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The Thermal Environment of an Inter-tidal Pacific Oyster Farm

W R Turrell, E Dalgarno, G Hermann, J Graham, J-P Lacaze and B Rabe

Marine Scotland Science, Marine Laboratory
375 Victoria Road, Aberdeen, AB11 9DB

Summary

This report describes the opportunistic deployment of recording temperature loggers at the seaward and landward edges of a Scottish inter-tidal shellfish farm between October 2016 and May 2018. The farm, situated in Loch Creran, Argyll, cultures the Pacific oyster *Crassostrea gigas* (Thunberg, 1793). Additionally, the presence of the non-native invasive sea squirt *Didemnum vexillum* (Kott, 2002) has been recorded on the farm since 2015 (Cottier-Cook et al., 2019).

Results presented here confirm that inter-tidal habitats periodically exposed to the air on the Scottish west coast experience greater temperature extremes than sub-tidal habitats which are only exposed to sea temperatures. Minimum temperatures experienced inter-tidally are established by air temperatures and maximum temperatures are the result of air temperature plus *in situ* solar heating.

Oysters, and colonies of the invasive sea squirt, experienced *in situ* temperatures of between -3.63º C and 34.02º C. The largest change of temperature over any 12 hour period was 19.3º C at the seaward edge and 29.1º C at the landward edge of the farm. During the observational period, *in situ* solar heating raised inter-tidal trestle temperatures on the shellfish farm by up to 20º C above air temperature. No widespread mortality was reported in either species during the observed period.

Using tidal data, and some assumptions about the upper and lower trestles, it would appear that the bulk of the shellfish farm lies (in the vertical) between 1.0 m (lower seaward edge) and 3.0 m (upper landward edge) above chart datum. From the tidal records, this would indicate that trestles on the farm are submerged in seawater between 96% (lower seaward edge) and 45% (upper landward edge) of the time over a year (2017). It would appear that both Pacific oysters and *Didemnum vexillum* can tolerate these exposure levels but growth of both species appears enhanced at the shorter exposure times (seaward edge of farm) compared to the longer exposure times (landward edge of farm).

The maximum number of degree-days (with T_{ref} = 10.55º C) in Loch Creran during the observational period was 461 and 510 for the lower and upper trestles respectively, and 449 days for sub-tidal conditions with no air exposure. These are less than the 592 degree-days (T_{ref} = 10.55º C) needed to achieve gametogenesis in Pacific oysters (Eno, 1994). Hence, for the observational period October 2016 to May 2018, thermal conditions in Loch Creran were never suitable for Pacific oyster reproduction.

If the biological reference temperature T_{ref} is reduced to 9º C, then conditions in Loch Creran during the observational period could be categorised as having recruitment potential following the definition used by Syvret et al. (2008). It is not known if thermal adaptation can result in such reduction in the biological reference temperature but the evidence for successful reproduction of Pacific oysters in Scottish waters (e.g. Shelmerdine et al., 2017) suggests that some thermal adaptation may already have occurred.

An alternative to adaptation in reaching the required thermal exposure for recruitment potential is if *in situ* temperatures increased by 1.5º C. The general consensus is that our waters will have warmed by between 1º C and 4º C by the year 2100, but much depends on how society curbs its greenhouse gas emissions (Tinker et al., 2020).

Results presented here suggest that estimating thermal exposure using climatological sea temperatures can underestimate the recruitment potential of Pacific oysters in a region. In Loch Creran in 2017, using monthly averaged sea temperatures underestimated thermal exposure by 18% compared to using measured temperatures as experienced on the farm.

At the Loch Creran shellfish farm in 2017, sea temperatures were suitable all year round for asexual reproduction of *Didemnum vexillum* (ie. >8º C). Sea temperatures were suitable for the production of *Didemnum vexillum* larvae (i.e. >14º C) only in August and September. *Didemnum vexillum* on the upper trestles was additionally exposed to temperatures >14º C while in the air inter-tidally from March to September.

Two research priorities were identified during this study:

1) In order to be able to manage the risk of naturalisation of the Pacific oyster in Scottish waters, a priority research area is understanding the thermal conditions

required for successful reproduction and recruitment using stock already exposed to Scottish conditions.

2) In order to understand the reproductive potential of *Didemnum vexillum* in Loch Creran, research is needed into the potential development of larvae under local conditions, as well as the effect of exposure to high temperatures in air inter-tidally on the sexual development of *Didemnum vexillum*.

Contents

1. Introduction

In 2015, the invasive non-native carpet sea squirt *Didemnum vexillum* (Kott, 2002) was confirmed on an inter-tidal shellfish farm situated in Loch Creran (Figure 1) on the west coast of Scotland (Cottier-Cook et al., 2019). Owing to the concern of the potential spread of *Didemnum vexillum* (D.vex) in Loch Creran, a series of surveys was established by Marine Scotland Science (MSS) of the farm itself (e.g. Turrell et al., 2018) and the surrounding environment (e.g. Brown et al., 2018a,b). The farm cultures Pacific oysters, *Crassostrea gigas* (Thunberg, 1793)[1]. This in itself is a non-native species to Scotland, and there are growing concerns that this species may become naturalised in Scottish waters (e.g. Shelmerdine et al., 2017).

As all invertebrates are ectotherms (i.e. they are dependent on the ambient temperature for their body heat) it was considered important to gather some information on the thermal environment experienced by both oysters and the carpet sea squirt D.vex on the farm. Additionally, it was thought that frequent temperature measurements might reveal details about how the farm was inundated by the tide.

This report describes the temperature data gathered between 2016 and 2018, and presents analyses of these data. Conclusions relevant to both the potential naturalisation of the Pacific oyster in Scottish waters, as well as the potential spread of D. vex in Loch Creran, are developed.

1.1 Pacific Oyster

The Pacific oyster, *Crassostrea gigas*, is native to Japan and east Asia (Jones et al., 2013) and the northwest Pacific (Russia to China, Carrasco and Barón, 2010). It was introduced to British aquaculture in the 1960/70s after studies demonstrated that conditions in UK seas were too cool for it to successfully reproduce (Syvret, 2008; Jones et al., 2013) and that it had a faster growth rate than the native oyster, *Ostrea edulis* (Shelmerdine et al, 2017).

After mussels, Pacific oysters are the second most farmed shellfish species in the UK (Collins et al., 2020). In 2018, Scotland produced over four million Pacific oysters with a value of £1.49 M (Munro and Wallace, 2019). Pacific oyster production represented, by value, approximately 16% of the total Scottish shellfish industry, including farmed mussels and harvested scallops and native oysters. Approximately

[1] A change of name of *Crassostrea gigas* (Thunberg, 1793) to *Magallana gigas* (Thunberg, 1793) was suggested by Salvi et al. (2014). However, this has been subsequently opposed by, for example, Bayne et al. (2017).

41% of the 2018 Scottish oyster production occurred in Argyll, where Loch Creran is situated.

1.1.1 Naturalisation

Despite the earlier indication that the Pacific oyster could not naturally reproduce in UK waters, there is growing evidence that this is no longer the case (Syvret, 2008). For example, in the UK naturalised Pacific oysters are found extensively along the coasts of Kent and Essex, and sporadically along the coasts of south and south-west England (Herbert et al., 2012). In Scotland, there was an early record of Pacific oysters found reproducing in the wild in Loch Sween (Spencer et al., 1994), and a more recent observation in Shetland (Shelmerdine et al., 2017), indicating that reproduction and recruitment is possible under some existing conditions within Scottish seas.

If established in natural habitats, Pacific oysters can cause morphological and ecological changes (e.g. Herbert et al., 2012), as well as economic damage to features such as natural habitats used for recreation (Carrasco and Barón, 2010). For a full review of the potential ecological and economic impact of naturalised Pacific oyster in the UK, and possible implications to nature conservation, see Herbert et al., 2012.

1.1.2 Inter-tidal exposure

In its native habitat and in aquaculture, the Pacific oyster is predominantly an inter-tidal species (e.g. Dunphy et al., 2006; Carrasco and Barón, 2010). Inter-tidal shellfish can be exposed to wide thermal fluctuations, and they show strategies for energy conservation and metabolic change to cope with these.

Carrasco and Barón (2010) recognised the importance of air temperatures as well as sea temperatures when studying the distribution of Pacific oysters globally and their recruitment success. They found that the native range of Pacific oysters lay within the range of sea temperatures of 14.0°C to 28.9°C for the warmest month of the year and from -1.9°C to 19.8°C for the coldest month. However, they found that settlement of oyster spat was successful for air temperatures within the range 15°C and 31° C for the warmest month and between -23°C and 14°C for the coldest month of the year.

1.1.3 Thermal range

The typical thermal range of Pacific oysters was described by Collins et al. (2020). They noted that 2ºC was the minimum temperature for growth to be possible, 20ºC was the optimum temperature for growth, and 27ºC was the maximum temperature for growth to occur.

1.1.4 Thermal dependency of recruitment

Several studies have focused on how the reproductive success of Pacific oysters depends on temperature (e.g. Mann, 1979; Eno, 1994). These studies have identified three phases which must be successfully achieved for recruitment to occur (Table 1).

Table 1

The three recruitment stages of Pacific oyster defined by Eno (1994) in relation to required thermal conditions. Gametogenesis, or the production of gametes, is an essential stage in oyster reproduction.

Stage	Biological process	Thermal Requirement	Regime
Stage 1	Achieve gametogenesis	Experience 592 degree-days above 10.55º	Sub-Tidal or Inter-tidal
Stage 2	Initiate spawning	Reach 18ºC	
Stage 3	Larval development prior to settlement	Experience 350-400 days above 10.55ºC	Sea

In Table 1, the parameter degree-day (${D_{day}}^{Tref}$) at time *t* is defined as:

$$ {D_{day}}^{Tref}(t) = \sum_{0-t} d.\ (T(t) - T_{ref}) $$

Where:

t = time in days from 1st January 00:00
d = measurement interval of $T(t)$ in days
$T(t)$ = *in situ* temperature at time t
T_{ref} = reference temperature

Note that using this definition of thermal exposure, a value in units of degree-days must always be cited along with the value of the reference temperature T_{ref}, otherwise it cannot be interpreted or reproduced.

Eno (1994) included times when inter-tidal oysters were exposed to air in her Stage 1 degree-days calculations, and also noted that the rapid thermal variations that oysters experience when exposed to air may contribute to triggering spawning once gametogenesis has been achieved as they provide the “thermal shock” that is needed in laboratory experiments to induce spawning.

Once spawned, Pacific oyster larvae are pelagic for between two to four weeks (Herbert et al., 2012) until the final larval stage which settles onto the foreshore or seabed and develops a hard shell that develops into a juvenile oyster. Hence, the thermal requirements of larval development (i.e. Stage 3) must be achieved within the sea.

While Eno (1994) suggested a minimum of 350 degree-days above 10.55ºC, Syvret et al. (2008) suggested it could be as short as 225 degree-days, and noted that this figure, and the associated trigger temperatures, were based on a few laboratory-based studies and need further work to confirm. They went on to define four recruitment conditions, each with different thermal requirements (Table 2).

Table 2
The four recruitment conditions used by Syvret et al. (2008) to assess different thermal regimes in UK waters and their related thermal requirements.

Condition	Recruitment Condition	Thermal Requirement	Regime
1	Conditioning Potential	600 degree-days relative to 10.55ºC	Sub-Tidal or Inter-tidal
2	Spawning Potential	600 degree-days relative to 10.55ºC attained by July to September	
3	Recruitment Potential	Condition 1 reached + 225 degree-days relative to 10.55ºC	Sea
4	High Recruitment Potential	Condition 2 reached + 225 degree-days relative to 15ºC attained by July to September	Sea

1.1.5 Climatological v. *in situ* temperatures

Eno (1994) used *in situ* measured temperatures recorded every 96 minutes, while Syvret et al. (2008) used regional monthly average temperatures obtained from published values and assumed these temperatures applied constantly to each 24-hour period of each respective month. These values were of variable accuracy, coming from a variety of sources around the UK including satellite-based data and long term monitoring data collated in regional climatologies. Such climatological data is often all that is available for a region, and was used for example by the study of Carrasco and Barón (2010) of the thermal range of oysters globally.

1.1.6 Other factors affecting growth and recruitment

While the thermal requirements described above are necessary for successful growth and/or recruitment, they are not sufficient, as other factors such as food availability, salinity, oxygen content etc. are also required to be favourable (e.g. Syvret et al., 2008; Malham et al., 2009). Disease can also be a factor in the life history of oysters, although the occurrence of disease and its impact itself can also be altered by temperature (Carrasco and Barón, 2010).

In Loch Creran, salinity variations are not considered an issue as Pacific oysters have been found to tolerate salinities from 15 to 44 psu (Malham et al., 2009). No reported issues of eutrophication have yet come from Loch Creran, and it has a largely upland catchment area devoid of urban development or intensive agriculture (e.g. Gowen et al., 1992).

1.2 *Didemnum vexillum*

Didemnum vexillum (D.vex) is a colonial ascidian tunicate, commonly known as a carpet sea squirt. It is characterised by a tough outer rubbery covering (it’s “tunic”) made from polysaccharide cellulose. Tunicates are filter feeders, sucking in water through one opening and expelling it through another. D.vex feeds on phytoplankton and suspended organic matter (Fletcher et al., 2013). Its colonies have been described in some areas of the world as “growing aggressively on all manner of substrates” (Carman et al., 2009). It can smother other sessile species, modify habitats it colonises and foul man-made structures, infrastructure and vessels.

1.2.1 Reproduction

D.vex can reproduce both sexually and asexually (Griffiths et al., 2009). Sexual reproduction involves the release of “tadpole-like” larvae into the plankton community. Larvae are about 0.6 mm long when immature and attached to a yolk mass. They are brooded within the tunic of the colony beneath the zooids (Fletcher and Forrest, 2011). It can take several weeks for larvae to fully develop and once released into the sea, the free-swimming larvae are about 1.4 mm long (Fletcher and Forrest, 2011). Larvae do not feed, and must attach to a suitable substrate within a few hours of release (Griffiths et al., 2009). Larval dispersion and subsequent settlement of D.vex over a distance of hundreds of metres up to several kilometres has been reported (Fletcher et al., 2013).

In a study in New England, larvae were only released when the temperature rose above 14-20°C (Valentine et al., 2009) and then continued to be released for 3.5 to 5 months even when temperatures dropped below these levels (Valentine et al., 2009). However, no larvae were released when temperatures dropped to 9-11°C (Valentine et al., 2009).

Valentine et al. (2009) thought that larvae were only released after a certain period of development rather than when a specific temperature was reached. Hence newly settled colonies may be releasing larvae at different temperatures under different environmental conditions. The period when recruitment via larvae is possible is dependent on when the development of larvae commences, how long it takes, and how long environmental conditions are right for them to survive (Valentine et al., 2009).

Asexual reproduction

Torn off fragments from many colonial marine invertebrates (e.g. sponges, corals, gorgonians, sea squirts) can remain viable, resettle onto a suitable substrate, and colonise new locations (Bullard et al., 2007), and D.vex is no exception. D.vex has been found on drifting flotsam, such as eelgrass rafts (Carman et al., 2014). Vercaemer et al. (2015) think floating fragments may have spread D.vex across Georges Bank, although this could also have been the result of vessel and offshore equipment (including marine renewables) movements. Storms can result in fragments being released from D.vex colonies (Carman et al., 2014), as can wave action (Reinhardt et al., 2012) and any human intervention such as mechanical cleaning.

Fragments of D.vex have also been observed to repair the torn edge of the fragment while drifting in suspension, and change shape (e.g. into spheres) possibly to aid dispersion (Morris and Carman, 2012). They suggested that even one or two zooids are capable of settlement and successful colonisation, and hence they emphasise the importance that cleaning operations do not release fragments of D.vex, however, small.

1.2.2 Environmental range

D.vex can tolerate a wide range of environmental conditions. Table 3 summarises some of the temperature ranges found in published studies. Globally, D.vex has been found in temperatures of -1°C to 25°C (Valentine et al., 2009).

Colonies “degenerate and all but disappear” in very cold conditions, but then “regenerate” when temperatures warm (Valentine et al., 2009). The degree to which they are “knocked back” by cold temperatures determines how quickly they can regenerate and reproduce when temperatures warm again (Valentine et al., 2009).

Fletcher et al. (2013) make the point that the response of D.vex to temperature (in absolute terms rather than in terms of temperature change) seems to be inconsistent between regions globally, and sometimes even within regions. They, therefore, conclude factors other than simple water temperature are responsible for the seasonal cycle D.vex undergoes within a region, including growth, sexual reproduction, and degeneration. They suggest light, nutrients, food availability and dissolved oxygen may all play a role, although the seasonal cycle of temperature is often a proxy for the seasonal cycle in these other factors.

In terms of salinity, Gröner et al. (2011) performed extensive laboratory experiments looking at the tolerance of D.vex to short term and long-term exposure to low salinities of TEN. D.vex, as an invasive species, showed higher growth and survival when exposed to low salinities than a native tunicate, both obtained from UK waters. However, Evans and Huntington (2005) suggest that ascidians in general have limited tolerance of low salinities, and hence they selected freshwater as a possible control treatment for D.vex.

1.2.3 Inter-tidal

D.vex may also be tolerant of short periods of low oxygen (Lambert, 2009). Conversely, it can tolerate up to six hours in air (Carman et al., 2010), although its survival in air also depends on factors such as humidity and temperatures, surviving longer in humid or cooler conditions. Hence D.vex can tolerate both sub-tidal (e.g. Georges Bank, Valentine et al., 2007a) and inter-tidal (e.g. rock pools, Valentine et al., 2007b) conditions.

1.3 Loch Creran

Loch Creran is a typical fjordic sea loch on the Scottish west coast (Figure 1, Landless and Edwards, 1976). As a side loch of Loch Linnhe it connects to the more exposed wider loch at the seaward western boundary. It is 12.8 km in length and is comprised of four basins, each separated by a shallow sill (Edwards and Sharples, 1985). It has a maximum depth of 49 m and a flushing time of around three days.

Loch Creran is designated as a Special Area of Conservation under the Habitats Directive (JNCC, 2020). The site was designated owing to the presence of biogenic reefs of the calcareous tube-worm *Serpula vermicularis*. These occur in shallow water around the shores of the loch. The species has a world-wide distribution but the development of reefs is extremely rare, and the reef in Loch Creran is the only known example in Europe. There are also reefs of the horse mussel *Modiolus modiolus*, and areas of bedrock reef, which support further species-rich assemblages.

The shellfish farm in Loch Creran where the temperature measurements were made is located on the southern shore of the most seaward basin (Figure 3).

1.3.1 Shellfish farm structure

The basic element of the farm layout is the table, a structure made from steel bar approximately 3 m long and 1.5 m wide (Figure 2a). Oysters are placed within bags made from plastic meshing. Bags are laid across a table, or slung in a row beneath a table (Figure 2b). Tables are lined up generally perpendicularly to the sea in rows of between three and six tables placed end-to-end. A row of tables is referred to as a trestle (Figure 2b). Trestles are typically grouped in blocks, with four trestles in a block being typical (Figure 2c). Finally, blocks of trestles are placed in rows parallel to the water's edge, separated by walk ways and roadways.

The satellite image in Figure 3 shows blocks of trestles organised into three main rows parallel to the water's edge at low tide, but with additional blocks also placed throughout the foreshore area. The farm location was chosen as it lies within a broad bay with a gently shelving seabed composed of soft but serviceable sediment, which is exposed at spring low tides.

Table 3

A selection of various thermal limits and ranges for other species relevant to Loch Creran

Temperature (°C)	Condition	Reference
	Native oyster *Ostrea edulis*	
15	Minimum for settlement and growth of larvae	Collins et al. (2020)
25-30	Optimum temperature for settlement and growth of larvae	Collins et al. (2020)
18-22	28-56 days to condition oysters for reproduction	Syvret et al. (2008)
	Salmon	
10-18	Good salmon growth	Collins et al., 2020
6	Minimum for salmon growth	Collins et al., 2020
	Mussels	
17-21	Optimal survival of *Mytilus edulis* larvae	Collins et al., 2020
12-16	Condition mussel prior to reproduction	Syvret et al. (2008)
5	Threshold temperature to undergo gametogenesis	Syvret et al. (2008)
	Didemnum vexillum	
-1-25	Temperature range D.vex found to tolerate in a review of global occurrence	Valentine et al. (2009)
> 8-12	Asexual budding initiated	Valentine et al. (2009)
14-20	Release of larvae and settlement of larvae	Valentine et al. (2009)
	Diseases and Parasites	
>16	*OSHV–1 μvar* causes mortality in oysters above this temperature	Collins et al. (2020)
13.5	*Vibrio* numbers increased in oysters above 13.5°C	Collins et al. (2020)
12-20	*Bonamia ostreae* prevalence is most likely	Collins et al. (2020)
4-8	*Paramoeba perurans* population growth occurs	Collins et al. (2020)

Figure 1: Location of Loch Creran in relation to the Scottish west coast. For this study tide gauge data was obtained from Tobermory, and meteorological data from Stornoway airport.

Figure 2\: Oyster farm components. a) two unused tables stacked together showing their construction, b) a single trestle consisting of three tables placed end-to-end, c) a block of four trestles, each of three tables in length, d) the whole farm at low tide

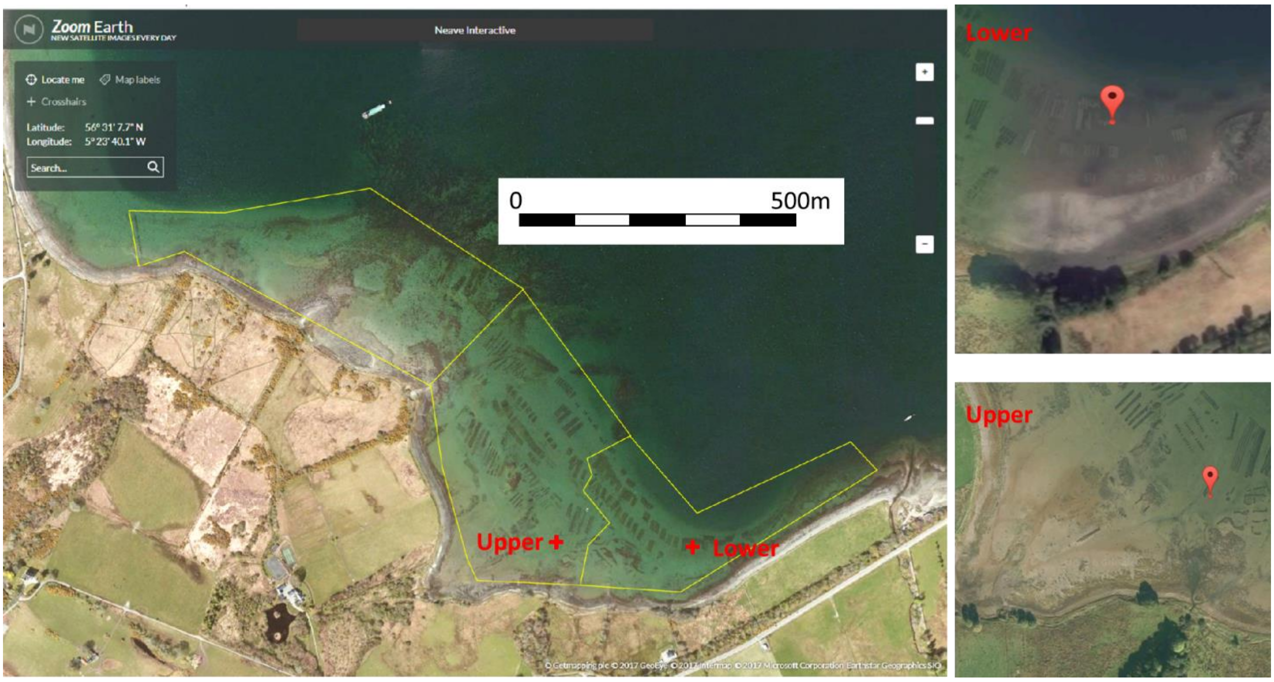


Figure 3: Location of two *in situ* temperature loggers on the shellfish farm. Left figure – overall position on farm. Upper right – detailed position of lower trestle. Lower right – detailed position of upper trestle. (All satellite images ©2018 Google. Data SIO, NOAA, US Navy NGA, GEBCO, Map Data ©2018 Google).

2. Methods

2.1 *in situ* Temperature

in situ temperature data was obtained using small self-recording digital temperature loggers (Vemco, 2020) mounted on two selected trestles within the shellfish farm, one at the seaward edge of the farm, and hence lower down the sloping foreshore, and the other near the landward edge of the farm, hence higher up the foreshore. Thus the lower logger was submerged for longer periods by the ingress of the tide than the upper logger. Table 4 gives the positions of the loggers, Figure 3 shows their location within the farm and Figure 4 shows views of the deployment locations.

Table 4
Positions of the two *in situ* temperature loggers.

Trestle	Latitude	Longitude
Lower Trestle	56° 30.6588’N	05° 22.9345’W
Upper Trestle	56° 30.6637’N	05° 23.1735’W

The relevant specifications of the loggers, as quoted by the manufacturer (Vemco, 2020) were as follows:

Temperature Range:	-30°C to +80°C
Temperature Accuracy:	± 0.1°C from -5°C to 35°C
Resolution:	0.01°C
Physical size:	Length 9.8 cm, diameter 2.3 cm, weight: 52.2 g
Clock Stability:	±1 minute per month

Table 5 presents the start and end dates of the two instrument deployments used in this study. The first deployment took place in October 2016 and the instruments were recovered in June 2017, and the second deployment lasted from May 2017 to May 2018. There was, therefore, a short overlap period in May and June 2017 (Table 5) when each trestle had two recorders present, and this period permitted some inter-comparisons and checks to take place.

Table 5
Start and end times of the two deployments, and the period these two data sets overlap with one another.

Data	Start		End		N Data
16 – 17	16:05	18/10/2016	09:00	26/06/2017	72,204
17 – 18	17:10	25/05/2017	08:00	16/05/2018	51,210
Overlap	17:10	25/05/2017	09:00	26/06/2017	

The logging interval for the 2016 deployment was five minutes, whereas it was ten minutes for the 2017 deployment. In order to allow the data to be merged, the 2016 record was converted to a ten minute logging interval by averaging consecutive readings using post-processing software (see data processing section below).

Figure 4: Views of the *in situ* temperature loggers and their locations. a) Detail of temperature logger attachment on upper trestle; b) view shore-wards along lower trestle; c) view shore-wards along upper trestle.

2.2 Tidal data

Hourly tidal height data was obtained from the standard UK tide gauge at Tobermory (56.62311ºN, 6.06422ºW) on the Isle of Mull (Figure 1). Data was obtained from the British Oceanographic Data Centre.

Analysis from published tide tables revealed a time difference of low water (LW) and high water (HW) at Tobermory and the head of Loch Creran of ten minutes and one hour respectively during spring tides. During neap tides, the time differences were between -6 and +20 minutes (Table 6). Tobermory lies at the northern end of the Sound of Mull, which connects to the southern end of the Loch Linnhe system; the distance between Tobermory and Loch Creran is about 50 km.

2.3 Meteorological data

Air temperature and sunshine duration were obtained from the UK Met Office coastal station at Stornoway airport (Met Office, 2019; 58.213ºN 6.319ºW, elevation 15 m). This was the closest station with a full set of hourly data, concurrent with the *in situ* temperature data. Stornoway airport is approximately 180 km north of Loch Creran, and 60 km to the west. Owing to the spatial scale of weather systems at this latitude, weather experienced at Stornoway will be representative of that experienced at Loch Creran.

2.4 Data analysis

Data analysis was performed using bespoke programmes written in the "R" language (R Core Team, 2019). Data was first complied into a single environmental data file, with a 10 minute record interval, with format:

$Y(t), M(t), D(t), H(t), Minute(t), DecYear(t), T_{upper}(t), T_{lower}(t), H(t), T_{air}(t), Sun(t)$

Where

t – time of recording

$Y(t)$ – year of recording

$Month(t)$ – month of recording

$Day(t)$ – day of recording

$Hour(t)$ – hour of recording

$Minute(t)$ – minute of recording

$DecYear(t)$ – time of recording expressed as decimal year

$T_{upper}(t)$ – *in situ* temperature at time t at upper trestle (º C)

$T_{lower}(t)$ – *in situ* temperature at time *t* at lower trestle (º C)

H(t) – height of the tide at Tobermory at time *t* (m)

$T_{air}(t)$ – air temperature at Stornoway airport (º C). Ten minute values interpolated from hourly values in original data.

Sun(t) – duration of sunshine at Stornoway airport (hours). Ten minute values interpolated from hourly values in original data.

In this file, the overlap period was removed and hence data was in ten minute intervals, without break, between 16:10 18/10/2016 to 08:00 16/05/2018.

Table 6

Predicted time and height differences between Loch Creran Head and Tobermory, from published tide tables

	Date	Range (m)	HW		LW		HW		LW	
			Time (HH:MM)	H (m)	Time (HH:MM)	H (m)	Time (HH:MM)	H (m)	Time (HH:MM)	H (m)
Spring Tides										
Winter	19/01/2020	3.0	+01:01	-0.3	+00:13	-0.7	+00:56	-0.3	+00:12	-0.7
Spring	02/04/2020	4.2	+00:57	-0.2	+00:14	-0.7	+01:04	-0.3	+00:12	-0.7
Summer	06/07/2020	3.2	+00:57	-0.3	+00:14	-0.7	+01:01	-0.3	+00:13	-0.7
Autumn	02/10/2020	3.3	+00:54	-0.3	+00:14	-0.7	+00:56	-0.3	+00:14	-0.7
Neap Tides										
Winter	26/01/2020	1.9	-00:08	-0.7	+00:00	-0.4	-00:06	-0.7	+00:04	-0.3
Spring	09/04/2020	1.0	+00:07	-0.4	-00:04	-0.7	-00:01	-0.4	-00:06	-0.7
Summer	13/07/2020	1.4	+00:13	-0.4	-00:06	-0.7	+00:12	-0.4	-00:09	-0.7
Autumn	10/10/2020	1.2	+00:19	-0.5	-00:04	-0.7	+00:09	-0.4	-00:04	-0.7

2.5 High water (HW) and low water (LW) sea temperatures

Times of high water (i.e. maxima in *H(t)*) and low water (i.e. minima in *H(t)*) in the Tobermory tidal height data were extracted. At these times values of other parameters (i.e., *in situ* temperature and tidal height) were extracted (Table 7).

2.6 Filtered air temperature

A reference data set for typical air temperature during the *in situ* logger deployments was needed, noting the geographical separation of Stornoway airport from Loch Creran. To provide this, the hourly meteorological data was filtered using a 48-hour wide moving average in order to remove high frequency variability, and to give an idea of general air temperature in the region. The filter resulted in ten minute values of the Stornoway air temperature averaged over the previous 48 hours prior to (and including) the record time *t* ($T_{air}^{FLT}(t)$).

Table 7
Parameters extracted from the environmental data set at high water (HW) and low water (LW).

Parameter	High Water Values	Low Water Values
Date	$Y^{HW}(t)$ $M^{HW}(t)$ $D^{HW}(t)$	$Y^{LW}(t)$, $M^{LW}(t)$, $D^{LW}(t)$
Time	$H^{HW}(t)$ $Min^{HW}(t)$,	$H^{LW}(t)$ $Min^{LW}(t)$,
Decimal Time	$DecYear^{HW}(t)$	$DecYear^{LW}(t)$
in situ Temperature	$T_{upper}^{HW}(t)$ $T_{lower}^{HW}(t)$	$T_{upper}^{LW}(t)$ $T_{lower}^{LW}(t)$
Tobermory Tidal Height (m)	$H^{HW}(t)$	$H^{LW}(t)$

2.7 Monthly average sea and air temperature

In order to examine the background cycles of average air and sea temperatures, a moving centred 28 day filter was applied to Stornoway air temperatures ($T_{air}(t)$) and to Loch Creran sea temperatures, using $T_{lower}^{HW}(t)$. The resulting data sets were monthly filtered air temperature $T_{air}^{MNT}(t)$ and sea temperature $T_{sea}^{MNT}(t)$. Additionally, averages for the calendar months of 2017 were calculated.

2.8 Temperature and tidal height frequency diagrams

Frequency diagrams were used to examine the range of variability in *in situ* temperature, air temperature and tidal heights. These diagrams show the proportion of each record that exists between certain ranges of parameter values. Typically the overall extreme range of a parameter is sub-divided into 20 sub-divisions and the proportion of the record in each sub-division estimated. The proportion is presented as a decimal fraction of one.

The frequency analysis involving temperature (sea, air or *in situ*) uses data from only one full calendar year to avoid aliasing issues for part years. The frequency analysis for tidal height uses all available data.

2.9 Thermal exposure – degree-days

The definition of degree-days as defined by Eno (1994) was used, as described in Section 1.1.4 above. Various values of T_{ref} and sources of $T(t)$ were used, as described in the results section below.

3. Results

3.1 Temperature data overlap period

The temperature records of $T_{lower}(t)$ *and* $T_{upper}(t)$ are virtually indistinguishable during the overlap period (Figure 5). This provides reassurance that the detailed position of a logger within the structure of a trestle was not of critical importance, and that recorded temperatures were representative of the environs of a trestle rather than very specific points within a trestle/oyster bag structure.

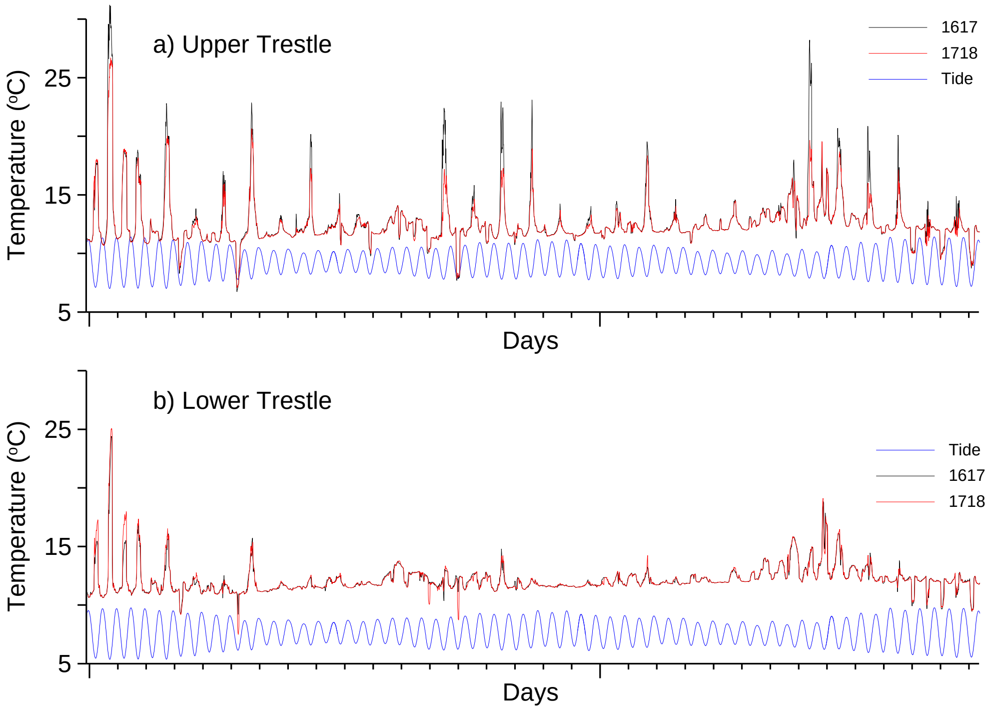


Figure 5: Comparison of the two deployment records, i.e. the 2016 deployment (*1617* – black line*)* and the 2017 deployment (*1718* – red line) during the overlap period. a) $T_{upper}(t)$, b) $T_{lower}(t)$. Values for tidal height *H(t)* are also shown (blue line). Horizontal axis is *DecYear(t).*

3.2 Environmental data set

Figure 6 presents time series of air temperature, sunshine hours, tidal height and *in situ* temperatures during the whole deployment period.

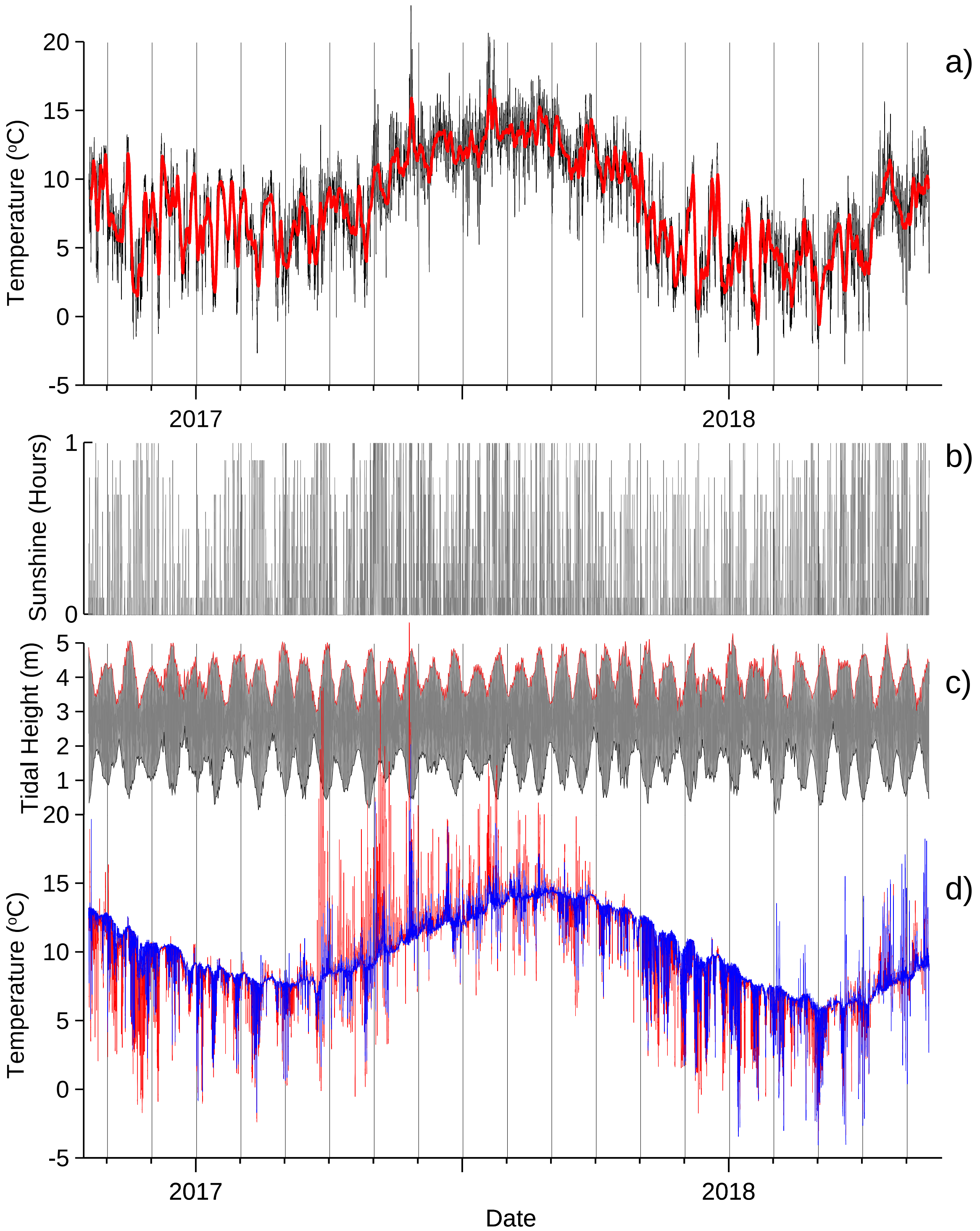


Figure 6: Summary plot of the environmental data. Panels from top to bottom: a) air temperature: black line - $T_{air}(t)$, red line $T_{air}^{FLT}(t)$; b) Sunshine hours (*Sun(t))*; c) Tidal height *H(t)* in grey, also shown are values of $H^{HW}(t)$ (red) and $H^{LW}(t)$ (black); d) *in situ* temperatures red line $T_{upper}(t)$, blue line - $T_{lower}(t)$. Horizontal axis shows deployment date(*DecYear(t)).*

3.3 *in situ* temperatures - High water (HW) and low water (LW)

In total, there were 1,111 high waters and 1,110 low waters over the record period (i.e. 18/10/2016 to 16/05/2018). Figure 7 shows the *in situ* temperature values at the upper and lower temperature loggers as recorded every ten minutes, compared to the values extracted for every high water. Figure 8 shows the same, but for low water values. Figure 9 presents the temperature differences at high water and low water between the upper and the lower *in situ* temperature loggers. Note that a negative difference implies that the temperature at the upper logger was cooler than at the lower logger.

As there was a time lag of up to one hour between Tobermory and Loch Creran, the values of $T_{upper}^{HW}(t)$, $T_{lower}^{HW}(t)$ and $T_{upper}^{LW}(t)$, $T_{lower}^{LW}(t)$ were not extracted exactly at the time of local high or low water at the shellfish farm. However, for high water the maximum ±1 hour difference most likely made little difference in water level, remembering the twelfths rule[2], and it was assumed that both the upper and lower trestles would have been fully submerged at these times. Similarly at low water, it was assumed that on most tides both loggers were exposed to the air (see section on farm inundation below).

A few summary statistics of the high water and low water temperature differences between the upper and lower loggers are presented in Table 8.

Table 8

Summary statistics of temperature differences between upper and lower temperature loggers at high and low water. Note that a negative difference implies that the temperature at the upper logger was cooler than at the lower logger.

Value	High Water	Low Water
Minimum difference	-3.66ºC	-10.07ºC
Maximum difference	+1.83ºC	+18.43ºC
Average difference	-0.05ºC	-0.32ºC

[2] The “twelfths” rule is an approximate rule of thumb that describes how water level change over a tidal period. From HW to LW the water level drops by 1, 2, 3, 3, 2, and 1 twelfths of the tidal range in the first, second, third, fourth, fifth and sixth hour of the falling tide respectively.

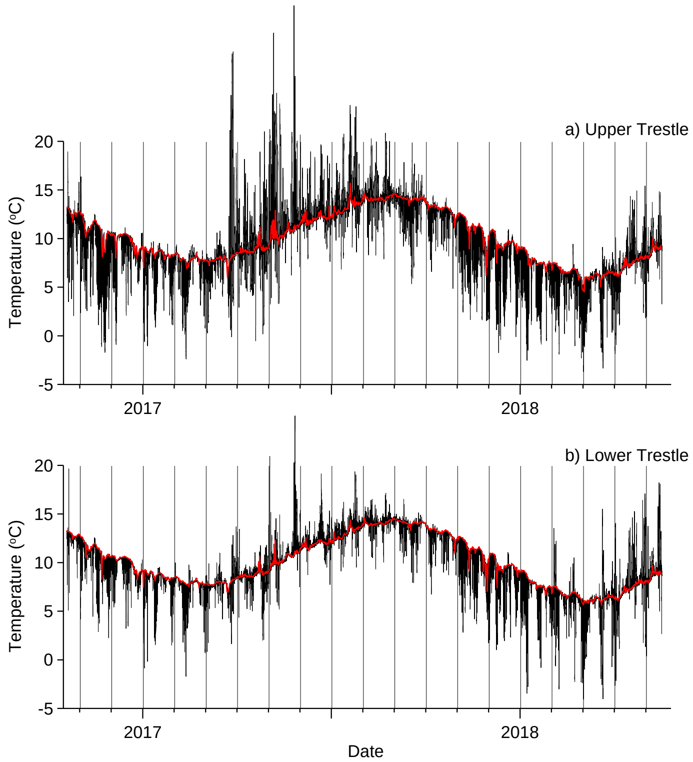


Figure 7: Comparison of the temperature on each trestle with the values extracted at high water, Tobermory (HW). a) Upper trestle, black line - $T_{upper}(t)$; red line - $T_{upper}^{HW}(t)$. b) Lower trestle, black line – $T_{lower}(t)$; red line – $T_{lower}^{HW}(t)$.

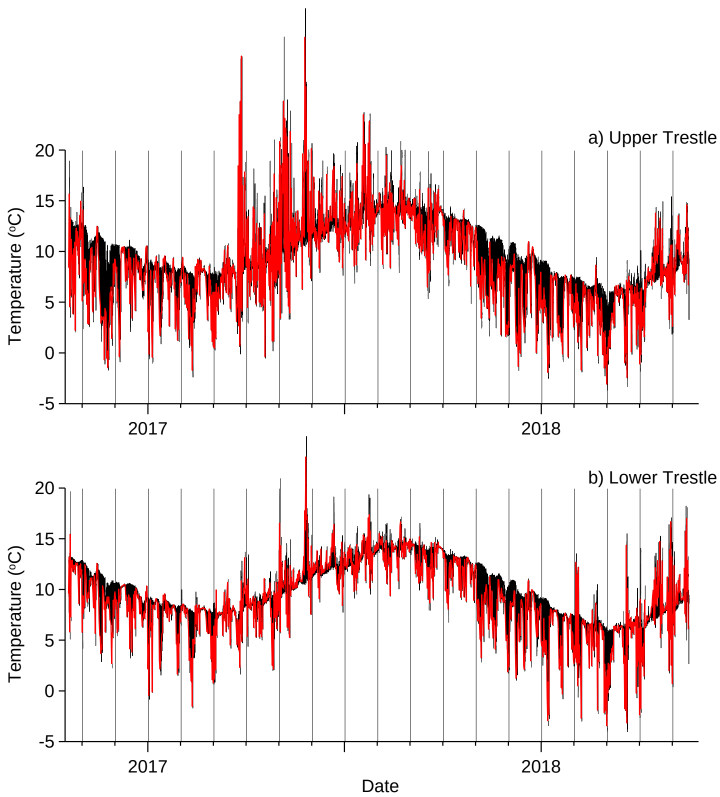


Figure 8: Comparison of the temperature on each trestle with the values extracted at low water, Tobermory (LW). a) Upper trestle, black line - $T_{upper}(t)$; red line - $T_{upper}^{LW}(t)$. b) Lower trestle, black line – $T_{lower}(t)$; red line – $T_{lower}^{LW}(t)$.

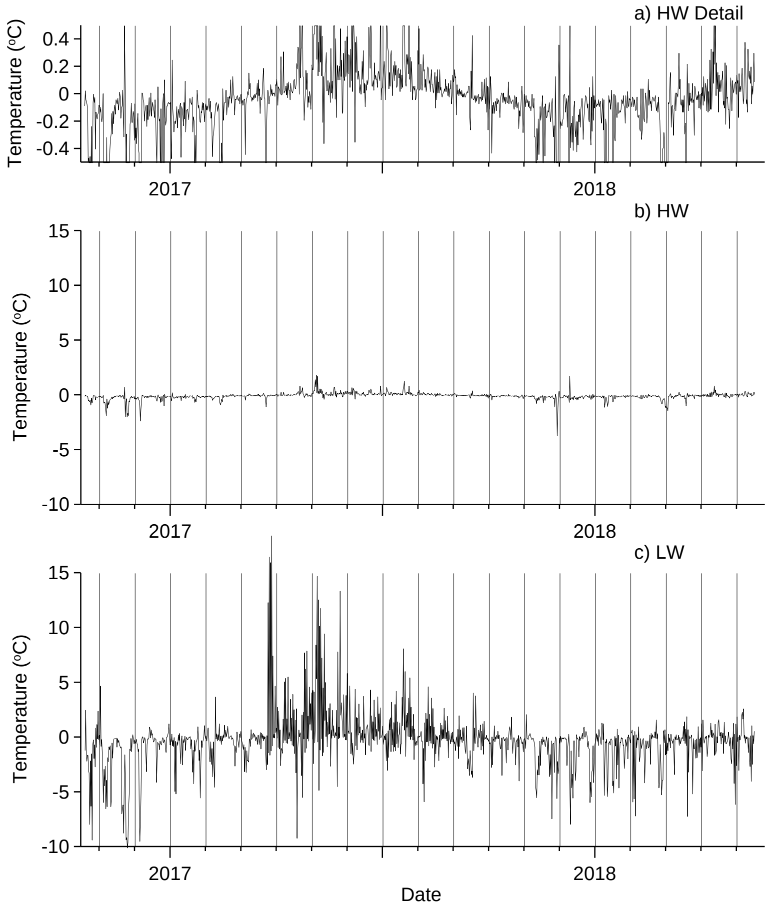


Figure 9: The temperature difference between the upper and lower *in situ* temperature loggers at high water (a, b) and low water (c). Figure a) provides an enhanced view of figure b) – note vertical scale. Data clipped to axis.

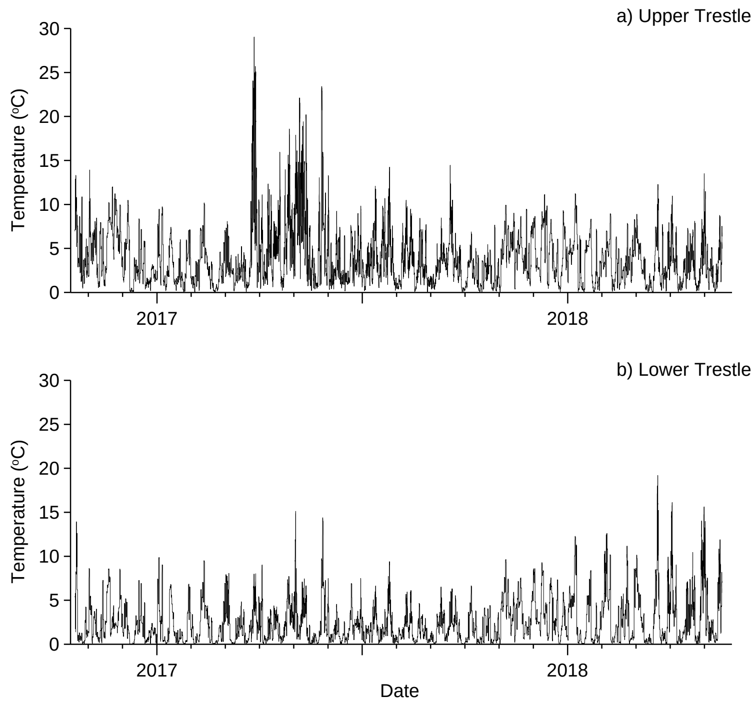


Figure 10: The maximum *in situ* temperature difference each trestle experienced in any one 12 hour period.

3.4 *in situ* temperatures – Changes over 12 hours

Figure 10 presents the maximum *in situ* temperature difference each trestle experienced in any one 12 hour period.

3.5 Air temperature – Filtered values

Figure 11 shows the filtered air temperature ($T_{air}^{FLT}(t)$) compared to the unfiltered air temperature ($T_{air}(t)$). It can be seen that the high frequency variability has been smoothed.

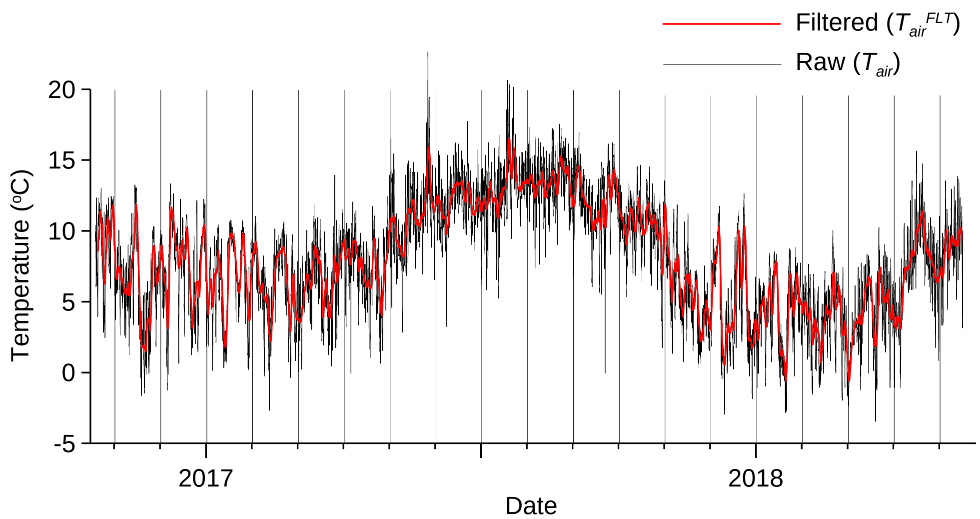


Figure 11: Comparison between the hourly air temperature at Stornoway ($T_{air}(t)$ – black line), and the filtered air temperature ($T_{air}^{FLT}(t)$ – red line).

3.6 Air and sea temperatures – Annual cycles

Figure 12 presents values of monthly filtered air temperature $T_{air}^{MNT}(t)$ and sea temperature $T_{sea}^{MNT}(t)$ and Table 9 presents monthly mean values for sea, air and *in situ* temperatures for the year 2017.

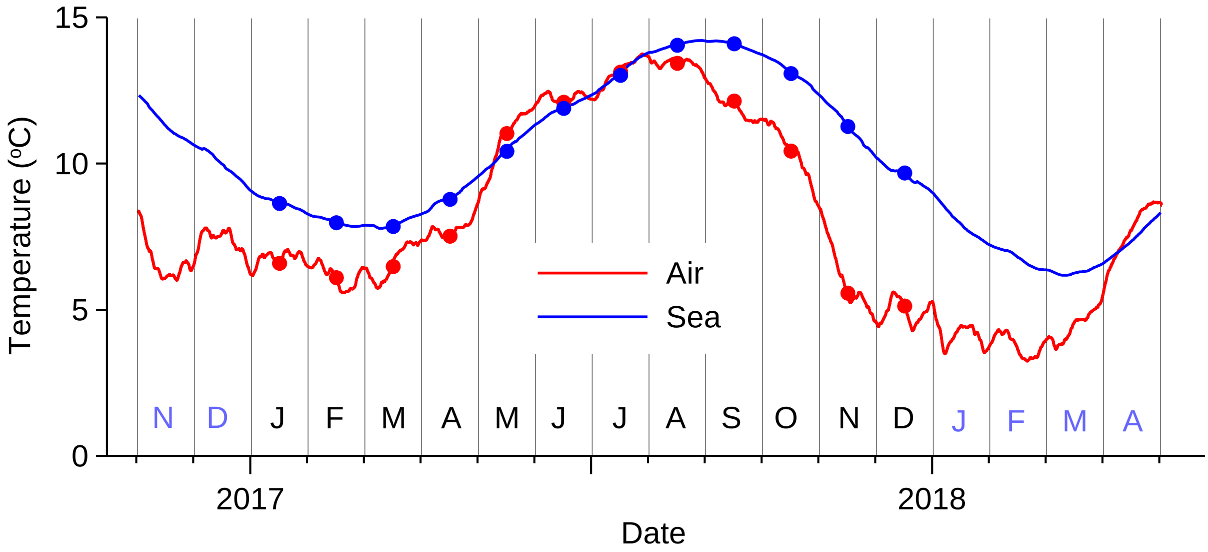


Figure 12: Monthly filtered air temperature $T_{air}^{MNT}(t)$ (red) and sea temperature $T_{sea}^{MNT}(t)$ (black). Symbols indicate monthly averages from Table 9.

3.7 Frequency diagrams – temperature and tidal height

Figure 13 presents the frequency diagram for sea (T_{lower}^{HW}), air, and upper and lower trestle *in situ* temperatures for the calendar year 2017. Figure 14 shows a similar analysis for HW (H^{HW}) and LW (H^{LW}) tidal heights, i.e. the tidal heights extracted by the high water and low water routines described in Section 2.5, but using all available data.

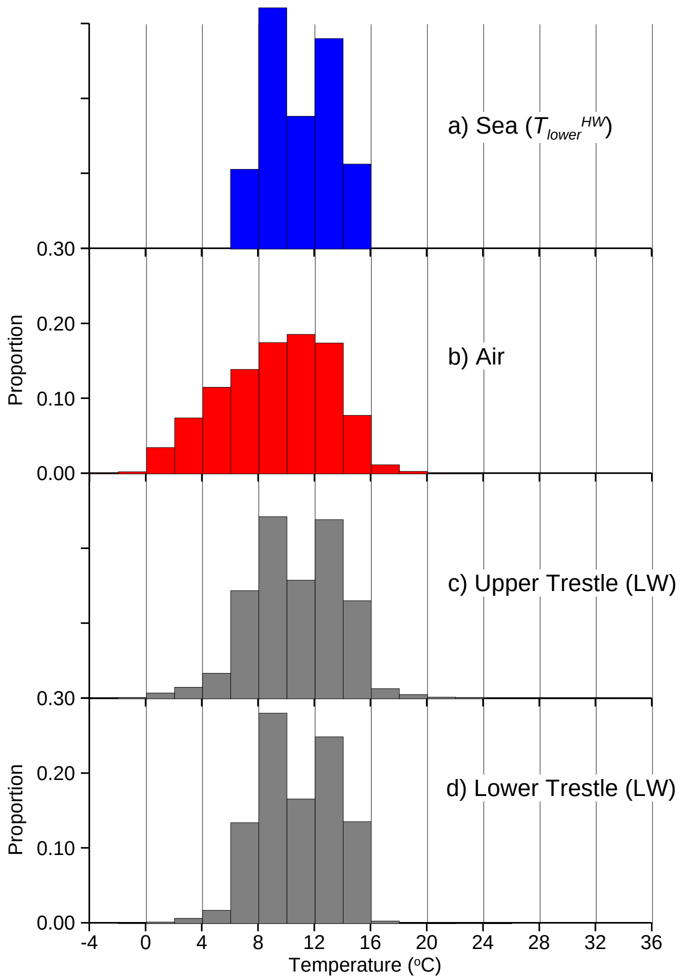


Figure 13: Frequency diagram for the calendar year 2017 a) sea (T_{lower}^{HW}), b) air, c) upper and d) lower trestle *in situ* temperatures.

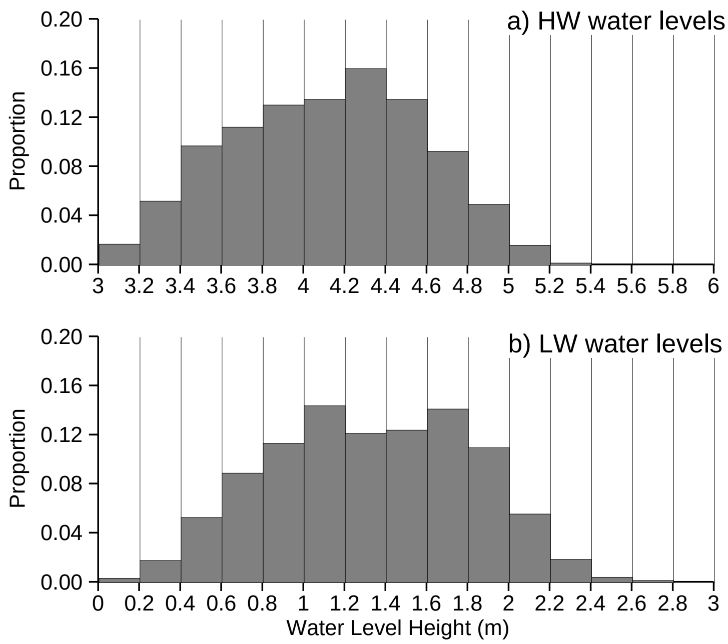


Figure 14: Frequency diagram for HW (H^{HW}) and LW (H^{LW}) tidal heights, i.e. the tidal heights extracted by the high water and low water routines described in Section 2.5.

3.8 Summary statistics

Table 10 presents summary statistics for the basic data sets described above over the full observational period 18/10/2016 to 16/05/2018.

3.9 Thermal exposure – Degree-days

Figure 15 presents the evolution of degree-days using the definition of Eno (1994), and the reference temperature of 10.55º C (See Section 1.1.4).

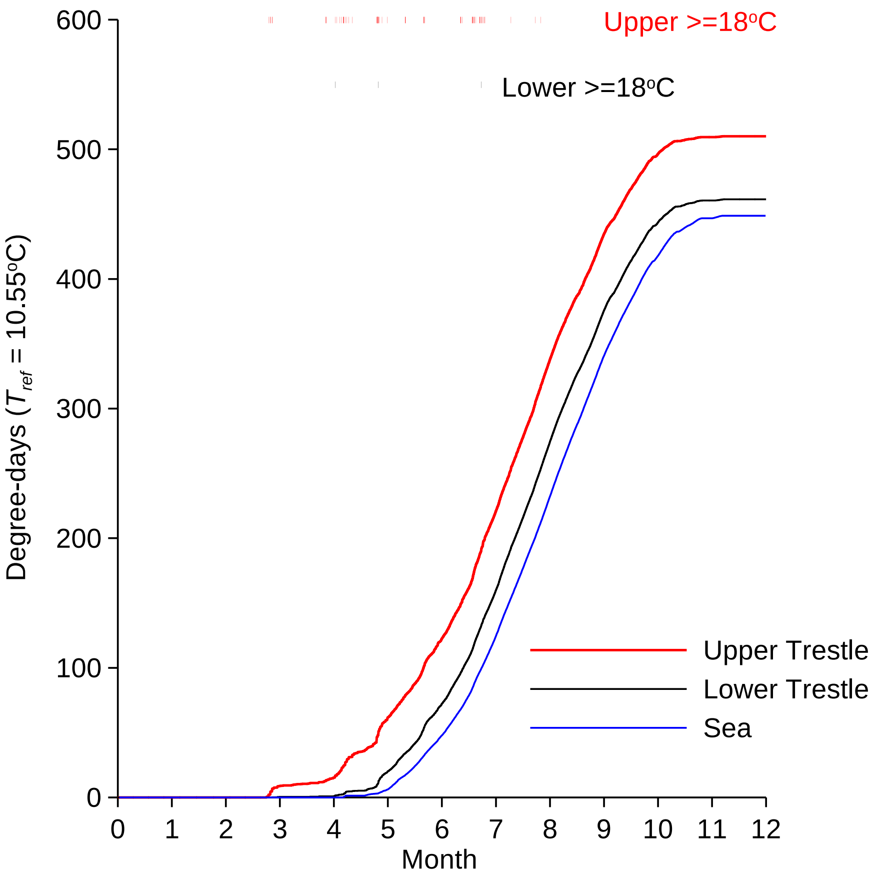


Figure 15: Evolution of degree-days using the definition of Eno (1994), and the reference temperature of 10.55ºC. Upper (red line) and lower trestle (black line) degree-days use *in situ* temperatures. Sea temperatures use T_{lower}^{HW}. The two horizontal lines at the top of the figure indicate when *in situ* temperatures equalled or exceeded 18ºC: upper trestle red; lower trestle black.

Table 9

Monthly averages of sea (T_{lower}^{HW}), air (T_{air}) and *in situ* temperatures (T_{upper} , T_{lower}) for the year 2017 in ºC.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Sea	8.67	8.01	7.88	8.81	10.45	11.92	13.05	14.08	14.13	13.11	11.30	9.71
Air	6.62	6.13	6.51	7.55	11.06	12.13	13.16	13.46	12.17	10.46	5.60	5.16
Upper	7.61	7.24	7.87	8.96	11.72	12.55	13.69	14.30	13.71	12.45	9.48	8.15
Lower	8.08	7.56	7.75	8.78	10.89	12.27	13.37	14.24	13.91	12.69	10.16	8.75

Table 10

Summary statistics of sea, air and *in situ* temperatures in ºC. Minimum (Min.) and maximum (Max.) values are for the observational period 18/10/2016 to 16/05/2018. Average (Avg.*) values are for the full calendar year 2017 in order to avoid aliasing issues.

Description	Parameter	Min.	Avg.*	Max.
in situ Upper Trestle	T_{upper}	-3.63	10.67	34.02
in situ Upper Trestle	T_{lower}	-4.00	10.72	25.15
Loch Creran Sea Temperature	T_{upper}^{HW}	4.52	10.95	15.69
	T_{lower}^{HW}	5.59	10.94	14.64
	T_{upper}^{LW}	-3.08	10.03	31.12
	T_{lower}^{LW}	-3.44	9.94	23.07
Stornoway Air Temperature	T_{air}	-3.40	8.07	22.7

4. Discussion

The aim of mounting temperature loggers on trestles on the oyster farm was to record a representative thermal environment an oyster, or a colony of D.vex, might experience, located within a bag mounted on one of the tables in the farm. Initial analyses performed in this study aimed to confirm how representative the *in situ* logger data was.

4.1 Overlap period

The analysis of the overlap period examined whether there was much local variation of temperature within the structure of a trestle. Two temperature recorders in each of the lower and upper trestles were present during the overlap period, located at different positions within each trestle. The comparison between records from within the same trestle table (Figure 5) showed that there was very little difference in temperature at different points within one trestle. Hence this suggests that the temperature records $T_{upper}(t)$ and $T_{lower}(t)$ were representative of the thermal environment within each respective trestle experienced over the recording period.

4.2 Sea temperatures

The aim of the selection of the upper and lower trestle locations was to monitor the temperature environment experienced at the seaward and landward edges of the farm. Several assumed conditions needed to be correct in order to meet this aim:

- The loggers were not located on a transect perpendicular to the water's edge (Figure 3), hence the lateral (i.e. along-shore) heterogeneity between the two logger locations (approximately 150 m along the water's edge) was assumed to be negligible.
- At high water, it was assumed that both the lower and upper trestles were fully submerged, and hence that high water (HW) *in situ* temperature values represented the temperature of the sea loch surface layer at that time, i.e. the local temperature of the sea.
- It was assumed that times of low water and high water at Tobermory could be used as proxies for those events within Loch Creran at the shellfish farm.

These three assumptions were tested using the analysis shown in Figure 9, where the differences between the upper and lower temperature loggers is shown. The differences between the loggers at high water (Figure 9b) were consistently small, with an average difference of -0.05ºC (i.e. upper logger cooler than lower logger).

Although there were a few occasions where temperature differences were larger (i.e. out of 1,111 high water values, just 23 exceeded a ±1ºC difference), the overall small degree of difference confirms several aspects of the experiment.

The consistently small differences between the two loggers at high water, compared to the much larger differences at low water, confirms:

- That using Tobermory tidal data is valid in that temperature values extracted from the farm loggers at times of HW Tobermory are consistent with them both being simultaneously submerged.
- That high water values can be used as representative of sea temperatures in Loch Creran during the experiment.
- That there are negligible spatial gradients within the sea on the scale of the logger deployments and hence the fact that the loggers were not located on a transect perpendicular to the shore was unimportant.

For the purposes of further analyses the temperature of the lower logger at high water (T_{lower}^{HW}) is taken as the local sea temperature in Loch Creran. From Figure 7b, the minimum sea temperature measured during the observational period was 5.59ºC and the maximum was 14.64ºC.

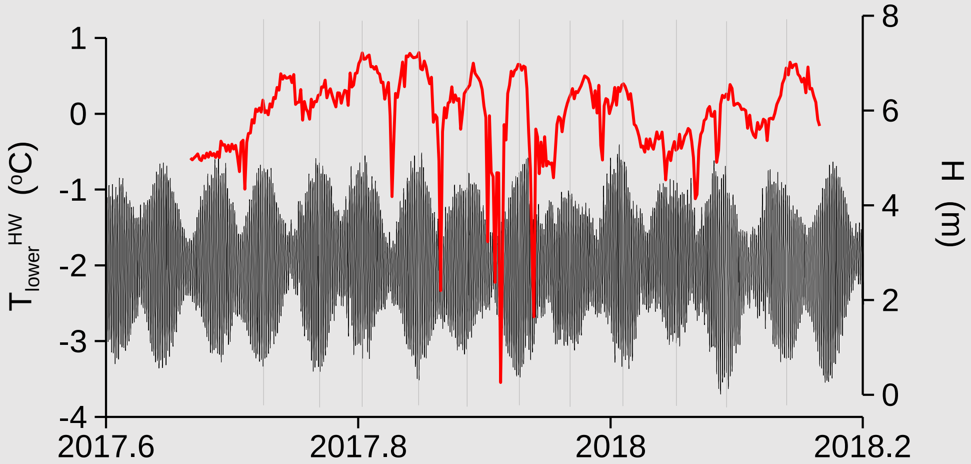
Aside - Seasonality in T_{upper}^{HW}-T_{lower}^{HW}

It is worth noting at this point that a close examination of Figure 9b suggests that the small differences that did exist between the upper and lower temperature loggers themselves had a seasonality. This is confirmed by Figure 9a, where the temperature scale has been enhanced. It can now be seen that there was a clear seasonal cycle, with an amplitude of about ±0.15ºC, which is just within the accuracy of the logger to resolve. In the winter the upper logger was consistently cooler by up to 0.15 C compared to the lower logger, and in the summer the upper logger was consistently warmer. This small seasonal change may reflect thermal inertia in the loggers and surrounding oyster bags, or small vertical temperature gradients in the surface layer of the loch at high water.

The magnitude of this seasonality does not alter the details of the subsequent analyses in any substantive way, or invalidate the use of the temperature of the lower logger at high water (T_{lower}^{HW}) as the local sea temperature in Loch Creran.

Aside – Neap/Spring Cycle in Loch Creran Sea Temperature

Examination of Figures 6 and 7 (using T_{lower}^{HW} as a proxy for sea temperature) suggests that there is a possible spring/neap cycle in sea temperatures for at least part of the deployment period. This is examined in this case study where the sea temperature has been extracted for the period 02:10 02/09/2017 to 17:30 28/02/2018, where the spring/neap cycle looks particularly evident (e.g. see Figure 7b). Over this period the mean sea temperature trend was removed, resulting in the figure below. Maxima in sea temperature variation appeared to occur when spring tide low tides were lowest, i.e. when the tidal range was largest, and hence when tidal exchange in the loch was at its most energetic. Cool anomalies occurred during neap tides, when tidal range was smaller, and exchange was less energetic. It is not yet clear what is resulting in the temperature variations.



4.3 Air and sea temperatures – annual cycles

When the monthly averaged sea temperature (T_{sea}^{MNT}) is compared to the monthly averaged air temperature from Stornoway (T_{air}^{MNT} – Table 9, Figure 11), some basic aspects of the thermal environment at the shellfish farm becomes evident.

Commencing in the winter of 2017, average air temperatures reached a minimum in mid-February (6.1ºC) and sea temperatures a minimum in early-March (7.9ºC). Both then began to warm, with air temperatures warming faster than sea temperatures. By early May, and from then until the end of July, average air and sea temperatures were approximately the same and warmed at the same rate. Note that, owing to the lack of difference between sea and air temperature during this period, *in situ* temperature alone cannot be used as an indicator of whether a temperature logger was submerged or not.

At the end of July 2017, average air temperatures reached their maximum value (13.5ºC) and subsequently began to cool. Sea temperatures, however, continued to warm, reaching a maximum value of 14.1ºC at the end of August, and then they too began to cool although at a slower rate than air temperatures.

By the end of November 2017, the difference between air and sea temperatures reached a maximum of about 5.5ºC (i.e. air 4.5ºC; sea 10.0ºC). Air and sea temperatures reached their annual minima again in February and March 2018 respectively, although these were cooler than in the winter of 2017, i.e. 2018 air minimum temperatures 3.4ºC and sea temperature minimum 6.0ºC.

4.4 Air and sea temperatures – short term variations

Figure 16 shows the comparison between the monthly averaged air and sea temperatures and the Stornoway air temperature ($T_{air}(t)$) and sea temperature ($T_{lower}^{HW}(t)$) respectively. It can be immediately seen that short-term variations in sea temperatures were much smaller than variations in air temperatures. Sea temperature departures from the long term average were less than 2.5ºC, and there were approximately ten such events during the recording period. In contrast, air temperatures were extremely variable, with departures from the average temperature occurring constantly, and up to a magnitude of 11ºC.

Hence it is clear that oysters, and D.vex, on the shellfish farm experienced much greater temperature variations when exposed to the air than when submerged.

We now examine whether air temperature itself is the only cause of variation.

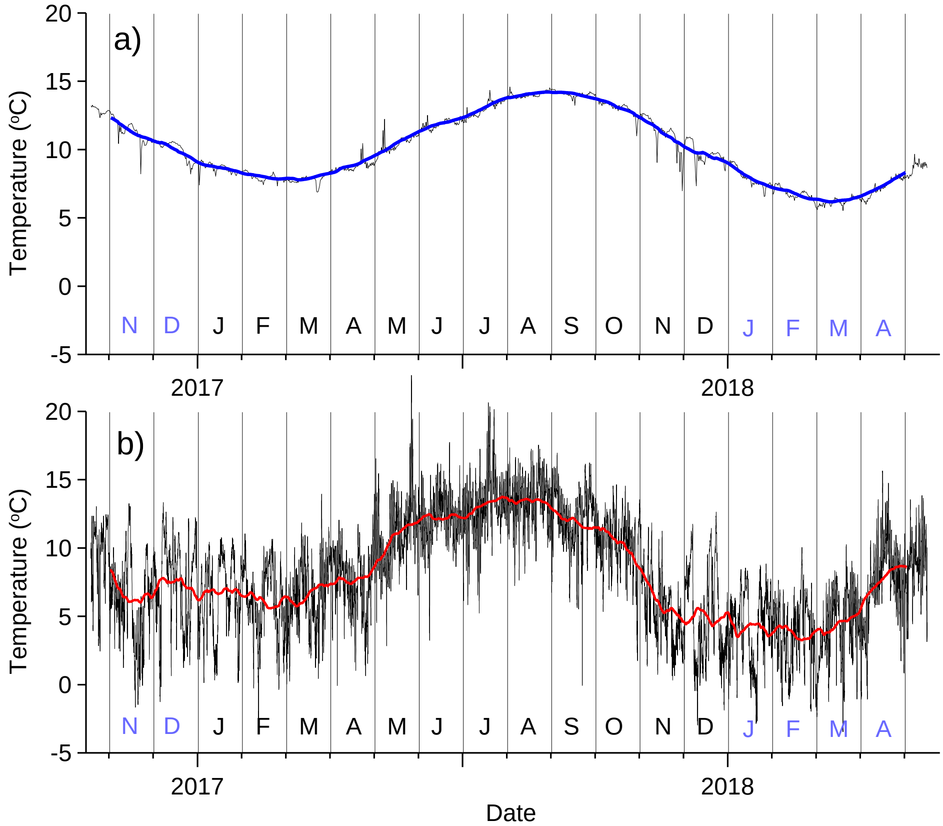


Figure 16: Comparison between a) the monthly averaged sea temperature (blue line - $T_{sea}^{MNT}(t)$) and the hourly sea temperature (black line - $T_{lower}^{HW}(t)$); b) monthly averaged air temperature (red line – $T_{air}^{MNT}(t)$) and the Stornoway air temperature (black line - $T_{air}(t)$). Note that air and sea temperature plots are to the same scale to permit inter-comparisons.

4.5 *In situ* temperatures - short term variations

Figure 16a demonstrates that for any sub-tidal location at the edge of the shellfish farm (i.e. below the lowest low tide), the minimum/maximum temperatures that would have been experienced during the measurement period would have been 5.59ºC and 14.64ºC respectively. In order to examine the minimum / maximum temperatures experienced by inter-tidal parts of the shellfish farm we may use the *in situ* temperatures recorded at low water, i.e. $T_{upper}^{LW}(t)$ and $T_{lower}^{LW}(t)$ (Figure 8 and Table 9).

Table 10 describes the extrema recorded during the full observation period, but does not indicate how frequently extreme temperatures occurred. In order to examine that aspect of temperature variation, Figure 13 presents the frequency analysis of temperatures for the calendar year 2017.

Examining first air temperatures, it can be seen that extreme warm temperatures (>16ºC) occurred less frequently (~1.6% of the annual record for 2017) than extreme cold temperatures (<6ºC, 23%). *in situ* temperature frequency distributions resemble that for sea water much more closely than they resemble air temperature distributions. However, both upper and lower *in situ* temperatures exhibited temperatures cooler than the minimum sea temperature and warmer than the maximum sea temperature.

Examining extreme warm temperatures, for 2017, the maximum recorded air temperature was 22.7ºC, and this was exceeded at the upper (lower) trestle by *in situ* temperatures during 139 (13) 10-minute logging intervals. The difference between air and *in situ* temperatures all year round cannot be used, as for part of the year sea temperatures are warmer than air temperatures. However, at least for the upper trestle we may examine LW *in situ* temperatures, as these were always obtained in air. For these, 239 out of 706 (34%) low water *in situ* temperatures were warmer than the air temperature at that time. For 44 low waters (9%), the temperature difference was greater than 5ºC.

Figure 10 summarises the temperature change an oyster, or a colony of D.vex may have undergone over any one 12 hour period. Short term changes at the lower trestle were smaller in magnitude than at the upper trestle. At the lower trestle, the average 12-hour maximum change was 2.6ºC, while the upper trestle it was 4ºC. Much larger changes could occur however, as the maximum 12-hour change at the lower trestle was 19.3ºC (0200 20/03/2018) while at the upper trestle it was 29.1ºC (1240 26/03/2017). From Figure 10 it can be seen that short term changes were influenced by the spring-neap tidal cycle, whereas no clear annual cycle was evident. However, at the upper trestle largest changes occurred in spring 2017 (March, April, May).

The conclusion from the analysis of extreme temperatures is that minimum temperatures were established by air temperatures and maximum temperatures were the result of air temperature plus *in situ* solar heating.

During the period of observation, no unusual oyster mortalities were reported by the farm, and no reduction in the invasive species *D.vex* was observed. Hence inter-tidal Pacific oysters mounted on aquaculture trestles in Loch Creran, as well as inter-tidal colonies of the invasive sea squirt, experienced temperatures between -4ºC and 34ºC without widespread mortality.

Malham et al. (2009) also reported that Pacific oysters in Irish aquaculture could be exposed to prolonged high *in situ* temperature (>21°C for 14 days) without experiencing mass mortalities. They also found that rapid temperature fluctuations (e.g. 8ºC to 24ºC in one tidal cycle) did not result in mortality in oysters. However, high temperatures combined with eutrophic conditions (high nutrients and enhanced phytoplankton densities) could trigger mortality.

4.6 Farm inundation

We now examine how the farm was inundated by the tide, as this influences how the different parts of the farm were exposed to sea temperatures, or air temperatures plus direct solar heating. Farm inundation can be deduced by combining the tidal records from Tobermory with aspects of the *in situ* temperature records described above and observations made on the farm.

The minimum tidal height in the Tobermory record was 0.04 m and the maximum height was 5.32 m (Figure 6). Thus the extreme range during the observing period was 5 m. Figure 6 confirms there is a strong neap/spring cycle in the region. Figure 13 confirms that low waters ranged between 0 m and 2.8 m and high waters between 3 m and 5.4 m.

While the upper and lower trestle temperature loggers were not surveyed in, in terms of vertical height, there are some estimates of their vertical position that can be made.

Upper trestle:

If it is assumed, from observation, that the upper trestle was submerged every tide but also accessible every tide, the logger must have been be located between the heights of 2.8 m (highest low tide) and 3 m (lowest high tide; Figure 14). As the upper trestle was selected at the upper extreme of the farm, we can deduce that the upper trestle lay at a height of 3 m above chart datum. At this height, from Figure 17, the upper trestle would have been exposed to the air for approximately 200 days (4800 hours) in one year, 55% of the time.

Lower trestle:

From observation at the farm, it can also be assumed that the lower trestle was only exposed at spring tides. Examining Figure 6, a height of 0.4 m would result in the trestle being exposed only at the lowest spring tide. The data shown in Figure 13d indicates that the lower trestle experienced temperatures less than the minimum sea temperature for approximately 3% of the record for 2017, i.e. for approximately 11 days in a year, and above the maximum sea temperature for approximately 1% of the year, i.e. four days. This suggests that the minimum time for the lower trestle to be out of the water was 4% (i.e. 3%+1% of the year, or 15 days). This amount of exposure was equivalent to the exposure of a height above datum of 1.0 m. The temperature extremes method only allows estimates of the minimum time exposed to the air. The actual exposure time may be longer, but not revealed by temperature differences between the *in situ* temperature and the sea temperature. However, the analysis confines the height of the lower trestle to be between 1.0 m and 2.8 m. For the purposes of this analysis we will assume that the lower trestle lay at a height of 1.0 m above chart datum, i.e. the lowest depth in the possible range.

Summary

Using tidal data combined with observations of temperature variation and some assumptions about the upper and lower trestles, it would appear that the bulk of the shellfish farm lay in the vertical between 1.0 m (lower seaward edge) and 3.0 m (upper landward edge) above chart datum. These heights refer to trestle heights, not seabed heights.

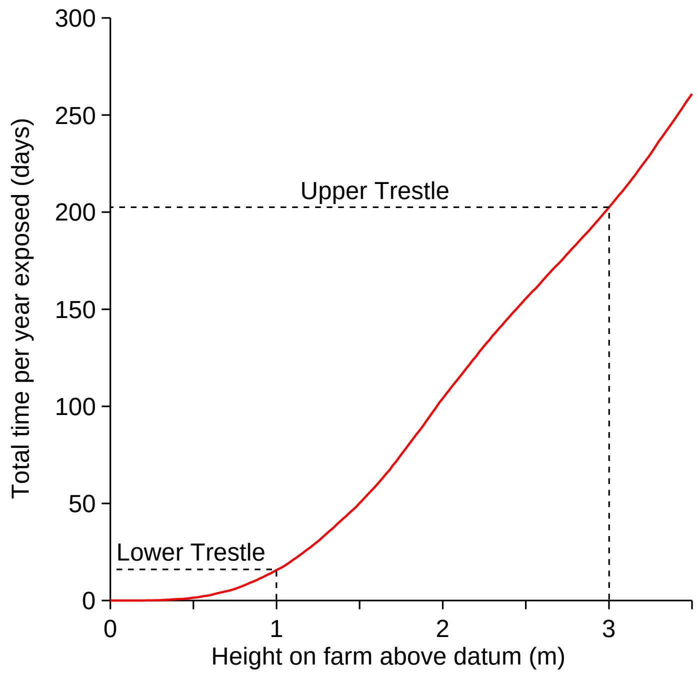


Figure 17: The number of days in a year (2017) a certain height above chart datum on the farm is exposed to the air.

4.7 Thermal exposure of Pacific oysters – degree-days

The analysis of the *in situ* temperature data using the conditions for recruitment for Pacific oysters published by Eno (1994) suggest that in Loch Creran conditions were never suitable for larval production (Figure 17) during the observational period. Using the reference temperature of 10.55ºC as the biological zero for gamete production (Eno, 1994; Herbert et al., 2012) resulted in conditions at neither upper or lower trestles exceeding the minimum of 592 degree-days (Eno, 1994). The maximum number of degree-days, with T_{ref}=10.55ºC, in Loch Creran was 461 and 510 for the lower and upper trestles respectively, and 449 days for sub-tidal conditions with no air exposure (Figure 17).

At the upper trestle, it is clear that the warmer temperatures that existed when the oysters were exposed to the air were essential for reaching the higher thermal exposure than at the lower trestle. Eno (1994) certainly included air exposure temperatures in her calculations of thermal exposure contributing towards gametogenesis, although Carrasco and Barón (2010) noted that exposure to high air temperatures in a typical inter-tidal semi-diurnal cycle can lead to stress in Pacific oysters.

Note that for completeness, Figure 17 also indicates when *in situ* temperature at the upper and lower trestles equalled or exceeded 18ºC, the temperature needed to initiate spawning, according to Eno (1994).

4.7.1 Underestimation of thermal exposure

Table 11 shows the difference in the estimation of thermal exposure using temperatures measured at the upper trestle on the Loch Creran farm *in situ*, and using monthly averaged sea temperatures as used, for example, by Syvret et al. (2008) and Carrasco and Barón (2010). The difference is caused by the extreme temperatures experienced while trestles were exposed to the air inter-tidally. The difference decreases as more months are included (i.e. T_{ref} decreases) and hence the influence of the extremes is diluted by more average conditions.

However, it must be emphasised that estimating thermal exposure using climatological sea temperatures can underestimate the recruitment potential of Pacific oysters in a region. In Loch Creran in 2017, using monthly averaged sea temperatures underestimated thermal exposure by 18% compared to using measured temperatures as experienced on the farm.

It should be noted here that temperature alone is not the sole influence over recruitment potential or recruitment success. Change in temperature has been used by a large body of marine ecological science as a proxy for changes in the biotic and abiotic processes in the sea which may affect rectuiment, and which may be altered by climate change. It is easily measured and the processes controlling it are readily modelled. Temperature change has successfully been used as a proxy indicator for ecological change as temperature directly controls the rate of many chemical and biological processes in the sea. Hence it is not temperature alone which will ultimately control recruitment success, but it does provide a primary indicator.

T_{ref}	**Degree-days using monthly average**	**Degree-days using *in situ* measured**	**% Difference**
10.55	418	510	-18.0
10	556	617	-9.9
9	793	833	-4.8
8	1063	1093	-2.7

Table 11 The difference in the estimation of thermal exposure using temperatures measured at the upper trestle *in situ*, and using monthly averaged sea temperatures. Detailed calculations are presented in the Appendix.

4.7.2 Thermal adaptation

Carrasco and Barón (2010) reviewed the evidence of Pacific oysters genetically adapting to specific thermal regimes. They noted that using strains which had been selected for the tolerance of a local thermal regime would increase the possibility of Naturalisation. From the evidence of Shelmerdine et al. (2017) we already know that a strain of Pacific oyster did adapt to some Scottish conditions, at least once. Additionally, Spencer et al. (1994) observed successful Pacific oyster spat settlement in Loch Sween (approximately 60km south of Loch Creran) and two other observations of wild Pacific oyster in Scottish waters have been reported by Cook et al. (2014) in the Solway Firth and by Smith et al. (2015) in the Firth of Forth.

The reference temperature and acclimatisation

The concept of "degree days" and "thermal exposure" is a mathematical concept, which requires a reference temperature. It records the length of time a location experiences temperatures above the reference temperature, and provides an indication of how warmer than that temperature the location was over that time, i.e. it is the time integral of temperature above the reference temperature.

The concept was first proposed for Pacific oysters by Mann (1979). The reference temperature of 10.55ºC was used by Eno (1994), citing Mann (1979). However, it was further discussed by Syvret et al. (2008) who dedicate a whole section to the subject of acclimatisation in relation to the reference temperature (their Section 2.2.3, p45). It might be argued that factors such as the temperature for gametogenesis is solely set by the genetics of the brood stock. However, Syvret et al. specifically note that "spawning potential can be raised in the life of the animal rather than over many generations of natural selection". They go on to suggest that "old mature wild stock may pose a higher level of risk in terms of wild settlement than cultured stock".

In a study of gametogenesis in Pacific oysters in France (Fabioux et al., 2005), the authors investigated the effect of cooler temperatures. They found "Moreover, gametogenesis initiation was demonstrated to begin under 10 °C unlike data given in previous studies (Mann, 1979)", also "This relation is in agreement with the "day-degrees" notion proposed by Mann (Mann, 1979) except that minimal temperature for oyster gametogenesis would be lower than 10 °C." and "We obtained ripe oysters at 8-10 °C in wintering conditioning"

Some have considered the value of 10.55ºC as the reference temperature for the thermal exposure calculation as an immutable natural constant. It is far from that. Indeed Syvret et al. (2008) themselves stated that “It is recommended that current on-going research […] is monitored closely in order to obtain a more relevant field study based conditioning value when it becomes available”. They also state in their recommendations (p92), “Further work is needed to refine the ‘biological zero’ and spawning and settlement degree days that are relevant to Pacific oysters in UK environmental conditions”.

Hence the reference temperature is simply a concept that can both vary in nature, and also be varied mathematically to investigate its effect on the data.

Finally, we note that the value of 10.55ºC comes from a single study using just 200 oysters performed 40 years ago, under hatchery conditions aimed at raising oysters in the thermal outflow of industrial plant in the USA, and with other factors, such as oyster nutrition, artificially controlled (Mann, 1979).

Conditions in L. Creran

Table 12 shows values of the annual degree-days, using the upper trestle *in situ* temperatures. If the biological reference temperature is reduced to 9ºC, then conditions in Loch Creran during the observational period could be categorised as having “recruitment potential” following the definition used by Syvret et al. (2008). It is not yet known conclusively if thermal adaptation can result in such reduction in the biological reference temperature (i.e. the temperature needed to initiate gametogenesis, Eno 1994), but the evidence for successful reproduction of Pacific oysters in Scottish waters suggests that this may have occurred. These results suggest that work is needed in order to better understand the thermal conditions required for successful reproduction and recruitment in Pacific oysters using stock already exposed to Scottish conditions.

The reference temperature of 10.55ºC may not be relevant to oysters in Scottish waters, and as we suggest, supported by Syvret et al., more work is needed to investigate this. Note that Shelmerdine et al. (2017) arrive at a similar conclusion, i.e. “The findings suggest that the species can spawn and survive at much lower temperatures than those used in current models”.

Table 12
Thermal exposure in degree-days based on different values of the reference temperature T_{ref} and using the upper trestle *in situ* temperatures T_{upper}.

T_{ref} (ºC)	Annual degree-days	Month reached 592 degree-days
10.55	510	Never
10	617	October
9	833	September
8	1093	August

4.7.3 Climate Change

In Table 12 the indication is that T_{ref} must decrease by approximately 1.5ºC before conditions in Loch Creran could be classified as having recruitment potential as defined by Syvret et al. (2008). The section above considers how this might be achieved through adaptation to thermal conditions. However, an alternative to reaching the required thermal exposure for recruitment potential is if *in situ* temperatures increased by 1.5ºC due to climate change. This possibility is further discussed below.

Scottish Waters Warming

The general consensus is that our waters will have warmed by between 1ºC and 4ºC by the year 2100 , but much depends on how society curbs its greenhouse gas emissions (MCCIP, 2020). The general observation of future warming of Scottish waters is evidenced as follows:

1) The UK Climate Projections programme is funded by the UK Government in order to provide managers and planners with the best possible consensus advice on future climate change in the UK. It is based on detailed ensemble model runs (i.e. taking into account uncertainty) of state-of-the art climate models developed by the UK Met Office Hadley Centre. The UKCP programme states that “the shelf seas around the UK are projected to be 1.5 to 4ºC warmer and ~0.2 practical salinity units (p.s.u.) fresher (lower salinity) by the end of the 21st century” (Jenkins et al., 2009). The Scottish Government uses the marine projections of the UKCP09 as a basis for their planning and forward looks. For example, 1) the basis of the Scottish Climate Change Adaptation Plan; 2) Please see the NMPi future climate change data layers (Turrell, et al., 2017) and associated report at: http://marine.gov.scot/data/nmpi-future-climate-change-data-layers. The UKCP09 climate change projections of warming in UK seas were also used by the Shellfish Association of Great Britain as

the basis of their own analysis of the risk of the wild establishment of Pacific oysters in UK waters (Herbert et al., 2012).

Note that the full marine UKCP09 report gives quantified probabilities associated with the ensemble model predictions of warming (Lowe et al., 2009).

2) The latest consensus view from 150 leading UK academic and government scientists from 50 organisations of climate change impacts prepared by MCCIP (MCCIP, 2020; Tinker and Howes, 2020) states that “there are a now a number of North-West European Shelf (NWS) seas climate projections for the end of the 21st century. There is good agreement on the sign of the temperature change on the NWS among the end of century climate projections. However, there is a spread in the magnitude of this warming. Most projections give a warming between 1–4°C.”

Will our waters warm or cool?

There have been some studies that suggest that rather than warming, Scottish seas may cool as a result of changes in the large scale circulation of the global ocean, and specifically as a result of changes to the Atlantic Meridional Overturning Circulation (AMOC). The AMOC, of which a component is referred to as “the Gulf Stream”, is partly responsible for our climate in Scotland. The AMOC is currently weakening, but the probability that it will shut down completely is judged by the IPCC as “very unlikely” (i.e. 0-10% probability). Most model projections of warming of Scottish waters include the effect of a weakening AMOC. This is best summarised by the UK Climate Projections briefing prepared for UK government by the Met Office (Jenkins et al., 2009):

“The North Atlantic Ocean Circulation (sometimes less precisely referred to as the Gulf Stream) maintains a warm North Atlantic, and as such is partly responsible for the climate of the UK being more temperate than other regions at the same latitude. If, hypothetically, the Atlantic Ocean Circulation were to be switched off the UK’s climate would be a few degrees colder than today’s. Climate models project that the Atlantic Ocean Circulation will weaken gradually in response to increasing greenhouse gases, by up to 50% by 2100, and the effect of such weakening is included in the UKCP09 Projections. However, no comprehensive climate model, when forced with the IPCC SRES emissions scenarios, produces a complete or abrupt shutdown in the 21st century. Although additional freshwater from rapid melting of the Greenland ice sheet could further weaken the Atlantic Ocean Circulation, IPCC AR4 concludes that it is very unlikely that an abrupt change will occur this century. We cannot comment with any confidence on changes that may have already happened, due to the lack of continuous, long term, robust, measurements”.

In summary, based on the work of the IPCC and the UKCP09, it would be very unwise to base climate change adaptation planning on a cooling scenario for Scottish waters.

Pacific oyster habitat response to warming seas

The observation that the thermal habitat suitable for reproduction of the Pacific oyster may move north owing to the effects of climate change, and specifically for Scotland warming seas, is far from new or novel. For example, the 2008 MCCIP peer-review of non-native species science in relation to climate change (Elliott et al., 2008) stated “as this species [Pacific oyster] has become established in other countries such as the Netherlands and Germany as well as areas in the south of the UK there is potential for an increased risk of the species becoming established in Scotland as water temperatures increase. This could lead to the out-competition of native filter feeders (Eno et al., 1997) and the extensive modification of estuarine habitats”.

In 2012, a report of the Shellfish Association of Great Britain (Herbert et al., 2012) noted that “An analysis of the effect of rising sea temperatures, under a medium emissions scenario, on the distribution of *C. gigas* and eight other marine invasive non-native species showed that they would all theoretically be able to expand their range by the 2080s to encompass the entire UK”.

A peer-reviewed study published in 2013 by authors from the School of Environmental Sciences (University of East Anglia), the Centre for Environment, Fisheries and Aquaculture Science (CEFAS, Lowestoft), the UK Tyndall Centre for Climate Change Research, and the University of British Columbia (Jones et al., 2013), concluded that “ensemble predictions made here suggest that Pacific oyster will experience an opening of suitable habitat in northern UK waters, reaching the Faroe Islands and the eastern Norwegian Sea by 2050” and that “an increase in habitat suitability is seen in the central North Sea, and around the northern coast of Norway and Scotland”

The 2013 MCCIP peer-review of non-native species science in relation to climate change (Cook et al., 2013) noted that “it is highly likely that self-sustaining populations of the molluscs *Crassostrea gigas* and *Crepidula fornicata* will also continue to expand northwards and become established on the west coast of

Scotland in the 2020s". In 2014, a similar report commissioned by the Scottish Aquaculture Research Forum (Cook et al., 2014) concluded that "periodic increases of 2ºC have been observed off the west coast of Scotland (Inall et al., 2009). This warming seawater has been linked to the northwards spread of certain non-native species in the UK, such as the Pacific oyster *Crassostrea gigas* (Thunberg, 1793) (Maggs et al., 2010). A recent report suggests that it is highly likely that 'wild' populations of *C. gigas* will continue to expand northwards and become established on the west coast of Scotland by the 2020s (Cook et al., 2013)".

Finally, in 2017 the MCCIP peer-review of non-native species science in relation to climate change (Cottier-Cook et al., 2017) noted that "this survey determined the current northern most limit for this species on the west coast of the UK (54.8° N). As more northerly populations are recorded, however, in Ireland (55.1° N) (Kochmann et al., 2013) and Scandinavia (60.0° N) (Wrange et al., 2010), it is highly likely that *C. gigas* will continue to spread further northwards (Cook et al., 2014)".

4.8 Thermal exposure of *Didemnum vexillum*

Table 3 suggests that there are two critical temperatures for D.vex reproduction. Temperatures greater than 8ºC can support asexual reproduction (e.g. through budding of zooids), and temperatures greater than 14ºC can support larval production.

Table 13 and Table 14 present the running totals of the time above 8ºC and 14ºC respectively at the lower and upper trestles, and in the sea in Loch Creran during 2017. Sea temperatures rarely fell below 8ºC, and this is confirmed by Figures 12 and 16a. Hence sea temperatures were suitable for D.vex asexual reproduction all year round.

In terms of sexual reproduction through the shedding of larvae, sea temperatures were above 14ºC only in August and September. At the upper trestle, in situ temperatures began to exceed 14ºC as early as March, but only for very short periods. In both June and July there were longer times above 14ºC, reaching running totals of about eight days in duration in both months. In total, by the end of September D.vex on the upper trestle would have experienced a total of 42 days in duration of temperature above 14ºC while submerged in sea water, and a further 15 days in duration above 14ºC while exposed to the air inter-tidally. While it is clear that exposure to air in an inter-tidal cycle does not kill D.vex, at this time it is not known how thermal exposure of D.vex while in air affects its development.

In summary, at the Loch Creran shellfish farm in 2017 sea temperatures were suitable all year round for asexual reproduction in D.vex (ie. >8ºC). Sea temperatures were suitable for the production of D.vex larvae (i.e. >14ºC) in August and September. D.vex on the upper trestles was also exposed to temperatures >14ºC while in the air inter-tidally from March to September.

Table 13

Running totals, to the end of each calendar month in 2017, of the time temperatures exceeded 8ºC. The symbol (-) indicates totals temperatures never exceed 8ºC. Sea – sea temperatures using T_{lower}^{HW}. Upper – *in situ* temperature at upper trestle (T_{upper}). Lower - *in situ* temperature at lower trestle (T_{lower}).

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Sea	29	42	53	83	114	144	174	204	234	265	295	325
Upper	18	28	39	65	95	125	156	187	216	247	270	291
Lower	23	34	45	73	103	133	164	195	225	256	282	306

Table 14

Running totals, to the end of each calendar month in 2017, of the time temperatures exceeded 14ºC. The symbol (-) indicates temperatures never exceed 14ºC. Sea – sea temperatures using T_{lower}^{HW}. Upper – *in situ* temperature at upper trestle (T_{upper}). Lower - *in situ* temperature at lower trestle (T_{lower}).

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Sea	-	-	-	-	-	-	1	17	42	-	-	-
Upper	-	-	1	2	5	8	17	39	57	-	-	-
Lower	-	-	-	-	1	2	8	31	51	-	-	-

5. Conclusions

- In Loch Creran during the observation period (18/10/2016 to 16/05/2018) sea temperatures varied between 4.52ºC and 15.69ºC (measured in Loch Creran) and air temperatures varied between -3.4ºC and 22.7ºC (based on records from Stornoway).

- Hence inter-tidal habitats periodically exposed to the air on the Scottish west coast experienced greater temperature extremes than sub-tidal habitats which only experience sea temperatures.

- Inter-tidal shellfish (Pacific oysters) mounted on aquaculture trestles in Loch Creran, as well as inter-tidal colonies of the invasive sea squirt *Didemnum vexillum* on the farm, experienced *in situ* temperatures between -3.63ºC and 34.02ºC. No widespread mortality was reported in either species.

- The largest change of temperature over any 12 hour period was 19.3ºC at the lower trestle and 29.1ºC at the upper trestle on the farm.

- Short term changes altered over the spring/neap cycle. Largest changes occurred in the spring (March, April, May). There was little difference between the other seasons.

- The minimum temperatures experienced inter-tidally were established by air temperatures and maximum temperatures were the result of air temperature plus *in situ* solar heating.

- During the observation period, *in situ* solar heating raised inter-tidal trestle temperatures by up to 20ºC above air temperature.

- Using tidal data and some assumptions about the upper and lower trestles, it would appear that the bulk of the shellfish farm lies in the vertical between 1.0 m (lower seaward edge) and 3.0 m (upper landward edge) above chart datum. These heights refer to trestle heights, not seabed heights.

- From the tidal records, this would indicate that trestles on the farm are submerged in seawater between 96% (lower seaward edge) and 45% (upper landward edge) of the time over a year (2017).

- Pacific oysters and *Didemnum vexillum* can both tolerate these exposure levels but growth of both species appears enhanced at the shorter exposure times (seaward edge of farm) compared to the longer exposure times (landward edge of farm).

- The maximum number of degree-days, with T_{ref}=10.55ºC, in Loch Creran during the observational period was 461 and 510 for the lower and upper trestles respectively, and 449 days for sub-tidal conditions with no air exposure. These are less than the 592 degree-days (T_{ref}=10.55ºC) needed to achieve gametogenesis in Pacific oysters.

- Hence, for the observational period 18/10/2016 to 16/05/2018, thermal conditions in Loch Creran were never suitable for Pacific oyster reproduction.

- Estimating thermal exposure using climatological sea temperatures can underestimate the recruitment potential of Pacific oysters in a region. In Loch Creran in 2017, using monthly averaged sea temperatures underestimated thermal exposure by 18% compared to using measured temperatures as experienced on the farm.

- If the biological reference temperature is reduced to 9ºC, then conditions in Loch Creran during the observational period could be categorised as having recruitment potential following the definition used by Syvret et al. (2008).

- It is not known if thermal adaptation can result in such reduction in the biological reference temperature (i.e. the temperature needed to initiate gametogenesis, Eno 1994), but the evidence for successful reproduction of Pacific oysters in Scottish waters suggests that this may have occurred.

- **In order to be able to manage the risk of naturalisation, a priority research area is understanding the thermal conditions required for successful reproduction and recruitment in Pacific oysters using stock already exposed to Scottish conditions.**

- An alternative to adaptation in reaching the required thermal exposure for recruitment potential is if *in situ* temperatures increased by 1.5ºC. The general consensus is that our waters will have warmed by between 1ºC and 4ºC by the year 2100, but much depends on how society curbs its greenhouse gas emissions.

- At the Loch Creran shellfish farm in 2017, sea temperatures were suitable all year round for asexual reproduction in D.vex (ie. >8ºC). Sea temperatures were suitable for the production of D.vex larvae (i.e. >14ºC) in August and September. D.vex on the upper trestles was also exposed to temperatures >14ºC while in the air inter-tidally from March to September.

- **In order to understand the reproductive potential of D.vex in Loch Creran, research is needed into the potential development of larvae under local conditions, as well as the influence of exposure to high temperatures in air inter-tidally on the sexual development of D.vex.**

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7. References

Bayne, B.L. and 26 co-authors (2017). The proposed dropping of the genus *Crassostrea* for all Pacific cupped oysters and its replacement by a new genus *Magallana*: a dissenting view. Journal of Shellfish Research 36(3), 545-547.

Brown, L., Gubbins, M., Donnan, D. and Dodd, J. (2018a) The Marine Invasive Non-Native Species Didemnum vexillum: Survey of inter-tidal and shallow sub-tidal artificial infrastructure in Loch Creran. Scottish Marine and Freshwater Science Vol 9 No 11, 20pp. DOI: 10.7489/12127-1

Brown, L., W.R. Turrell, J. Graham, M. Gubbins, G. Hermann, J.-P. Lacaze, and I. Matejusova (2018b) The Marine Invasive Non-Native Species Didemnum vexillum: Loch Creran Surveys - October 2016. Scottish Marine and Freshwater Science Vol 9 No 10, 40pp. DOI: 10.7489/12126-1

Bullard, S.G., B. Sedlack, J.F. Reinhardt, C. Litty, K. Gareau, R.B. Whitlatch (2007). Fragmentation of colonial ascidians: Differences in reattachment capability among species. Journal of Experimental Marine Biology and Ecology 342:166-168. doi:10.1016/j.jembe.2006.10.034.

Carman, M. R., J.A. Morris, R.C. Karney, and D.W. Grunden (2010). An initial assessment of native and invasive tunicates in shellfish aquaculture of the North American east coast. Journal of Applied Ichthyology, 26:8-11.

Carman, M.R. and D.W. Grunden and D. Ewart (2014). Coldwater reattachment of colonial tunicate Didemnum vexillum fragments to natural (eelgrass) and artificial (plastic) substrates in New England. Aquatic Invasions, 9(1):105-110. doi: http://dx.doi.org/10.3391/ai.2014.9.1.09.

Carrasco, M.F., and P.J. Barón (2010). Analysis of the potential geographic range of the Pacific oyster Crassostrea gigas (Thunberg, 1793) based on surface seawater temperature satellite data and climate charts: the coast of South America as a study case. Biological Invasions, 12: 2597-2607.

Collins, C., Bresnan, E., Brown, L., Falconer, L., Guilder, J., Jones, L., Kennerley, A., Malham, S., Murray A. and M. Stanley (2020). Impacts of climate change on aquaculture. MCCIP Science Review 2020, 482–520.

Cook, E. J., Jenkins, S., Maggs, C., Minchin, D., Mineur, C., Nall, C., and Sewell, J. 2013. Impacts of climate change on non-native species. MCCIP Science Review 2013: 155-166.

Cook, E. J., Beveridge, C. M., Lamont, P., O'Higgins, T., and Wilding, T. 2014. Survey of Wild Pacific Oyster Crassostrea gigas in Scotland. Scottish Aquaculture Research Forum Report, Scotland. www.sarf.org.uk/cms-assets/documents/207056-140687.sarf099.pdf.

Cottier-Cook, E.J., D. Minchin, R. Giesler, J. Graham, A.M.O. Mogg, M.D.J. Sayer, and I. Matejusova (2019). Biosecurity implications of the highly invasive carpet sea-squirt Didemnum vexillum Kott, 2002 for a protected area of global significance. Management of Biological Invasions, 10: 311-323.

Cottier-Cook, E. J., Beveridge, C., Bishop, J. D. D., Brodiec, J., Clark, P. F., Epstein, G., Jenkins, S. R., et al. 2017. Non-native species. MCCIP Science Review 2017: 47 - 61. doi:10.14465/2017.arc10.005-nns.

Dunphy, B.J., Wells, R.M., and A. G. Jeffs (2006). Oxygen consumption and enzyme activity of the subtidal flat oyster (Ostrea chilensis) and inter-tidal Pacific oyster (Crassostrea gigas): responses to temperature and starvation. New Zealand Journal of Marine and Freshwater Research, 40: 149-158.

Edwards, A. and Sharples (1985). Scottish sea loch catalogue. SAMS.

Elliott, P., Reid, P. C., Edwards, M., and McCollin, T. (2008). Non-native species. MCCIP Annual Report Card 2007-2008 Scientific Review.

Eno, C. (1994). Monitoring the temperature regimes of which the Pacific oyster Crassostrea gigas is subject to in coastal inlets in Britain (The Fleet lagoon and Teign and Dart estuaries) in relation to their reproductive success. Report to Joint Nature Conservation Committee.

Evans, B.K. and R. Huntington (2005). Environmental factors affecting ventilation in the ascidian, Pyura praeputialis. Journal of Experimental Zoology, 262(4): 462-468.

Fabioux, C., Huvet, A., Le Souchu, P., Le Pennec, M., and Pouvreau, S. 2005. Temperature and photoperiod drive Crassostrea gigas reproductive internal clock. Aquaculture, 250: 458-470

Fletcher, L.M. and B.M. Forrest (2011). Induced spawning and culture techniques for the invasive ascidian Didemnum vexillum (Kott, 2002). Aquatic Invasions, 6(4): 457-464. doi: 10.3391/ai.2011.6.4.11

Fletcher, L.M., B.M. Forrest and J. J. Bell (2013). Natural dispersal mechanisms and dispersal potential of the invasive ascidian Didemnum vexillum. Biological Invasions, 15: 627-643. doi 10.1007/s10530-012-0314-x.

Fletcher, L.M., B.M. Forrest, J. Atalah, and J.J. Bell (2013). Reproductive seasonality of the invasive ascidian Didemnum vexillum in New Zealand and implications for shellfish aquaculture. Aquaculture Environment Interactions, 3: 197-211. doi: 10.3354/aei00063.

Gowen, R., Tett, P., and Jones, K. (1992). Predicting marine eutrophication: the yield of chlorophyll from nitrogen in Scottish coastal waters. Marine Ecology Progress Series: 153-161.

Griffiths, K. S., Mowat, R.H.F. Holt, K. Ramsay, J.D.D. Bishop, G. Lambert and S.R. Jenkins (2009). First records in Great Britain of the invasive colonial ascidian Didemnum vexillum Kott, 2002. Aquatic Invasions, 4(4): 581-590.

Gröner, F., M. Lenz, M. Wahl, and S.R. Jenkins (2011). Stress resistance in two colonial ascidians from the Irish Sea: The recent invader Didemnum vexillum is more tolerant to low salinity than the cosmopolitan Diplosoma listerianum. Journal of Experimental Marine Biology and Ecology, 409(1): 48-52.

Herbert, R.J.H., Roberts, C., Humphreys, J., and S. Fletcher (2012). The Pacific oyster (*Crassostrea gigas*) in the UK: Economic, legal and environmental issues associated with its cultivation, wild establishment and exploitation. Report for the Shellfish Association of Great Britain.

Jenkins, G. J., Murphy, J. M., Sexton, D. M. H., Lowe, J. A., Jones, P., and Kilsby, C. G. 2009. UK Climate Projections: Briefing report. Met Office Hadley Centre, Exeter, UK.

Jones, M.C., Dye, S.R., Pinnegar, J.K., Warren, R., and W.W.L. Cheung, (2013). Applying distribution model projections for an uncertain future: the case of the Pacific oyster in UK waters. Aquatic Conservation: Marine and Freshwater Ecosystems, 23: 710-722.

JNCC (2020). Loch Creran. Designated Special Area of Conservation (SAC).

https://sac.jncc.gov.uk/site/UK0030190

Landless, P. and Edwards, A. (1976). Economical ways of assessing hydrography for fish farms. Aquaculture, 8:29-43.

Lambert, G. (2009). Adventures of a sea squirt sleuth: unravelling the identity of Didemnum vexillum, a global ascidian invader. Aquatic Invasions, 4(1): 5-28.

Lowe, J. A., Howard, T. P., Pardaens, A., Tinker, J., Holt, J., Wakelin, S., Milne, G., Leake, J., Wolf, J., Horsburgh, K., Reeder, T., Jenkins, G., Ridley, J., Dye, S., Bradley, S. (2009), UK Climate Projections science report: Marine and coastal projections. Met Office Hadley Centre, Exeter, UK.

Mann, R. (1979). Some biochemical and physiological aspects of growth and gametogenesis in *Crassostrea gigas* and *Ostrea edulis* grown at sustained elevated temperatures. Journal of the Marine Biological Association of the UK, 59: 95-110.

Met Office (2019). Met Office MIDAS Open: UK Land Surface Stations Data (1853-current). Centre for Environmental Data Analysis. Accessed 12/05/2020. http://catalogue.ceda.ac.uk/uuid/dbd451271eb04662beade68da43546e1

MCCIP (2020) Marine Climate Change Impacts: Marine Climate Change Impacts Report Card 2020 (Stoker, B., Turrell, W.R., Robinson, K.A., Howes, E.L., Buckley P., Maltby, K. and Matear L., eds.) Summary Report, MCCIP, Lowestoft, 28pp. doi:10.14465/2020.arc00.000-000

Morris Jr, J.A. and M.R. Carman (2012). Fragment reattachment, reproductive status, and health indicators of the invasive colonial tunicate Didemnum vexillum with implications for dispersal. Biological Invasions, 14(10): 2133-2140.
Munro, L. and S. Wallace (2019). Scottish Shellfish Farm Production Survey Data. DOI: 10.7489/1917-1

Ordóñez, V., M. Pascual, M. Fernández-Tejedor, M.C. Pineda, D. Tagliapietra, and X. Turon (2015). Ongoing expansion of the worldwide invader Didemnum vexillum (Ascidiacea) in the Mediterranean Sea: high plasticity of its biological cycle promotes establishment in warm waters. Biological invasions 17(7): 2075-2085.

R Core Team, (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Reinhardt, J.F., Gallagher, K.L., Stefaniak, L.M., Nolan, R., Shaw, M.T. and R.B. Whitlatch (2012). Material properties of Didemnum vexillum and prediction of tendril fragmentation. Marine Biology, 159(12): 2875-2884.

Salvi, D., Macali, A., Mariottini, P. (2014). Molecular phylogenetics and systematics of the bivalve family *Ostreidae* based on rRNA Sequence-Structure Models and Multilocus Species Tree Associated Data. PLOS ONE, 9, Article Number: e108696.

Smith, I. P., Guy, C., and Donnan, D. 2015. Pacific oysters, Crassostrea gigas, established in Scotland. Aquatic Conservation: Marine and Freshwater Ecosystems, 25: 733-742

Shelmerdine, R.L., Mouat, B. and R.J. Shucksmith (2017) The most northerly record of feral Pacific oyster Crassostrea gigas (Thunberg, 1793) in the British Isles. BioInvasions Records, 6: 57–60.

Spencer B.E., Edwards D.B., Kaiser M.J., C.A. Richardson (1994). Spatfalls of the nonnative Pacific oyster, *Crassostrea gigas*, in British waters. Aquatic Conservation-Marine and Freshwater Ecosystems, 4:203-217.

Syvret, M., Fitzgerald, A., and A.P. Hoare (2008). Development of a Pacific Oyster aquaculture protocol for the UK: Technical Report. FIFG Project No: 07/Eng/46/04. Sea Fish Industry Authority Technical Report.

Tinker, J.P. and Howes, E.L. (2020). The impacts of climate change on temperature (air and sea), relevant to the coastal and marine environment around the UK. MCCIP Science Review 2020, 1–30

Turrell, W.R., Cox, M., Mason, L., Milne, D. and Watret, R. (2017) NMPi Future Climate Change Data Layers. Scottish Marine and Freshwater Science Vol 8 No 11, 44pp. DOI: 10.7489/1975-1

Turrell, W.R., Brown, L. and Mayes, A. (2018). The marine invasive non-native species *Didemnum vexillum*: Loch Creran Surveys - September 2016. Scottish Marine and Freshwater Science Vol 9 No 9, 55pp. DOI: 10.7489/12125-1

Valentine, P.C., Collie, J.S., Reid, R.N., Asch, R.G., Guida, V.G. and D.S. Blackwood (2007a). The occurrence of the colonial ascidian Didemnum sp. on Georges Bank gravel habitat—ecological observations and potential effects on groundfish and scallop fisheries. Journal of Experimental Marine Biology and Ecology, 342(1): 179-181.

Valentine, P.C., M.R. Carman, D.S. Blackwood, E.J. Heffron (2007b). Ecological observations on the colonial ascidian Didemnum sp. in a New England tide pool habitat. Journal of Experimental Marine Biology and Ecology, 342: 109–121. doi:10.1016/j.jembe.2006.10.021

Valentine, P.C., Carman, M.R., Dijkstra, J. and D.S. Blackwood (2009). Larval recruitment of the invasive colonial ascidian *Didemnum vexillum*, seasonal water temperatures in New England coastal and offshore waters, and implications for spread of the species. Aquatic Invasions, 4(1): 153-168.

Vemco (2020). Minilog II-T Temperature Data Logger. https://vemco.com/wp-content/uploads/2014/01/minilog2t.pdf

Vercaemer, B., Sephton, D., Clément, P., Harman, A., Stewart-Clark, S. and C. DiBacco (2015). Distribution of the non-indigenous colonial ascidian Didemnum vexillum (Kott, 2002) in the Bay of Fundy and on offshore banks, eastern Canada. Management of Biological Invasions, 6(4): 385-394.

8. Appendix

Table A1

Detail of degree-day calculations. MEAS – *in situ* measured temperatures at the trestles. AVG – monthly average sea temperatures as presented in Table 9.

		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec			
	Sea T	8.67	8.01	7.88	8.81	10.45	11.92	13.05	14.08	14.13	13.11	11.3	9.71			
	Days	30	28	31	30	31	30	31	31	31	31	30	31	AVG	MEAS	%
MEAS	10.55				15.4	46.4	60.9	98.2	116.9	96.8	61.8	13.0	0.6		510	
AVG	10.55						41.1	77.5	109.4	111.0	79.4			418	510	18.0
AVG	10					14.0	57.6	94.6	126.5	128.0	96.4	39.0		556	617	9.9
AVG	9					45.0	87.6	125.6	157.5	159.0	127.4	69.0	22.0	793	833	4.8
AVG	8				24.3	76.0	117.6	156.6	188.5	190.0	158.4	99.0	53.0	1063	1093	2.7

Marine Scotland Science
Marine Laboratory
375 Victoria Road
Aberdeen
AB11 9DB

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