England. These sub-populations were predicted to be distinct and, therefore, not connect with one another. Isolation of each sub-populations was caused by their relatively large geographic distance apart, barriers in advection (e.g., residual currents), or geographic barriers (e.g., the Llŷn Peninsula). Potential connectivity within each sub-population was evident, and depended upon hydrodynamics and seasonal variability in atmospheric forces. The result of Cardigan Bay isolation is corroborated by an observed decrease in abundance in this area alone in the past 50 years (Bush, *draft*). In consequence, according to our simulations, the sub-populations listed above should be considered as separate systems

and managed accordingly, under the EU Habitats Directive (Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora) and UK's Biodiversity Action Plan (1994).

We simulated larval dispersal from 26 known *S. alveolata* populations within the eastern Irish Sea. However, populations that may exist elsewhere, such as along the coast of Ireland, were not considered in our model. It is possible that larvae from Ireland, or from other abundant locations that were not simulated (e.g., the English Channel and Scotland) could contribute to the Irish Sea *S. alveolata* metapopulation. However, our modelling results suggest that such outlying populations would only connect with the eastern Irish Sea populations if their pelagic larval duration (PLD) were longer than the 30-day PLD simulated here. The impact of additional , potential persistent, populations of low abundance has not been considered.

Model assumptions

There are several significant assumptions to our biophysical modelling methodology, which should be taken into account when considering our results. The spawning season and spawning patterns of *S. alveolata* larvae appear to vary considerably across the European shelf seas. There are reports of early season spawning in March and April (e.g., Gruet and Lasse 1983) and spawning late in the season, in October (e.g., Dubois et al., 2007). Spawning happen in batches or cohorts, or semi-continuously (Dubois et al., 2007). However, our study only considered batch-spawning during the most common spawning period from May through to August, as was observed in the larval monitoring surveys. With this in mind, future studies may wish to simulate *S. alveolata* larval dispersal throughout the year, and inter-annually, to fully appreciate the potential inter-seasonal and inter-annual variability in dispersal and connectivity. Indeed, several observational studies note high inter-annual variability in recruitment success (Wilson 1971; Gruet 1986). Although modelling such variability will only be achieved at a considerably higher computational cost, which was beyond the scope of this study.

Since it is still unclear what the dominant behavioural characteristics of *S. alveolata* are during their pelagic larval duration (PLD), we chose to simulate passive larval transport in this study, where larvae behave as neutrally buoyant

particles and their trajectories are controlled solely by hydrodynamics. Sensory vertical swimming behaviour, such as tidally-synchronised transport or daily vertical migration, are common larval traits that enable the larvae to remain close to shore or to avoid predation (Levin 2006), and can drastically alter their larval trajectories (Ayata et al., 2009; 2011). Indeed, Robins et al., (2013) found that, for the Irish Sea region, behavioural traits were equally as influential on larval transport patterns as hydrodynamics. A PLD of 30 days was simulated here as the minimum time to settlement. However, PLDs up to 3 months have been observed during winter months (Cazaux 1970), and 1-2.5 months during summer months (Dubois et al., 2007). In these circumstances, our study would considerably underestimate potential dispersal distances and levels of connectivity. Therefore, as the larval behavioural characteristics and PLD of *S. alveolata* become more understood, it is important that these are incorporated into particle tracking algorithms.

We do not take into account larval mortality in our PTMs. The only potential source of larval mortality is lack of settlement at reef habitats. Larval mortality is thought to be high in another tube building polychaete, *Pectinaria koreni* (Ellien et al., 2004), and so, it is reasonable to assume larval mortality will be high in *S. alveolata* larvae. Therefore, we over-estimate connectivity. However, mortality should not change connectivity patterns unless it varies in space (Paris et al., 2007). This is a common modelling approach (e.g., North et al., 2008; Ayata et al., 2010; Sundelöf and Jonsson 2012) and allows us to examine how dispersion alone can lead to connectivity, rather than other factors such as predation and food limitation.

4.5. Conclusions

Sabellaria alveolata is a vital component of rocky coastal habitats, adding topographical complexity to the shoreline and building high levels of biodiversity in many habitats that are otherwise low in diversity. As a result of their protective status and current contradictions in the literature, more information is required to understand recruitment and dispersal of released larvae in UK shores, their associated behaviour and adult fecundity. This study has helped achieve this.

The results highlighted distinct population isolation between the three study sites (Llanddulas, Aberarth and Dunraven Bay) through the biophysical model, temporal differences in larval release into water column and differences in timing and scale of adult worm gamete production.

Additionally, egg size measurements of ripe female adult *Sabellaria alveolata* revealed these separate populations undergo similar gamete trade-offs in relation to eggs size, concentration and sperm count. Despite a peak in egg and sperm production during the summer period, timing and duration of these peaks was not consistent across sites or between sexes within sites. Llanddulas for example featured a single synchronised peak in both egg and spawn count in the summer months, whereas both Aberarth and Dunraven demonstrated a weak bimodal peak supporting the theory of a bimodal peak spawning pattern at southern sites.

Basic understanding of *Sabellaria alveolata* larval behaviour has improved considerably with results revealing that early trochophore larvae are indifference to light exposure. However, following eyespot development, larvae become negatively phototactic, indicating light is likely to influence dispersal. This information can be used for future larval dispersal models wishing to factor vertical migration of larvae. Future studies need to consider the extent to which larvae are influenced by light by exposing larvae to a variety of light intensities. The quantified horizontal swimming speeds reflect the dispersal and settlement stages of the larvae, with speeds increasing until the settling erpochete stage is reached, where speed drops substantially and the larvae are searching for suitable settlement substrate.

A key finding from this report concerns the distinct sub-populations of reef within the Irish sea: along the coast of Southern England, Bristol Channel, South Wales, Cardigan Bay, North Wales and Northern England. Considering the geographic and advection barriers and isolation between main study sites, isolation between sub-populations was predicted. Despite this, strong inter-connectivity between these sub-populations was found to be evident, but dependence on hydrodynamics and seasonal variability in meteorological events. Consequently, for the purposes of environmental management and the long term protection of *S. alveolata* particular attention should be paid to the

dominant source site in each of distinct subpopulation. In South Wales, Dunraven, Porthcawl, Swansea Bay and Limeslade Bay all show strong interconnectivity, and consequently protecting one would benefit all. Some larvae from Limeslade Bay are predicted to spread west along the Gower increasing its value as a source site. However, both Dunraven and Porthcawl show strong self-recruitment, increasing resilience and thus persistence naturally, and potentially facilitating environmental management. In Mid Wales, Barmouth, Shell Island and Criccieth show strong interconnectivity, whilst some larvae are predicted to disperse south to numerous sites. As with South Wales, protecting one site would protect all. In contrast, Llanddulas in North Wales is a predominantly self-recruiting site, and a source site to all transient populations on this coastline.

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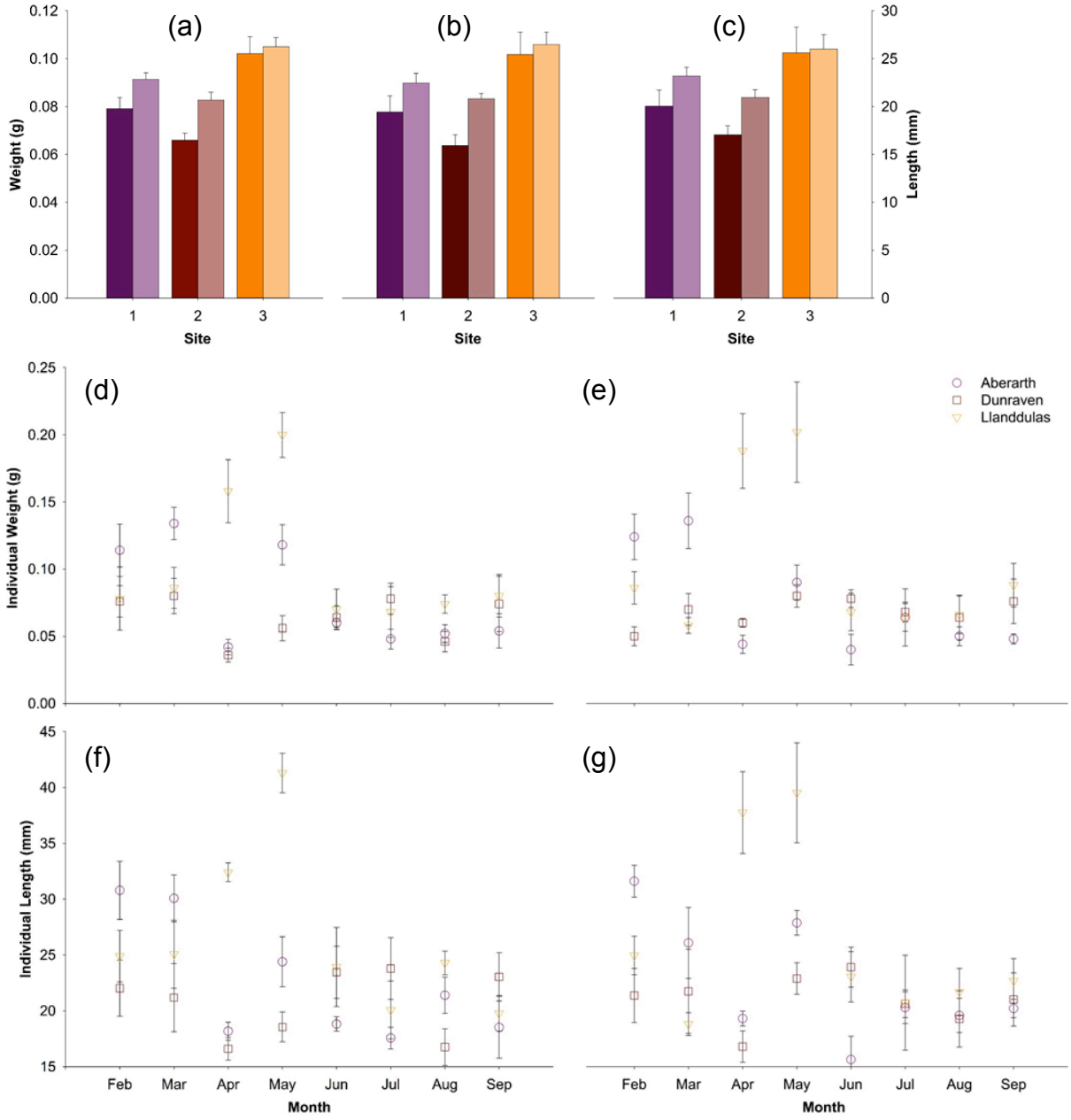
7. Appendices

7.1. Appendix 1

There are difference both between sites and within sites when individual length and weight are considered. Between sites there is a gradient in both weight and length of worms of both sexes from the smallest at Dunraven, through Aberarth, to the largest at Llanddulas (Appendix 1a to c). There is a substantial difference in size between mean weight and length of worms collected at Dunraven and Llanddulas in particular. There is also a within site difference at all 3 sites with variability in the weight and length of both collected males and collected females through time (Appendix 1d to f). Both male and female worms collected from Llanddulas in April and May were substantially heavier and longer than their equivalents at the other 2 sites, male worms collected in February were substantially different in weight, and female worms collected in August were substantially heavier than those collected at both other sites. Males collected in June and September were substantially heavier than those collected at Aberarth. Both males and females collected at Aberarth in February were substantially longer than both other sites and substantially heavier than their equivalent collected from Dunraven. Both sexes were substantially heavier than their equivalents at both other sites in March, with females substantially longer than worms collected from Dunraven and males than worms collected from Llanddulas. Females were substantially different in both length and weight to their equivalents in May. Males were also substantially different in length to both other sites, and in weight to Llanddulas. In June males collected from Aberarth were substantially shorter and lighter than from either Llanddulas or Dunraven, and females were also substantially shorter. Worms collected from Dunraven in May were substantially shorter than their counterparts at both other sites, and females weighed substantially less. Males also weighed substantially less than those collected from both other sites in February and were substantially shorter in April.



Appendix 1: Differences in the weight and length of donor *Sabellaria alveolata* collected monthly from all 3 sites. (a, b, c) Mean weight and length at each site where Aberarth is site 1 (purple), Dunraven is site 2 (maroon) and Llanddulas is site 3 (orange). Error bar represents standard error. Weight is depicted on the right (dark), length on the left (pale). (a) Mean weight and length of both sexes. (b) Mean weight and length of just females. (c) Mean weight and length of just males. (d, e) monthly mean weight of donor worms where (d) depicts female weight and (e) female weight. (f, g) monthly mean length of donor worms where (f) depicts female length and (g) depicts male length.

7.2. Data Archive Appendix

Data outputs associated with this project are archived as project 460, media 1521 on server–based storage at Natural Resources Wales.

The data archive contains

1. The final report in Microsoft Word and Adobe PDF formats.
2. Particle tracking model simulation for May. Digital attachment, avi format.
3. Particle tracking model simulation for June. Digital attachment, avi format.
4. Particle tracking model simulation for July. Digital attachment, avi format.
5. Particle tracking model simulation for August. Digital attachment, avi format.

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