Current status of the Pacific oyster, *Crassostrea (Magallana) gigas,* in the Menai Strait, spawning potential and potential mitigation using triploid oysters.

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Key Findings

There is very limited evidence of recent settlement of Pacific oysters in the Menai Strait in recent years, no expansion of feral populations over the last 30 years and reduction in abundance since the previous survey in 2019.

- There is no evidence to date of establishment of an invasive population of Pacific oysters derived from aquaculture operations within the Menai Strait. The observed numbers of Pacific oysters are very low and sparsely distributed. Field assessment in 2021 showed the overall number of feral Pacific oysters has declined at a range of sites previously surveyed in 2019 and 1994. This is consistent with a pattern of inconsistent and weak recruitment and survival of Pacific oyster within the Menai Strait, despite the presence of large areas of suitable intertidal habitat and spawning age animals held at aquaculture sites and among the feral oyster population.

- Overall, these results confirm a pattern of sporadic, low-level recruitment of Pacific oysters within the Menai Strait. This is potentially combined with a high level of post-settlement mortality, with declines in abundance between surveys. Reproductive success of both aquaculture and feral oysters does not appear to be significant, given the lack of any significant increase in feral oyster numbers over a 27-year period, and more specifically in the last 3-4 years. The survey did not identify any newly settled oysters, with the dominant age being 2-4 years old or more at the study sites.

Aquaculture of diploid Pacific oysters in the Menai Strait is not currently expected to contribute significantly to feral populations, as the temperature regime currently only rarely provides suitable conditions for spawning activity and larval settlement.

- A key factor in explaining the lack of population growth is likely to be the temperature regime within the Strait. The low density of feral oysters may also limit their reproductive capacity, as an inter-individual distance greater than 1.5m in natural populations of diploid oysters can drastically reduce likely reproductive potential. Also, any successful settlement may be masked by high early mortality in newly settled oyster spat.

- The spring-summer seawater temperatures (modelled and historically observed) in the Strait appear sufficient to support gonad development in Pacific oysters in all but three of the years considered (1992-2021). However, estimated cumulative seasonal temperature conditions (measured as degree-days) sufficient for larval development to settlement occurred in only four years, supporting the explanation that sporadic inconsistent spawning and settlement underlies the failure to support population growth.

- Spawning events are likely to be more constrained by peak summer seawater temperatures than by the cumulative seawater degree-days through the year. The threshold seawater temperature reported to be required for spawning at other comparable locations in England and France (18 – 20 °C) is rarely reached within the Menai Strait. In only two years out of the past 27 years have seawater temperatures exceed the 18 °C threshold required for spawning to occur.

- Previous experimental studies have shown that larval development at 17 °C is very extended (>30days) and settlement at this temperature is relatively low compared to higher temperatures (>20 °C). At summer seawater temperatures in the Menai Strait at generally around 17-18 °C. This may also contribute to the low recruitment success, over and above the cumulative temperature environment calculated as degree-days (above).

- Over the period modelled (1992-2021) there has been a trend of increasing average seawater temperatures in the Menai Strait. Future increase in temperatures expected with climate change may result in increased occurrence of conditions suitable for spawning and settlement of Pacific oysters, though the scale of these changes is difficult to project/predict over relatively short time scales. This may become more feasible as improved ocean models which incorporate near future projected climate change become available. Previous modelling studies over longer timescales (several decades) have shown that with increased seawater temperatures, northward recruitment and establishment of feral Pacific oyster populations across the UK may be expected. This implies potential for future recruitment from distant sources irrespective of aquaculture operations in the Menai Strait.

Literature evidence shows that the risk of aquaculture contributing to feral oyster populations can be effectively mitigated by use of triploid stocks, that are functionally sterile.

- The production of triploid oysters from cross-breeding of tetraploid and diploid parents is highly effective in reducing the reproductive potential for farmed Pacific oysters.
- Adoption of triploid oysters at existing and new aquaculture sites has the potential to reduce larval supply because they are functionally sterile, thus (a) reducing any additional impacts from aquaculture production and (b) having potential to contribute

to a decline in existing feral populations, by not contributing potential larvae to the system where the feral population is low and may not be self-sustaining on its own.

- Triploid oysters have a very low reproductive capacity; review of literature suggests between 0.0002% and 0.032% compared to diploid oysters. On this basis a theoretical farm stocked with 250,000 triploid oysters would have the reproductive capacity equivalent of between only 0.25 - 40 female diploid oysters.

Context for the project

The eastern Menai Strait is the most important aquaculture site in all of Wales, and the single largest mussel farming area in the whole of the UK. The main mussel fishery takes place within the Menai Strait Oyster and Mussel Fishery Order (1962), which provides legal protection of shellfish stocks owned by the leaseholders who farm mussels within the area. The Menai Strait Fishery Order Management Association (MSFOMA) was established in 2010 by the Welsh Government to promote and manage sustainable shellfish farming in the Menai Strait, acting as the grantee for the Menai Strait Oyster and Mussel Fishery Order. The Fishery Order is in the process of renewal in March 2022 and approval of the application will help secure investment and jobs in the mussel industry. MSFOMA is also the applicant for the renewal of the Menai West Oyster, Mussel and Clam Fishery Order 1978 (lapsed). The application for renewal of this order has been in progress for over a decade, with several shellfish businesses dependent on its approval to allow them to invest in and develop their businesses. Extramussel Ltd is a producer of mussels, operating leased areas with the Menai Strait Several Order and also a prospective leaseholder for a mussel and oyster production area in the Menai West Fishery Order. Menai Oysters and Mussels Ltd is an established shellfish producer, established in 1994 within the Menai West Fishery Order. The company initially concentrated on Pacific oyster production, with later expansion into mussel production. Investment in further expansion of the business is restricted by the ongoing uncertainty regarding the status of the Fishery Order.

Development of aquaculture is embedded in the objectives of the Welsh National Marine Plan, which also aligns with the principles of the Wellbeing of Future Generations Act (2015) and the Environment (Wales) Act (2016) in its objectives to facilitate the development of sustainable aquaculture in Welsh waters, supporting economic development in rural and coastal communities as well as contributing to food security. Increasing supply and consumption of sustainable healthy shellfish also aligns with the Food Strategy for Wales 2010-2020, and its four key principles ‘Sustainability, Resilience, Competitiveness and Profitability’. In this context, industry’s narrative is a desire for increased production of pacific oysters, supporting local income generation and improved protein supply using a species that is positive for the environment (Ferreira et al., 2018; van der Schatte Olivier et al., 2020) through provision of provision of important ecosystem services such as water quality improvement. In the UK, like other countries, this must be balanced against the fact that *Crassostrea gigas* can become established in the wider environment beyond aquaculture sties, where environmental conditions support reproduction and population growth.

The Menai Strait (West) Several Order

In 2008, the previous 40-year lease for a mussel, clam and oyster fishery order, Menai Strait (West), expired. Preparations for the renewal of this lease began some years before this date, with the proposed Order the same as the original, except clams were not included. CEFAS (2008) undertook an Appropriate Assessment concluding that the renewed order "will have no significant adverse effects alone or in-combination on the interest features of the Menai Strait and Conwy Bay SAC and EMS". In addition, staff at North Western and North Wales Sea Fishery Committee had undertaken consultations with local stakeholders and no objections had been raised. Despite this, the lease had not been renewed before fisheries were devolved to Wales. Welsh Government started the process again and a draft document providing scientific evidence for an Appropriate Assessment was completed in 2011 and finalised in 2013. Based on this, the required Screening Matrix, carried out by Welsh Government in June 2013, concluded that "No likely significant effect has been identified that could result from the granting of this order" and in 2015 a draft Fishery Order was produced. MSFOMA were invited to undertake the necessary public consultation in 2015, and this took place in October/November of that year. Following the consultation, a liaison group was established with local stakeholders to deal with objections. At the end of a lengthy discussion period, agreement was reached with the liaison group in June 2017. However, a revision of the Habitats Regulations Assessment was required (consisting of a summary of likely significant effects and an appropriate assessment). Based on this, further work was recommended to provide information on the potential for Pacific oyster aquaculture to cause significant effects to designated conservation features. Bangor University was commissioned to assess effects and did so (Robins *et al.* 2019). At that time, the Several Order renewal was not granted, and due to the time since the initial application was submitted, an updated review of scientific evidence is required. The present study follows on from the Robins *et al.* (2019) report and provides additional updated evidence.

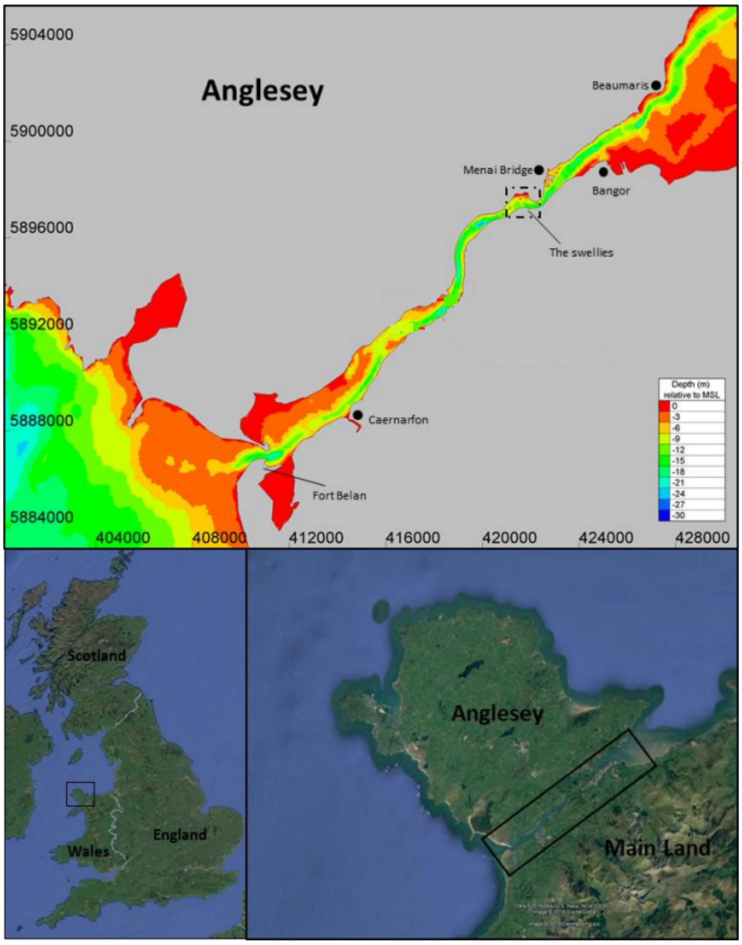

Figure 1. *Map of the Menai Strait (top), North Wales (bottom panels). Bathymetry contours and key towns/ areas are shown. (Survey locations are shown in detail in Fig. 2).*

This project is focused on Pacific oysters, as the delay in renewal of the Menai West fishery order is to a great extent the result of lack of evidence to support policy development on the future of Pacific oyster aquaculture in Wales. Pacific oysters are an introduced non-native species, which can become invasive where conditions are suitable for population expansion, though the Robins *et al.* (2019) report found no evidence of invasive population growth in the Menai Strait. Specific areas of uncertainty are the need for information on the current status of *C. gigas* populations in the Menai Strait, and the extent to which reproduction of farmed oysters may act as a source of self-sustaining escaped (feral) populations across marine habitats, especially those comprising designated conservation features. The work in this project, and this report, adds to the information available for consideration in the context of the environmental setting of the Menai Strait. This uncertainty also affects the Welsh shellfish sector more generally, and results will help inform policy development that is relevant to all potential oyster production areas in Wales.

Aims

This collaborative research project between the Shellfish Centre and the Menai Strait Fishery Order Management Association, Extramussel Ltd and Menai Oysters & Mussels Ltd, focused on the Menai Strait, an area with history of Pacific oyster aquaculture including a current production site in the lapsed Menai West Several Order Area. There is industry interest in potential for expansion of oyster production at these sites. The work follows from a study commissioned by Welsh Government (Robins *et al.* 2019) with the aim of providing additional and updated evidence.

The aims of the project were to:

1. Assess the current status of feral Pacific oyster populations in intertidal habitats of the Menai Strait, including comparison with historical survey data to allow estimation of change over time, stability of the feral population and any evidence of recruitment.

2. Investigate the spawning potential of diploid Pacific oysters in the Menai Strait based on seasonal seawater temperature conditions and the range of air/water temperatures experienced by intertidal animals over the tidal cycle. Future potential for spawning and larval development to settlement is considered in relation to climate change model projections for future seawater temperatures in the region.

3. To review the literature on the effectiveness of triploid introduction, as a method to reduce reproduction in oysters and to quantify the relative spawning potential of triploid and diploid farmed stock, in comparison with the potential reproductive output from the observed feral population within the Menai Strait.

Background on Pacific oysters

The Pacific oyster is recognised as a non-native species within UK and European waters. The decline of the European flat oyster *Ostrea edulis* was the motivation behind the introduction of the Pacific oyster *Crassostrea* (*Magallana*) *gigas* (hereafter referred to as *Crassostrea gigas* or *C. gigas*) into European waters (Guy and Roberts 2010). Pacific oyster translocations were taking place in Europe as far back as the late 1800s (Wolff and Reise 2002). Indeed, the practice of importing and laying *C. gigas* was commonplace along the Menai Strait in the 1890s, with Beaumaris in particular accommodating a considerable expanse of Pacific oyster lays (White 1894). It was believed at the time that *C. gigas* was environmentally benign and offered the perfect vehicle back to restabilising the lucrative oyster fishery which *O. edulis* once provided (Humphreys *et al*. 2014). The reproductive curbs placed on *C. gigas* by low European water temperatures acted as a preventive barrier to successful spawning and settlements of the Pacific oyster for > 70 years (Carrasco and Barón, 2010). However, with decades of conditioning to the extremes of the European marine environment and an ever-rising increase in sea temperature the first reports of *C. gigas* spat settlement were recorded in the 1970s in the Oosterschelde (Smaal *et al*. 2008).

Indeed, the species is now considered naturalised at many locations in Europe. Initially it was thought *C. gigas* was incapable of spawning in the non-native low temperature waters of the UK. However, after a century of environmental conditioning there have been successful spawning and settlement events in the lower latitude regions of the UK, particularly in the South of England. The aquaculture industry views Pacific oyster production as one of the mainstays of shellfish aquaculture in the UK, which can provide additional environmental benefits in the form of ecosystem services such as nutrient reduction (van der Schatte Olivier et al 2020).

Mitigation measures to limit the potential seeding of wild *C. gigas* populations from spawning of aquaculture stock have included development of triploid Pacific oysters. This method has proved largely successful in curbing the spawning capabilities of *C. gigas*. Nonetheless, concerns still exist within government and environmental stakeholders that rising sea temperatures and incomplete triploid transformation may lead to population spread of this species from aquaculture sites. This represents a barrier to expansion of the shellfish industry in the Menai Strait (and across Wales), where uncertainty and lack of evidence is delaying both renewals of fisheries orders and approval of new production areas.

Assessment of feral populations of *C. gigas* in the Menai Strait

MAFF reintroduced the Pacific oyster to the Menai Strait in 1964. The sub-surface sea temperature within the Menai Strait has experienced some significant peaks over the past 58 years since the reintroduction, with a temperature of > 19°C recorded in the summer of 2018. A survey by Spencer *et al.* (1994) recorded feral *C. gigas* at several northerly sites along the Strait and a recent survey by Robins *et al.* (2019) identified 9 settlement sites in the southern end of the Strait (Figure 2). The number of oysters recorded was < 10 per site, except for Abermenai Point (33) and Tŷ Calch (26) (Table 1). It is likely that these occurrences reflect some successful larval settlements, because of >50 years of environmental conditioning in combination with rising sea temperatures.

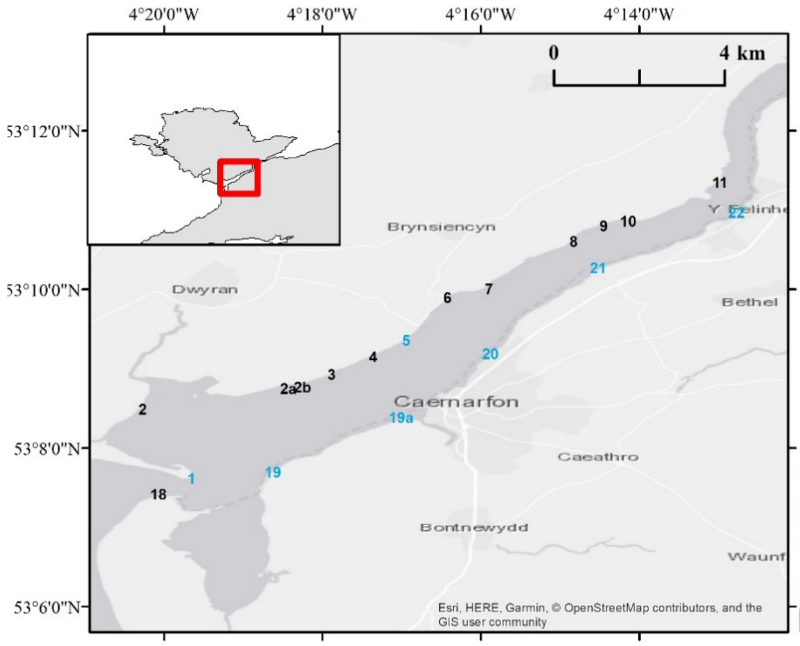

Figure 2 *Survey locations in the Southern Menai Strait , located in North Wales (top panel). Key towns/ areas are shown. (Survey locations and results are shown in Table. 1).*

***Table 1** Intertidal C. gigas density per 160m2 plot, as reported in Robins et al. (2019)*

Site	Name	C. gigas (Live)	C. gigas (Dead)	Shell (mm)	Avg Shell (mm)
1	Abermenai Point	33	18	62-115	88.4 ± 3.07
2	Traeth Melynog	0	0	0	0
2_a	Holiday Home	1	1	55	55
2_b	Old Boat House	0	0	0	0
3	Stud Farm	0	0	0	0
4	Cae Aur	1	1	55	55
6	MAFF	0	0	0	0
7	Plas Trefarthen	8	5	39 - 101	62.4 ± 9.57
8	Lianidan	3	0	40 - 55	48.3 ± 4.03
9	Mussels	5	3	41 - 108	73.4 ± 11.57
10	Castell Gwylan	2	0	92 - 94	93 ± 1
11	Moel Y Don	0	0	0	0
18	Fort Belan	0	0	0	0
19_a	Tŷ Calch	26	2	55 - 85	58 ± 12.7
19_b	Bath Cottage	7	0	54 - 97	73.1 ± 5

Site Selection

The survey region was divided into intertidal stretches along either side of the Menai Strait; east (53.134282, -4.329986 to 53.277161, -4.086653) and west (53.124386, -4.328050 to 53.238184, -4.088762). A total of 15 sites were selected based on suitable *C. gigas* settlement substrate availability and the previous oyster survey locations: Spencer et al. (1994), Morgan and Richardson (2012) and Robins *et al.* (2019). The highest densities were reported at Abermenai Point (0.825 m^{-2}) and Tŷ Calch (0.087 m^{-2}).

Survey Techniques

Surveys were undertaken at low water, when tidal height was 0.5m below datum. At each site, oyster densities were estimated using counts in replicated quadrats and in timed searches. Quadrat surveys followed an 80m transect recording oysters in a 0.25 m^2 quadrat every 5m on either side of the transect line (Figures 3-5). Timed searches involved recording oysters in two 10 min searches 1.5 m either side of the 80m transect. Density estimates using both techniques showed a highly significant positive relationship ($r^2 = 0.999$; $p < 0.0001$).

During the surveys, substratum composition was recorded for each location. Digital stills of littoral zone transect substrate were taken to allow the relative percentage coverage of different substrata to be determined. To establish the population dynamics of *C. gigas,* live oysters were measured to 0.25 cm *in-situ* at each site using a Vernier© calliper. Oyster densities were expressed for both quadrat/transect and timed search methodologies in number of oysters per m^2. Data was also converted into number 100 m^{-1} length of shore so that a direct comparison could be made between the current *C. gigas* densities and those presented by Spencer *et al.* (1994) and Robins (2019).

Population Model and Larval Settlement Forecast

A Gunderson (1993) population model and an Allen and Burnett (2008) post-settlement mortality correction factor was used to predict the larval output and post- settlement densities from the most abundant sites from the Robins *et al.* (2019) survey. These were revisited to ascertain if the current *in-situ* oyster population numbers match those of the model.

Population Modelling

The Gunderson population model was used to estimate the abundance of Pacific oysters, based on available area of suitable substrate at each site. This modelling approach has proved extremely accurate when assessing sessile or regionally limited fishery stocks (Kennedy and Roberts, 1999; Smyth *et al.* 2009). For each site, the proportion of total area accommodating the appropriate settlement substrate was determined by examining biotope classifications (Phase 3 EUNIS biotope coding). This allows for application of a correction factor, restricting abundance estimates to only the area within each survey site that is suitable for the settlement of oysters.

The model does not account for post settlement mortality within oyster populations. It is therefore important to consider that Cheney *et al*. (2000) showed many *C. gigas* age classes can be affected, with the most severe mortalities occurring among gravid and rapidly growing animals. Allen & Burnett (2008) estimated the average post-settlement mortality within intertidal *C. gigas* as being 40%. However, this can be as high as 60% during environmental pulse events such as heatwaves, freshwater run-offs, or prolonged cold spells. As no site-specific records of pulse events were available for this analysis, a mortality correction factor (c.f.) was set at 0.4 and applied to all Gunderson population outputs.

Age cohorts were determined as per Guy & Roberts (2010) via acetate peel and shell length records. Feral intertidal *C. gigas* stocks of diploid oysters in Menai Strait were ascertained as per methods described by Smyth *et al* (2009), with the total number of potential *C. gigas* estimated using the following formula which was adapted from Gunderson (1993):

$$P = \sum_{i=1}^{h} \left(R_i . F/a \right) C_i$$

Where:

P= Total population resident in full survey area.
Ri= Area of region I in m2.
a= Area sampled within a single sampling unit.
F= Correction factor estimating substratum types.
Ci= Mean number of oysters observed per sampling unit in the region i based on n samples.
h= Number of regions composing the survey.

The total population resident in the entire survey area, 'P', was determined using an estimate of the area of each survey region in m^2. The surface area, 'Ri', for the regions was estimated using scaled images of the Menai Strait from Global Lab image analysis software (Table 3). A proportionally weighted correction factor, 'F', was then applied to 'Ri' to account for the amount of suitable oyster settlement substratum present in the region. This factor was derived from survey results (Robins *et al*. 2019). Value 'a' is a constant which refers to the area sampled within a single sampling unit (9m). 'Ci' refers to the mean number of oysters observed per sampling unit in region 'i' based on 'n' samples.

Figure 3 *Laying a transect line at Llanidan.*

Figure 4 *Intertidal C. gigas collected within 20m2 at Abermenai Point 2019*

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Figure 5 *C. gigas collected at Tŷ Calch 2019, sizes range from 48-97 mm.*

Results

In 2019 feral *C. gigas* were recorded at nine sites, with Abermenai and Tŷ Calch the most abundant (Figures 6 and 7). The 2021 survey only identified *C. gigas* settlement at two sites out of the 15 assessed, notably Abermenai Point and Tŷ Calch (Figure 6). In 2021, both sites showed a reduction in oyster numbers compared to 2019. Abermenai Point dropped from 33 to 6 and Tŷ Calch from 26 to 11 (Figure 7). No meaningful change in oyster size was recorded between 2019 and 2021. The majority of feral *C. gigas* continue to be made up of the 61-90mm size cohort, which equates to year class 2-4 (Figure 7 and Table 2). Only two oysters recorded in 2021 were in the year 1-2 recruitment class, both found at Abermenai Point (Table 3).

Table 2 *In-situ feral C. gigas numbers and average shell length for 2019 and 2021*

Site	Name	*C. gigas* Counts 2019	Mean shell length ± std dev. (mm) 2019	*C. gigas* Counts 2021	Mean shell length ± std dev. (mm) 2021
1	Abermenai Pt.	33	88.4 ± 3.07	6	62.1 ± 4.08
2	Traeth Melynog	0	0	0	0
2_a	Holiday Home	1	55	0	0
2_b	Old Boat House	0	0	0	0
3	Stud Farm	0	0	0	0
4	Cae Aur	1	55.0	0	0
6	MAFF	0	0	0	0
7	Plas Trefarthen	8	62.4 ± 9.57	0	0
8	Lianidan	3	48.3 ± 4.03	0	0
9	Mussels	5	73.4 ± 11.57	0	0
10	Castell Gwylan	2	93.0 ± 1.0	0	0
11	Moel Y Don	0	0	0	0
18	Fort Belan	0	0	0	0
19_a	Tŷ Calch	26	58.0 ± 12.7	11	66.0 ± 5.7
19_b	Bath Cottage	7	73.1 ± 5.0	0	0

Table 3 *Number of oysters per age category, as determined following Guy & Roberts (2010)*

Size Cohort	Age	Abermenai 2019	Abermenai 2021	Tŷ Calch 2019	Tŷ Calch 2021
0-30mm	0-1	0	0	0	0
31-60mm	1-2	0	2	3	0
61-90mm	2-4	19	4	19	3
91-120mm	4-6	13	0	4	7
121-150mm	6-8	1	0	0	1

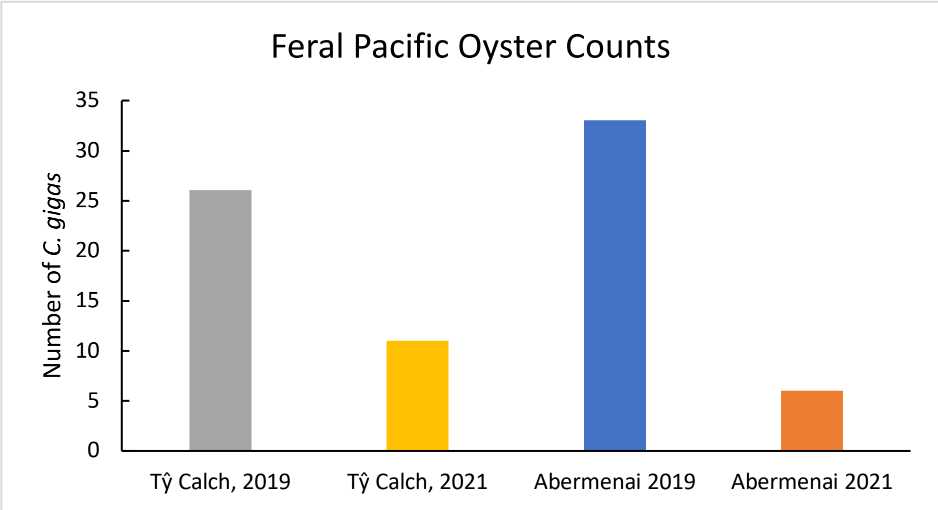

Figure 6 *Number of feral Pacific oysters recorded at the two most abundant sites in Menai Strait*

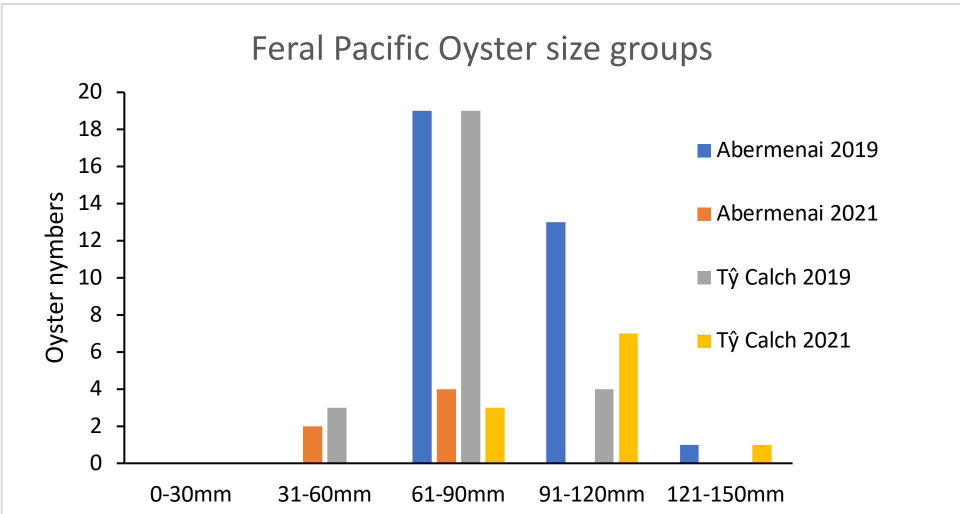

Figure 7 *Number of oysters in recorded in recognised age size cohorts (Guy and Roberts 2010)*

The Gunderson population model was constructed on a site-specific basis as only Abermenai Point and Tŷ Calch could be directly compared with the 2019 data (Table 3). Correction factors were applied to the model for each site based on an *in-situ* suitable settlement percentage calculated from substrate types within gridded quadrat transect surveys (Table 4). The predictive model used 2019 data with an additional post settlement mortality correction factor added to produce a more accurate estimate of population numbers in 2021 (Table 5). The model predicted a total *C. gigas* settlement of 1,627 for the combined sites. The 2021 survey recorded an *in-situ* total of 17 *C.gigas*. A Chi^2 test between the predicted data and the actual *in-situ* data produced a highly significant difference $P < 0.0001$.

Table 4 *Site specific dimensions of survey areas where population modelling was applied*

Region	Intertidal area m^{-2} (x10)	Total suitable area m^{-2} ($x10^3$)
Abermenai Point	18.090 *c.f. 0.27	6.587
Tŷ Calch	22.509 *c.f. 0.32	7.292

*c.f. correction factor based on substratum settlement suitability

Table 5 *Gunderson prediction for change in oyster abundance from 2019 to 2021, applying a correction factor (c.f) for post settlement mortality to total numbers of oysters in-situ 2019 (Allan and Burnett, 2008).*

Site	***Observed C. gigas*** **2019**	**Modelled 2021 (c.f. 0.4)**	***Observed C.gigas*** **2021**
Abermenai Point	33	869	6
Tŷ Calch	26	758	11
Total	**59**	**1,627**	**17**

There is no evidence of expansion of Pacific oyster populations in the Menai Strait in recent years. Indeed, the Pacific oyster populations at the sites surveyed have declined in the four years since the previous survey, and no specimens were found in the 0-30mm size range with most specimens greater than 2 years old. The Menai Strait has substantial areas of suitable intertidal substrate for *C. gigas* larvae to settle in high densities and predictive population modelling of the 2019 feral oyster assemblages recorded at Abermenai Point and Tŷ Calch provided estimates of an expected total population of 1,627 in 2021. However, this was clearly not the case, as only 17 oysters were recorded in 2021. The age of the oysters recorded in both 2019 and 2021 indicate that some spawning had been occurring over the preceding 2–4-year periods. However, based on actual occurrence of juveniles, the combined effect of larval output, settlement and juvenile survival was negligible, compared to the theoretical reproductive potential of the oyster population.

However, the likely very weak recruitment within the Menai Strait may be attributed to infrequent occurrence of spawning events, rather than larval dispersal to other areas, because hydrodynamic modelling by Robins *et al.* (2019) demonstrated the potential for retention of a significant proportion of larvae released from existing populations to remain within the Strait. The combination of a low fertilization rate and the low abundance of parent oysters could contribute to low larval output, and hence a small pool of larvae to supply retention and recruitment within Menai Strait. Moreover, research has not yet explored the role of larval mortality and post-settlement mortality in reducing recruitment success, and this may also contribute to the sparse numbers of early age-class oysters. Following various introductions over more than 60 years the Pacific oyster has spread throughout Europe from Portugal in the south to Norway and Shetland in the north, with several areas having naturalised populations (Reise *et al.*, 2017). The oysters found within the Menai Strait by Spencer *et al.* (1994) were predominantly at the northern end of the Strait with only a few at the southern end. The more recent surveys of 2019 and 2021 have recorded only small 'feral' assemblages in the southern end of the Strait. This suggests that environmental conditions in the Menai Strait are currently sub-optimal for Pacific oyster reproduction and population expansion, with lower reproduction and potentially higher mortality than for other more suitable settings.

Overall, these results confirm a pattern of sporadic and low levels of recruitment of Pacific oysters within the Menai Strait. This is potentially combined with a high level of post-settlement mortality, with declines in abundance between surveys. There is no evidence to date of establishment of an invasive population of Pacific oysters derived from aquaculture operations within the Menai Strait.

Spawning potential of diploid Pacific oysters in the Menai Strait based on temperature conditions

The frequency of successful spawning events is an important determining factor in the persistence of Pacific oyster populations (Alves *et al.,* 2021). The timing of spawning varies between years and locations with water temperature generally accepted as the most important factor for inducing reproductive activity. Using a technique called the "temperature sum" the larval peak can be predicted for a specific location. The temperature sum, also known as growing "degree-days", "heat units" or "thermal time", is the cumulative sum of daily water temperature above a threshold temperature. Sufficient cumulative exposure to water above a threshold temperature is required to enable gametogenesis and maturation prior to spawning. The gametogenesis process in *C. gigas* requires a minimum seawater temperature of 10.55 °C and cumulative 600-degree days above that threshold to reach the point of potential spawning (Mann 1979; King *et al.* 2020). Most studies consider seawater temperature only, however the effect of exposure to air temperatures on the gametogenesis can be significant, particularly within the intertidal during low tides (King *et al.,* 2020).

After temperature conditioning and environmental triggers of spawning events, the released larvae require an appropriate seawater temperature range and food supply to enable development of larval stages to settlement. Exact temperature requirements for larval development are less well known but previous studies have assumed a further 225 degree-days above 10.55 °C from spawning to settlement (Syvret *et al.,* 2008; King *et al.,* 2020). Hence overall, around 825 degree-days above 10.55 °C are required for Pacific oysters to achieve spawning and larval development to settlement. There is some uncertainty in this figure with Mills' (2016) investigation of feral and farmed Pacific oysters on the south coast of England, having found that gonad development was initiated at a lower threshold temperature of 9.5 °C, for example. In addition, successful spawning and larval survival and development will also depend on other critical environmental factors, especially food supply.

Seasonal temperature is not the only environmental influence which can initiate spawning in *C. gigas.* Spawning events can also be triggered by temperature spikes during tidal exposure, tidal state and food supply (e.g., Bernard *et al.,* 2016; Mills 2016). Bernard *et al.* (2016), in their long-term study in Archachon in France, indicated that spawning tends to occur on ebb flow following spring high tides, following a 2-4 °C amplitude in water temperature (i.e. highest temperature minus lowest temperature) over the preceding 24 h, when the baseline temperature that prompts spawning is more than 20 °C. They also noted a relationship between the air temperature range of the preceding 24h, with an amplitude of more than 12 °C associated with spawning events. Mills' (2016) investigation of feral and farmed Pacific oysters on the south coast of England, found that feral intertidal Pacific oysters spawned at lower temperatures, when seawater exceeded 18 °C, following a trigger of temperature shocking during tidal emersion.

We considered observed and modelled seawater and intertidal temperatures in Menai Strait, with the aim of addressing three questions:

- Are seasonal seawater temperatures sufficient to support gonad maturation, spawning and successful larval development in Pacific oysters?

- Are seawater temperatures and daily/tidal temperature variations experienced in intertidal oyster beds likely to trigger spawning events?

Observed seawater temperatures in the Menai Strait

Observational sea surface temperature (SST) data were obtained from a temperature logger (Cefas Data Storage Tag G6, temperature sensing) attached to Menai Bridge Pier (approx. 53.23°N, -4.13°E; maintained by Bangor University) storing daily mean temperatures at a water depth of 1 m (the logger was attached to a pier which floats, thus maintaining the logger at a constant depth below surface). The data ranges from January 2011 to the end of 2018 with ~85% of data present. Data collection via these loggers was discontinued in early 2019. These data are used to validate the use of the simulated temperatures, described below.

Modelled seawater temperatures: past-present

The reanalysis SST data has been attained from the Atlantic-Iberian Biscay Irish (IBI) Ocean Reanalysis/Analysis and Forecast systems. The numerical core of the IBI model is based on the NEMO v3.6 general ocean circulation model. The IBI model is described in more detail in Gutnecht et al. (2019) and the model outputs are available for download from the Copernicus Marine Service ([https://resources.marine.copernicus.eu/products]). These simulated data from the IBI models comprise 3D daily ocean fields, such as temperature, from 01/01/1993-24/12/2019 (for the Realanysis system, 1/36° horizontal resolution ~2-3 km) and from 01/05/2020-present (for the operational Analysis and Forecast System, 1/12° horizontal resolution). Since the Menai Strait was not fully-resolved in either model because of the limited spatial resolution, the temperature was extracted from the model at the grid cell closest to the southwestern end of the Menai Strait, for closest proximity to the area of interest (for consistency the same location was used in the higher resolution model: 53.0833°N, 4.4167°W). The two timeseries of simulated SST were combined to span 1993-2021 (inclusive) although there was a gap in the data from 24/12/2019-01/04/2020.

Present day seawater temperatures

Daily mean temperatures from 2011-2019 in the Menai Strait from both temperature logger data ('observed') and the IBI reanalysis model ('modelled') are given in Figure 8. The root mean square error (RMSE) between the model and observational timeseries is 0.8 °C which corresponds with a scatter index of 7%, where the scatter index is the RMSE normalised by the mean of the observational data. Note that the IBI model has a slight cold bias for temperatures >16 °C (relevant to the degree day calculations below) but overestimates the coolest temperatures (a warm bias for temperatures <8 °C). The RMSE given above is deemed to be sufficiently low (<1 °C) to use the output here for consideration of interannual temperature variability. Annual mean SST temperatures from the IBI model output at the point of interest from 1993-2021 (inclusive) are presented in Figure 9 (note, 2020 is not included as 3 months are missing). The line of best fit indicates that annual mean SST is gradually rising at the location of interest.

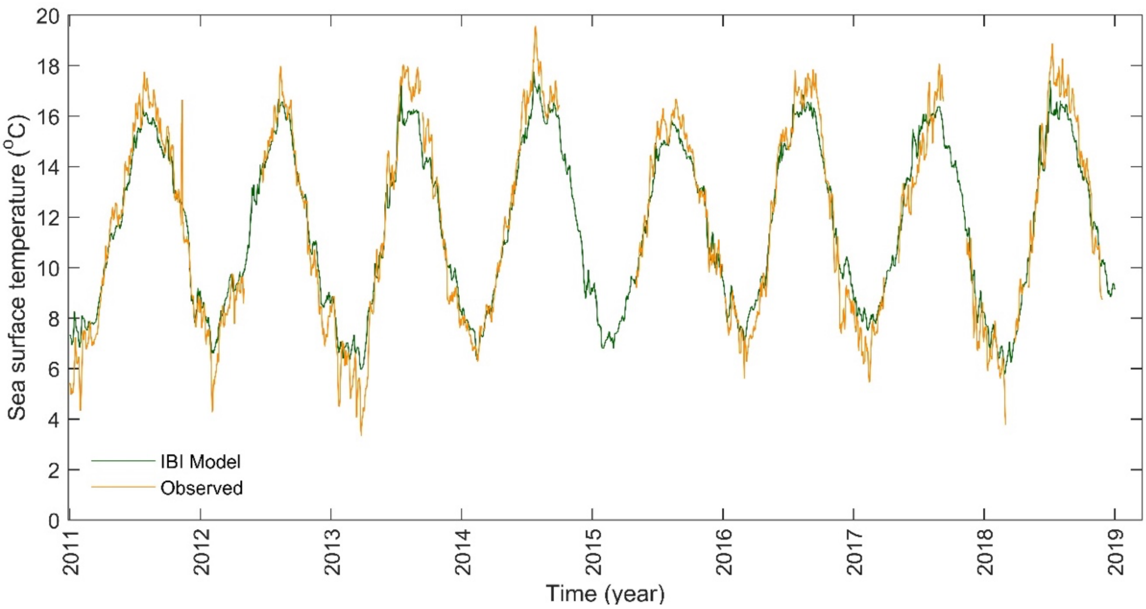

***Figure 8** Menai Strait sea surface temperature from January 2011 to January 2019. Temperature logger data from Menai Bridge Pier ('observed') is plotted in orange and IBI reanalysis/forecast temperatures ('modelled') are plotted in green.*

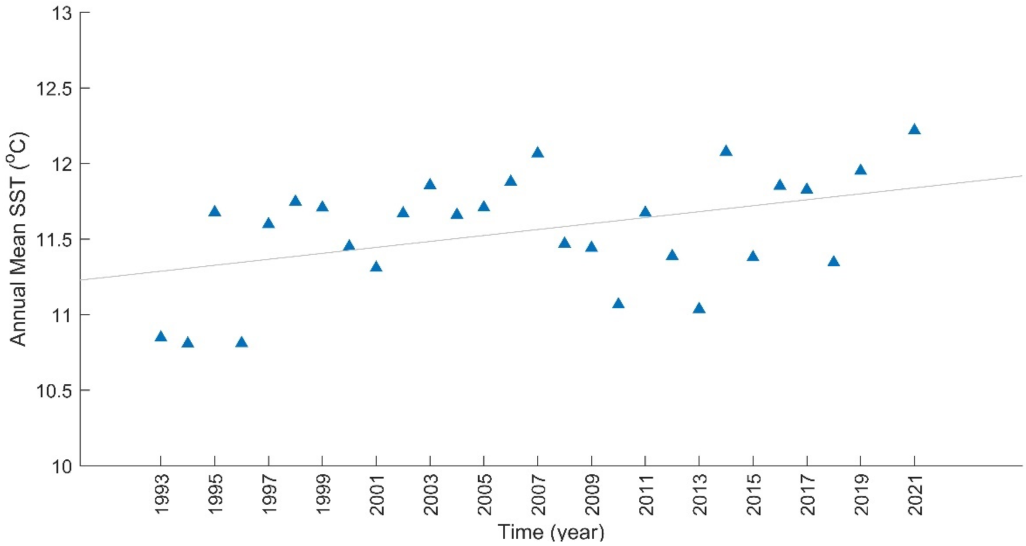

Figure 9 *Annual mean sea surface temperature from 1993-2021 from IBI reanalysis and IBI forecasting models combined, for a point location to the southwest of the Menai Strait. The grey line is the line of best fit, and indicates an overall increase in SST with time.*

Calculation of degree days required to support oyster spawning and larval development to settlement

The following formula was used to calculate degree days, based on the annual number of days when temperatures are above a threshold for gonad and larval development:

$$D = \int (t - t0)\, dt \text{ for } t > t0$$

Where *D* is the degree-day requirement, *d* is the number of days required to attain a ripe state, *t* is the ambient temperature that the animal is exposed to (only accounting for those SST warmer than the threshold temperature), and *t0* is a threshold temperature below which no evidence of gonad development (i.e., 10.55 °C). Here it was assumed that Pacific oysters need 600 cumulative degree days above a threshold temperature of 10.55 °C to mature and spawn (Mann, 1979; Syvret *et al.*, 2008; Robins *et al.*, 2019), with 825-degree days above the same threshold required to achieve larval development to settlement.

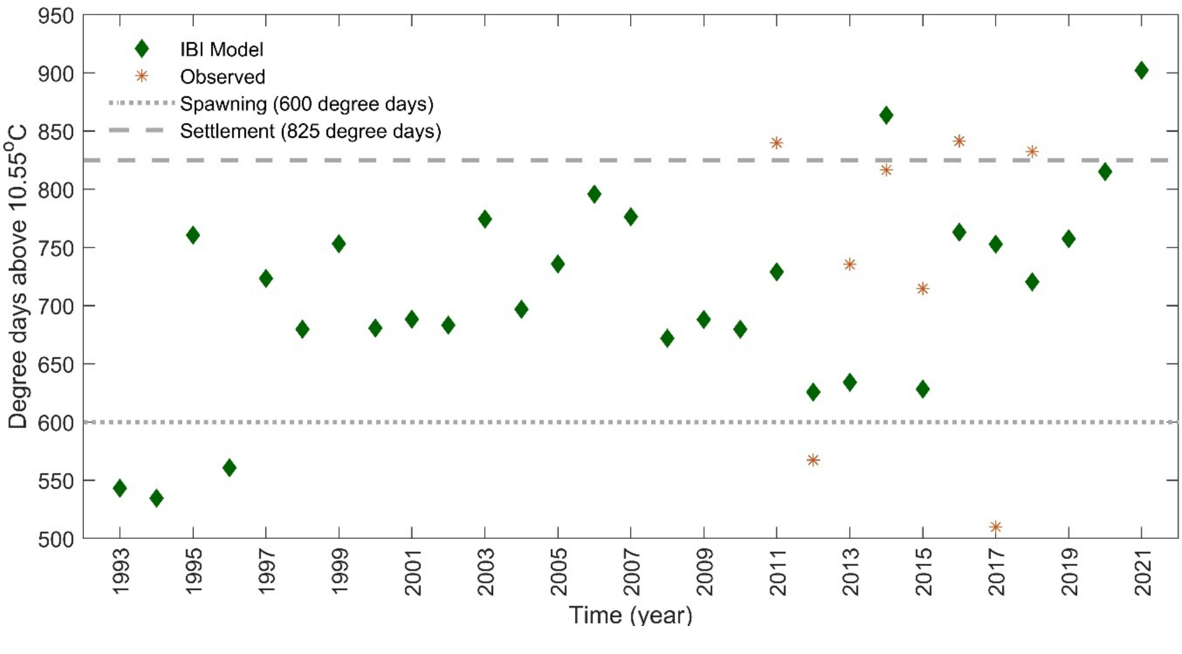

Figure 10 *Degree days above 10.55°C for each year calculated from the IBI reanalysis/forecast data (1993-2021, green diamonds) and calculated from the observational data (2011-2018, orange stars). The spawning and settlement thresholds are indicated by the dotted and dashed lines, respectively (600 and 825 degrees days; Mann, 1979; Syvret et al., 2008).*

Degree-days per year above the temperature threshold of 10.55°C are shown in Figure 10. For the simulated data, in all but three years (1993, 1994, 1996), the spawning threshold of 600 degree-days is exceeded. However, the threshold required for successful larval development to settlement (825 degree-days) was exceeded in only two of 29 years considered. Note, this is likely to be an overestimation of periods suitable for spawning, as previous studies have shown that seawater temperatures more than 18-20°C are required for spawning to be initiated, which occurs even more rarely in the Menai Strait (e.g., this occurred in only four years between 1993-2021, at this location). The degree days calculated using the observed SST data are significantly different to those calculated using the simulated SST, despite the latter being the best available product for such a long time period. This highlights the importance of both long-term monitoring and the use of site-specific data for such calculations, since small changes in observed vs simulated SST can lead to large differences in calculated cumulative degree days. Despite these limitations the simulated data are important as they show the general trends of degree days increasing with time, as SST gradually rise.

Intertidal temperatures experienced by oysters

Successful spawning events are an important determining factor in the persistence of feral Pacific oyster populations (Alves *et al.,* 2021) and temperature plays a critical role in determining reproductive potential of feral oysters. The degree-day thresholds applied in the analysis above are derived from Mann's (1979) study, which undertook experiments in which oysters were continuously immersed in seawater. However, this does not fully reflect the conditions experienced by intertidal oysters, which may be exposed to a wider range of temperatures during emersion at low tide. Internal shell temperatures in *C. gigas* can influence gonad development and fecundity in the oyster and the effect of exposure to air temperatures in the intertidal during low tides may affect gonad development and stimulation of spawning events (King *et al.,* 2020).

As previously discussed, the initiation of spawning events can be triggered by a range of environmental factors, including temperature spikes during tidal exposure, tidal state and food supply (Bernard *et al*., 2016; Mills, 2016). With these considerations in mind, we investigated the internal temperature of loggers designed to mimic internal conditions experienced by intertidal *C. gigas* in the Menai Strait.

Body temperatures of intertidal ectotherms such as *C. gigas* when aerially exposed, are driven by various factors and can be different to air or water temperatures (Helmuth *et al.*, 2016). Adapted loggers were used to investigate temperature ranges experienced in intertidal *C. gigas* in the study area during summer months. iButton© DS1922L temperature loggers were encased in Pacific oyster shells. The oyster mimics were constructed by setting the micro-loggers in silicone sealant, encased within a Pacific Oyster shell, so having similar thermal properties. This approach has been validated in the literature (Helmuth and Hofmann, 2001; Fitzhenry, Halpin & Helmuth, 2004; Helmuth *et al.*, 2016). They were deployed on oyster trestles from 1st May 2021 at the Menai Oysters & Mussels production site at Brynsiencyn, with logger resolution set at 15min data capture intervals. The recorded data provides a proxy value for the actual body temperatures experienced by Pacific Oysters at this location of the Menai Strait during the spawning months of 2021.

The data shown in Figure 11 demonstrates that intertidal oysters may experience a wide range of temperatures over relatively short diurnal and tidal cycles. In periods where low water coincides with warm air temperatures or high solar irradiation, internal temperatures experienced by oysters can be considerably higher than the prevailing seawater temperature at the time. The mimic oyster loggers recorded occasions in May to July 2021 when temperatures spiked to above 20 °C, with a sustained period of several days above 18°C in late July, when spikes reached to between 25-30 °C. There were extended periods in July-September where the oysters experienced peak daily temperatures of 18 °C or above.

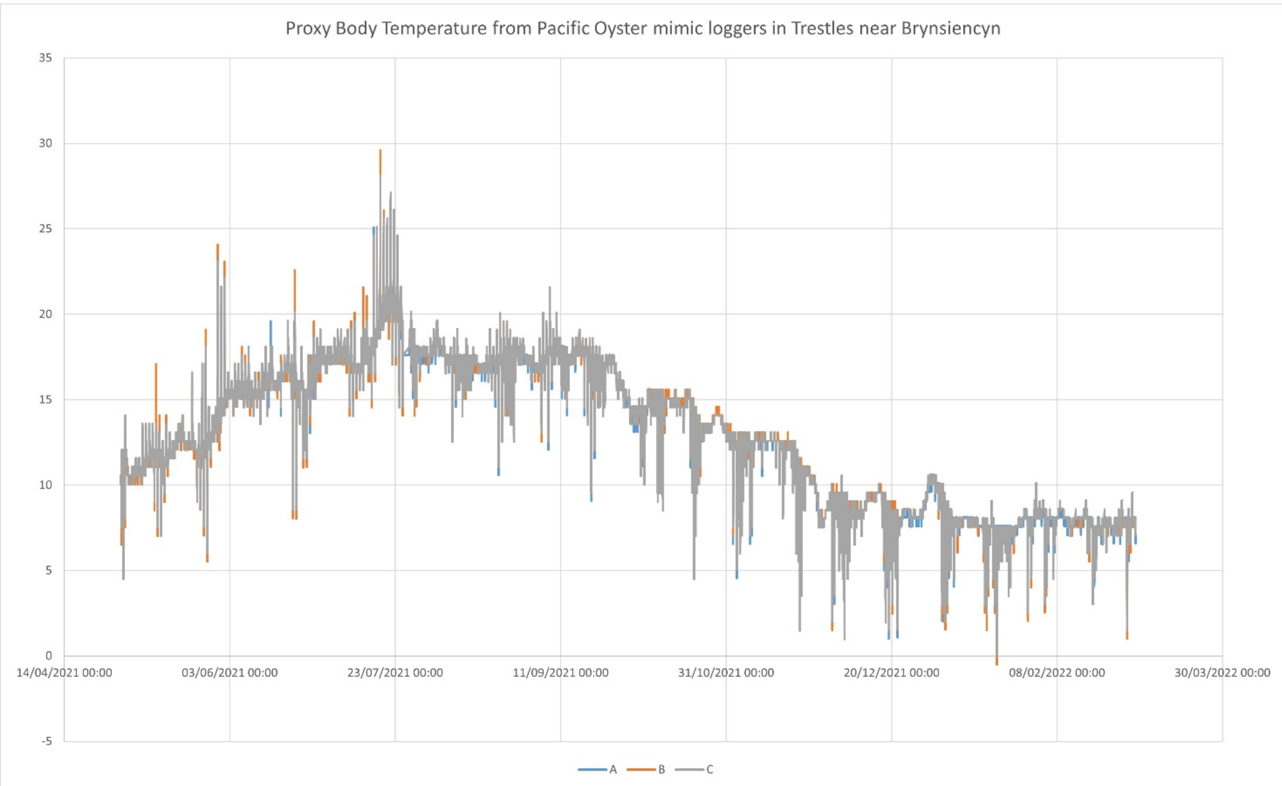

Figure 91 *Proxy body temperatures for Pacific Oysters cultured in Trestles in the Menai Strait (see text for explanation). X-axis shows time/date and Y-axis shows temperature (°C).*

Discussion

Pacific oysters tolerate varying abiotic conditions including wide water temperature variations from sub-zero to >30 °C. *C. gigas* thrives in intertidal areas with fast growth, large size, longevity and high fecundity (Reise *et al.*, 2017). Niche research modelling has suggested a thermal regime of between -2 and 29 °C sea surface temperature and air temperature of between and -23 and 31°C (Carrasco and Baron, 2010; Strand, 2012). Natural recruitment has been linked to exceptionally warm summers such as 1975 and 1976 and various years in the 80s (Drinkwaard, 1999; Diederich *et al.*, 2005) and into the 90s with natural spat fall recorded in the Menai Straits following the warm years of 1989 and 1990 (Spencer *et al.*, 1994).

Winter mortality has been observed during unusually cold winters although elimination of the species is unlikely with a number of individuals protected in deeper 'refuge' areas in the winter and larval dispersal in the summer (Strand, 2012). Currently the lower air or sea surface temperature for survival is unclear with suggestions for temperatures below freezing for 37 days (Diederich *et al.*, 2005) or water temperatures below 3 °C for 3-7 weeks (Child and Laing, 1998). Observations suggest that Pacific oysters stop filtration and feeding at water temperatures below 2 °C (Bernard *et al.*, 2011).

Based on the observed and modelled temperature conditions in the Menai Strait, seasonal seawater temperatures can support gonad development to spawning in nearly all years, but do not appear to be doing so given the population seen in 2021. While experiencing sufficient cumulative degree-days are a pre-requisite for gonad development, it does not mean that spawning will occur. In field studies of spawning in Pacific oysters, a minimum seawater temperature of 18 °C (Mills, 2016 – South of England) and 20 °C (Bernard *et al.,* 2016 – Arcachon, France) has to be exceeded before spawning will occur. Recent modelling studies, such as Alves *et al*. (2021), have used a higher threshold temperature for spawning of 19.5 °C. Over the period studied in the present report (1992-2019) peak summer seawater temperatures have only exceeded 18 °C in two years, indicating that while maturation up to spawning may occur, opportunities for spawning are much more restricted than suggested by the degree-day modelling.

Both Mills (2016) and Barnard *et al*. (2016) studies were for intertidal oysters, which would also experience the higher daytime air emersion temperatures such as recorded in the Menai Strait. This suggests that intertidal temperature peaks alone may not be sufficient to trigger spawning unless ambient seawater temperatures are also above a threshold of at least 18 °C and possibly higher. It therefore seems likely that the summer seawater temperatures in the Menai Strait are, in most years, below the threshold required to initiate spawning events, even if seasonal temperature regimes are sufficient to support gonad development and when average intertidal temperatures exceed 18 °C.

The degree-day modelling also indicates that years with sufficient cumulative temperatures to support larval development are very limited. The observed and modelled temperature data for the Menai Strait to 2019 also indicate that summer seawater temperatures may greatly restrict larval development, even in the warmest years. Reported temperatures for successful larval development for oysters in hatcheries is above 20 °C (Dutertre *et al*., 2009). Oyster larvae are thought to remain in the plankton for between 14 and 30 days before settlement (Quayle, 1988; Herbert *et al.*, 2012) potentially requiring a water temperature higher than 22 °C for at least two weeks with survival dependant on food availability (Dutertre *et al.*, 2009). Larvae will develop faster with higher water temperature and can survive for > 50 days at low temperatures (Herbert *et al.*, 2012). The observed and modelled seawater temperature data for the Menai Strait show that there are very few years in which temperatures reach 18 °C. Rico-Villa *et al.* (2009) studied the effect of temperature on larval development of *C. gigas* larvae, and at the lowest temperature tested (17 °C) larval survival was high but development to settlement was slow (>30 days) and successful settlement was much lower than for larvae reared at higher temperatures (at just 16%). From 1992 to 2019 peak summer seawater temperature conditions observed in the Menai Strait only reached 17 °C for >30 consecutive days in one year (2019), indicating that temperature conditions may constrain successful larval development to settlement to a greater extent than the degree-day calculations indicate.

Future modelling (UKCP18) suggests that water temperatures from late spring to autumn are likely to increase by around 1.9°C over the next 40 – 50 years, with larger than average changes in late summer and autumn, in line with previous estimates (Olbert *et al*., 2012). Our modelling shows a historical trend for increasing seawater temperatures in the Menai Strait from 1992-2021. Hence it is likely that temperature conditions will move towards the optimal range for reproduction and settlement of *C. gigas* more frequently than currently occurs. Based on the conditions reviewed here for current and retrospective constraints of temperature on reproduction of Pacific oysters, these projections indicate that (i) the proportion of years with cumulative degree-days sufficient to support gonad development, spawning and larval development may increase (ii) the peak summer seawater temperatures may exceed the 18-19 °C threshold required more frequently, to initiate more spawning events (iii) the duration of peak summer seawater temperatures may increase the potential for larval development to settlement. Currently it is difficult to predict the likely scale of these effects over the relatively short term (10-20 years), though this may be attempted as improved climate models become available.

Review of evidence for effectiveness of triploid oysters in reducing risk of recruitment from spawning of stocks held at aquaculture sites

Background

The development of triploid oysters, first reported by Stanley *et al.* (1981), was intended to have several positive outcomes for aquaculture, such as increased growth performance, improved product quality, and sterility or significant reduction in reproduction potential (Guo *et al.*, 2009). In the case of C*. gigas*, farmers may benefit from having marketable oysters in the summer months, without the gonad development and spawning that may occur in diploids. The use of triploids in oyster aquaculture has become widespread and is the norm in growing locations that are non-native for the Pacific oyster, (Degremont *et al*., 2016).

Where non-native oyster species are farmed, the use of cultured triploids has been advocated as a mitigation measure specifically to reduce the risk of establishment of feral populations. Adoption of triploid oysters at existing and new aquaculture sites has the potential to reduce larval supply because they are functionally sterile, thereby reducing any additional impacts from aquaculture production and potential contributions to existing feral populations.

Where new non-native species are being considered for introduction to an environment, elimination of reproductive potential in that stock is desirable and the use of triploids aims to achieve this. Methratta *et al*. (2013) noted that elimination of diploid offspring from the process of producing triploids is not absolute but has a very low probability of diploids being unintentionally introduced to an environment. In this review, we will consider the effectiveness of triploid induction methods in reducing reproductive potential in Pacific oysters.

What are triploid oysters and how are they produced?

Oysters, like most other higher organisms, are diploid (2n) with each cell having two sets of matching chromosomes. Diploid Pacific oysters have 20 chromosomes (Ahmed and Sparks, 1967). Oysters which have an extra set of chromosomes (three sets, 30 in total) are termed triploid, and those with double the normal number (four sets, 40 in total) are termed tetraploids. Oysters with any other chromosome number are termed aneuploids. Triploid and tetraploid oysters tend not to occur naturally, or if they do rarely occur are eliminated because they are functionally sterile. They can be produced in hatcheries, and there are two routes to production of triploids in a hatchery:

i) *Application of physical or chemical interventions to interfere with meiosis during production of gametes (resulting in "induced triploids").*

Sexual reproduction involves the division of a germ cell into four gametes resulting from two fissions of the nucleus in the process of meiosis, which itself is divided into two stages: meiosis

I and meiosis II. In diploid parents, each gamete will have a single set of chromosomes (termed haploid), so that these will combine during fertilisation to form diploid embryos with the same number of chromosomes as their parents. In oysters, and similarly for other molluscs, meiosis is completed after fertilization and a range of treatments can be used to prevent completion of one of the meiosis stages in fertilized eggs, whereby a female gamete can retain a full diploid set of chromosomes that can be combined with the haploid set of chromosomes from the male gametes, resulting in triploid embryos when combined (Stanley *et al*., 1981) (Figure 12). Physical interventions used to induce triploidy include application of high pressure or temperature shock with chemical treatment, including use of cytochalasin B, caffeine and others (Yang *et al*., 2018). Although an option, use of physical and chemical induction is less commonly used in oyster hatcheries because it is not completely effective, with up to 20% of offspring not being triploid and larval survival can be low (Yang *et al*. 2018).

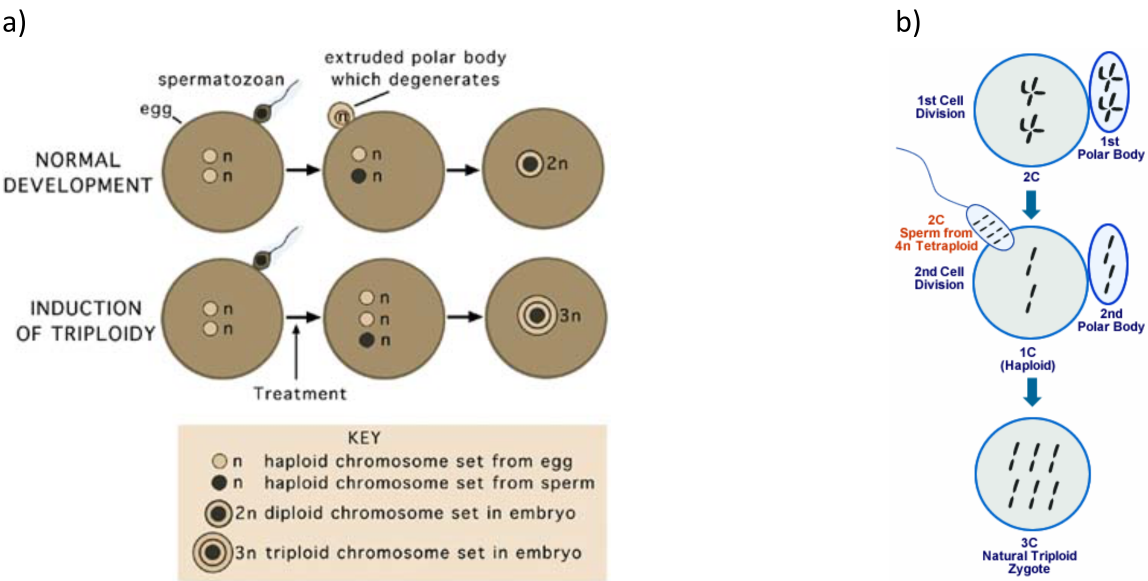

Figure 102 Schematic illustrations of (a) the production of triploid oysters by physical or chemical induction (from Helm et al., 2006), (b) production of " mated" triploids from crosses of tetraploid and diploid oysters (https://theoystersmyworld.com/tag/tetraploid-oysters/)

ii) *Mating of diploid females with tetraploid males (resulting in "mated" or "natural" triploids).*

Tetraploid oysters can be produced by applying a similar approach to the chemical induction described above but applied to eggs stripped from triploid females instead of diploids. When fertilised with haploid sperm, the resulting offspring have four sets of chromosomes (Guo &

Allen 1994a). Once grown to reproductive age, sperm from tetraploid males can be used to fertilise eggs from diploid females, with the resulting offspring being triploids. The process is much more effective than chemical induction, with a 100% reduction in diploid offspring (Guo *et al*., 1996).

How stable are triploids? Evidence of potential for reversion of triploids to functionally reproductive diploids

A range of studies have investigated chromosome instability in triploid oysters and the potential for reversion to a diploid state. While there is evidence for reversion, typically these studies show that individual oysters may have tissue that contains a mixture of triploid, diploid and aneuploid cells (termed "mosaics"). Frequency of occurrence of mosaics tends to be low, for example 0 – 5% of two-year old triploid *Crassostrea ariakensis* were found to be mosaics and these contained only 10% diploid cells (Zou 2002). In Pacific oysters, gill tissue from mosaic individuals arising from reversion of triploids has been found to contain between 19-31 chromosomes, with less than <10% being diploid (Zhang *et al.*2010).

It is important to note that mosaic reversion is very unlikely to result in recovery of reproductive potential, as reversion of a sufficient proportion of gonad tissue to produce haploid gametes would be required. Given the low rate of occurrence of mosaic oysters due to reversion, the low percentage of diploid cells within mosaic individuals and a lack of evidence of production of haploid gametes, the risk of recovery of reproductive potential through reversion of triploids can be considered extremely low or effectively zero (Methratta *et al*., 2013). Also, as presence of mosaic cells within individual oysters develops over time (Zou, 2002), appropriate management and timing of harvest of farmed triploid stocks can reduce any risk even further.

Effectiveness of triploids in reducing reproductive potential

i) maturation and spawning

Triploid oysters can undergo gonad maturation under natural on-farm conditions (Normand, 2008), though studies report a range of values from relatively very low rates of maturation to close to equivalent diploids (Normand *et al*., 2008) and with considerable differences between individuals within the same study (Jouaux *et al*., 2010). There is limited direct evidence of release of gametes by triploid pacific oysters under natural conditions, though there is indirect evidence of successful spawning in some females, based on drops in condition index and gonad histology, with fecundity estimated to be lower (25-80%) than seen in diploid females (Susquet *et al.,* 2016; Normand ~~et al~~ *et al*., 2008). In studies that have directly observed gamete production, egg output in triploids has been reported to be substantially lower, ranging from 0.5% - 13% that of diploids (Guo & Allen 1994b; Gong *et al.,* 2004; Susquet *et al.,* 2016). Sperm output has not been assessed in most studies of triploids, though Susquet *et al.* (2016) found that sperm production was 1.5% that observed in diploids.

ii) reproductive potential of triploid-triploid crosses

Guo and Allen (1994b) showed that while all triploids tested were capable of egg production, survival of fertilized eggs to larval settlement was very low compared to diploid-diploid crosses, in the order of (0.0085% versus vs 21% for diploids). The overall relative reproductive potential of triploids and diploids can be compared as the product of fecundity (egg production per female) and survival of fertilized egg to larval settlement. Hatchery and lab-based studies conducted by Guo & Allen (1994b) and Gong *et al.* (2004) are the most comprehensive investigations of reproductive potential, based on stripping of eggs from females. The relative reproductive potential of triploid-triploid crosses was found to be extremely low, at about 0.0008% of that achieved by diploid-diploid crosses. Based on fecundity and larval survival data from a range of studies, estimates of reproductive potential for triploids are shown in Table 6, ranging from 0.0002% - 0.032% that of diploids.

iii) ploidy of offspring from triploids, and triploids breeding with diploids

Triploid-triploid crosses have been found to produce mostly triploid offspring (90% according to -Guo & Allen 1994b), so that the low rates of successful reproduction described above would also apply to the few offspring that may survive to breeding age The proportion of triploids and diploids in offspring from diploid-triploid and triploid crosses varies between studies. Diploid-triploid crosses resulting in either mostly diploid or mostly aneuploid (intermediate chromosome number) offspring, while triploid-diploid crosses may produce a mixture of triploid, diploid and aneuploid offspring, implying some recovery of reproductive potential in subsequent generations (Guo & Allen 1994a; Gong *et al., 2004*).

iv) potential for cross fertilization of triploid oysters with wild/feral diploids

Where cross-fertilization is achieved in experimental laboratory conditions, Guo & Allen (1994b) showed that breeding between triploid and diploid oysters can occur but that survival to settlement is very low (0.0007% for diploid-triploid female-male crosses, 0.0463% for triploid-diploid). Also under laboratory conditions, Gong *et al.* (1994) found slightly higher reproductive potential in triploid-diploid crosses. Fecundity of triploid females was 13% that of diploids, and relative survival of fertilized embryos to settled spat was 0.035 and 1.57%, respectively, of the normal diploid rate (20%). Based on fecundity and larval survival data from a range of studies, estimates of reproductive potential for triploid cross fertilization with diploids are shown in Table 6, ranging from 0.0003% to 0.18% that of diploids. In addition, reproductive interaction between feral and farmed oysters will depend on proximity and relative population densities (see following section)

Estimation of relative potential reproductive output from farmed triploid oysters (modelled for a farm site stocked with 250,000 oysters)

Based on an example farm stocked with 250,000 triploid oysters (125,000 spawning females), the combination of reduced fecundity and low survival to settlement is estimated to reduce reproductive output of 125,000 farmed female triploid oysters to that of between 0.25 to 40 female diploid oysters, based on the rates defined from several studies (Table 6). Hence, the potential contribution of farmed triploid Pacific oysters to overall reproduction and subsequent recruitment is considered to be very low, and in management terms would need to be compared to the potential reproductive output of feral diploid oysters in any given area.

Table 6 *Summary of experimental studies showing reproductive potential of triploids and crosses between triploid and diploids, showing the relative reproductive potential, and modelled reproductive equivalence (in terms of number of diploid females) for an oyster farm holding 250,000 individuals.*

Cross	Relative fecundity, egg production per ♀	Relative survival to settlement	Relative reproductive potential*	Example farm size (total number of stocked oysters ♀ & ♂)	Modelled reproductive potential of a 250,000 oyster farm (equivalent number of diploid ♀)
Diploid-diploid	100%	100%	100%	250000	125,000
Triploid-triploid	0.5%- 80%[1-5]	0.04%[2]	0.0002% - 0.032%[1-4]	250000	0.25 – 40.5
Triploid ♀ - diploid ♂	2% - 13%[2-3]	0.33%*- 1.5%**	0.007%*- 0.18%**	250000	0.41 – 41.2
Diploid ♀ -triploid ♂	1.5-100% [1-3] (estimated)	0.0003% - 0.03%[2-3]	0.0003%- 0.18%[2-3]	250000	0.38 – 225

[1] Suquet *et al.*2016, [2] Guo & Allen (1994b), [3]Gong *et al.* (2004),[4] Normand *et al.* (2008), [5] Jouaux *et al.* (2010) [6] Methratta *et al.* (2013)

**Note that actual reproductive potential in natural environments will be lower, limited by fertilization success which is determined by population density, distance between spawners and hydrodynamics[6]*

Based on laboratory studies (Table 6) the relative contribution from triploid-diploid interbreeding would be similar, rising to equivalence of 0.38 – 225 diploid females for diploid-triploid crosses. However, the potential for interbreeding between farmed triploid oysters and feral diploids is considered extremely low due to a combination of very low fecundity in triploid oysters and the relatively very sparse occurrence of feral oysters in the Menai Strait. Models for fertilization success between broadcast spawning marine invertebrates show a high dependence on density of parent populations, which may also be expressed as distance between individuals (Levitan, 1991). Modelling of farmed densities of oysters indicates that fertilization rates of around 43% may occur between oysters stocked on trestles at a density of 500 m^{-2} (Methratta *et al.,* 2013). However, this would only apply to oysters that are closely co-located. When applied to oyster populations in natural environmental conditions modelling studies have assumed that an inter-individual distance of <1m is required for effective fertilization (Methratta *et al.,* 2013, for *C. ariakensis*), with field data showing less effective fertilization at an inter-individual distance of >1.5m for native oysters *Ostrea edulis (*Guy *et al.,* 2019*).*- This suggests that the risk of cross-fertilization between wild/feral diploid and triploid farmed oysters at existing sites in Menai Strait is likely to be much lower than indicated by experimental laboratory studies, especially if the area is managed (for example maintaining a buffer zone by clearing any feral oysters from ground around the trestles).

Conclusions

There is no evidence to date of establishment of an invasive population of Pacific oysters derived from aquaculture operations within the Menai Strait. The observed numbers of Pacific oysters are very low and sparsely distributed. Field assessment in 2021 showed the overall number of feral Pacific oysters has declined at a range of sites previously surveyed in 2019 and 1994. This is consistent with a pattern of inconsistent and weak recruitment and survival of Pacific oyster within the Menai Strait, despite the presence of large areas of suitable intertidal habitat and spawning age animals held in aquaculture sites and among the feral oyster population.

Overall, these results confirm a pattern of sporadic, low-level recruitment of Pacific oysters within the Menai Strait. This is potentially combined with a high level of post-settlement mortality, with declines in abundance between surveys. Reproductive success of both aquaculture and feral oysters does not appear to be significant, given the lack of any significant increase in feral oyster numbers over a 27-year period, and more specifically in the last 3-4 years. The survey did not identify any newly settled oysters, with the dominant age being 2-4 years old or more at the study sites.

A key factor in explaining the lack of population growth is likely to be the temperature regime within the Strait. The low density of feral oysters may also limit their reproductive capacity, as an inter-individual distance greater than 1.5m in natural populations of diploid oysters can drastically reduce likely reproductive potential. Also, any successful settlement may be masked by high early mortality in newly settled oyster spat. In our modelling approaches, we have not explored here the potential role of larval mortality and post-settlement mortality in reducing recruitment success, which will also contribute to the low numbers of early age-class oysters. We did, however, include 40% mortality as a correction factor (0.4) as part of our assessment of the previous and current populations in the comparison between surveys in 1998 (CCW, 1998) and our survey at the same sites in 2021, based on Alan and Burnett (2008), although these authors also note potentially higher post-settlement mortality under specific circumstances and this could be a contributory factor in the weak persistence of oyster populations in the Menai Strait.

Using the degree calculation approach, the spring-summer seawater temperatures historically observed and modelled in the Strait are sufficient to support gonad development in Pacific oysters in all but one of the years studied (1992-2019). However, degrees days sufficient for larval development to settlement occurred only in only 4 years, supporting the explanation that sporadic inconsistent spawning and settlement underlies the failure to support population growth.

In-situ microloggers set in silicone sealant encased within a Pacific Oyster shell, showed that higher temperatures (>25 °C) may be experienced by oysters on intertidal trestles, and indeed such peaks in temperature may be important triggers of spawning events.

However, spawning events are likely to be more constrained by peak summer seawater temperatures than by either the cumulative seawater degree days or intertidal temperature spikes. The threshold seawater temperature reported to be required for spawning at other comparable locations in England and France (18 – 20 °C) is rarely reached within the Menai Strait. In only 2 years out of the past 27 years have seawater temperatures exceed the 18 °C threshold required for spawning to occur.

In the longer term, we modelled future seawater temperature based on climate predictions and showed that from 2060 to 2099 it is likely that seawater temperatures would suit reproduction of Pacific oyster in an increasing proportion of years (and potentially in all years). Although climate predictions have a degree of uncertainty and depend on which climate scenario is applied to present data, our data is broadly in line with a report produced by Herbert *et al.* (2012), which suggested the southern UK seawater temperature would permit increased likelihood of spawning, settlement and recruitment by 2040 and to the whole UK by 2080, without being specific on precisely when the temperature threshold will be surpassed on a regular basis. Increasing seawater temperature in the future will permit the naturalised, if not native, populations of Pacific oysters in Europe and currently on the south coast of England, to shift northwards and expand feral populations, irrespective of aquaculture operations. A future increase in recruitment as a result of climate change may result in increased Pacific oyster densities within the Menai Strait, with the potential for reaching a numbers threshold at which cross-fertilisation becomes more successful, further contributing to recruitment success. However, arrival of recruits from elsewhere may be anticipated (not necessarily from aquaculture operations) as Pacific oyster range limits extend northwards and populations expand.

Aquaculture of diploid Pacific oysters in the Menai Strait is not currently expected to contribute significantly to feral populations, as the temperature regime currently does not provide suitable conditions for spawning activity. The risk of aquaculture contributing to feral oyster populations can be further mitigated by use of triploid stocks, that are functionally sterile. The production of triploid oysters from cross-breeding of tetraploid and diploid parents, is highly effective in reducing the reproductive potential for farmed Pacific oysters. They have a very low reproductive capacity of between 0.0002% and 0.032% compared to diploid oysters. On this basis a theoretical farm stocked with 250,000 triploid oysters would have the reproductive capacity equivalent of between only 0.25 and 40 female diploid oysters, which is likely fewer than the current resident population of feral Pacific oysters within the Menai Strait and outside aquaculture sites.

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