

Analytical Methods

Accepted Manuscript



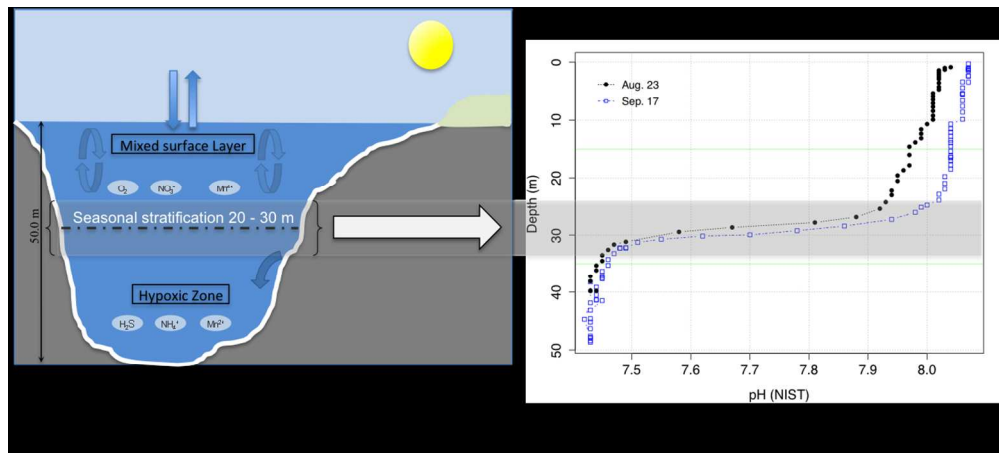
This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



228x101mm (150 x 150 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Determination of spatial and temporal variability of pH and dissolved oxygen concentrations in a seasonally hypoxic semi-enclosed marine basin using continuous monitoring.

Timothy Sullivan¹, Ciara Byrne¹, Luke Harman², John Davenport², Rob McAllen² and Fiona Regan^{1*}

^{1*} Corresponding Author:

Fiona Regan
Marine and Environmental Sensing Technology Hub,
School of Chemical Sciences,
Dublin City University,
Glasnevin, Dublin 9,
Ireland
Email: fiona.regan@dcu.ie

² School of Biological, Earth and Environmental Sciences (BEES),
University College Cork, Cork, Ireland

Abstract

Measurement and prediction of ocean acidification effects for nearshore marine ecosystems is currently a major objective of climate change research. This study examines the scales of pH variability in both surface waters (< 10 m depth) and deeper waters associated with localised seasonal hypoxia over two years at Lough Hyne marine reserve, Cork, Ireland. Lough Hyne, a semi-enclosed marine system, experiences localised seasonal (approximately May to September each year) hypoxia and eventual anoxia below a depth of circa 25 m, due largely to its bathymetric profile. We report the relationships between pH and other environmental parameters such as dissolved oxygen (DO) and temperature at this location, and we provide data, obtained using continuous autonomous sensing technology, which demonstrates that the variability in pH measurements in the waters of Lough Hyne marine reserve are comparable to predictions of future ocean acidification. This is of interest as comparatively few directly measured time series of pH in marine waters are reported, and additionally the first pH measurements were reported from Lough Hyne over 6 decades ago in 1952. This work also measures for the first time the steep chemical gradients associated with the transition from anoxia to normoxia that result in profound pH changes at Lough Hyne, providing insight into natural variability in pH experienced by marine organisms at such locations.

1 Introduction

There is currently considerable concern amongst the scientific community regarding the extent and likely impacts of increasing atmospheric CO₂ concentrations on the marine carbonate system^{1,2}. The likely reduction in calcite and aragonite saturation states resulting from increased pCO₂ driven shifts toward lower seawater pH are of particular concern^{3,4}, and recently published time-series have confirmed that localised inter-annual decreases in pH levels are being recorded which might well be linked to increasing atmospheric pCO₂. For example, time-series of direct pH measurements in temperate regions of the Eastern Pacific indicate a 0.045 pH unit decline per annum⁵. Similarly, direct measurement of pH between 1995 and 2004 from the European Station for Time Series in the Ocean (ESTOC), near the Canary Islands, has shown average inter-annual reductions of 0.0017 ±

1
2
3 0.0004 pH units per annum ⁶ . Seawater CO₂ data from the subtropical gyre of the North Atlantic
4
5 indicates a decline of 0.0017 ± 0.0001 pH units annually since 1983 ⁷ . Winter observations in
6
7 Icelandic waters from 1983 to 2008 indicate local rates of decline at 0.0014 pH units per annum for
8
9 the Northern Irminger Sea and 0.0024 units per annum for the Iceland Sea ⁸ . Calculation of pH in
10
11 subsurface waters of the Rockall Trough off Ireland's Atlantic coast in 2009 and 2010 indicate pH
12
13 decreases of 0.040 ± 0.003 units when compared with data from the World Ocean Circulation
14
15 Experiment (WOCE) surveys throughout the 1990s, while water from the Labrador Sea has reduced
16
17 by 0.029 ± 0.002 pH units from 1991 to 2010 ⁹ . While changes in pH of 0.1 pH units or less may not
18
19 appear substantial, the pH scale is logarithmic which means that a unit decrease in pH equals a ten-
20
21 fold increase in acidity. It is also of interest to note that the maximum feasible change in oceanic pH
22
23 has been calculated to be limited to a maximum average reduction of 0.77 pH units, which would only
24
25 occur if all of the calculated remaining fossil fuels were burnt ³ .
26
27

28
29 Understanding the rates and scales of anthropogenic change in oceanic pH is essential, however the
30
31 scales of natural variability in pH are rarely considered when discussing the effects of ocean
32
33 acidification on marine ecosystems to date ¹⁰ . This seems particularly relevant to coastal systems,
34
35 where ranges and rates of change in pH on a daily, tidal or seasonal basis for example, may be much
36
37 greater than predicted changes in oceanic pH related to increases in atmospheric CO₂ concentrations
38
39 ¹⁰ . Therefore, ambient fluctuations in pH may have provided marine organisms with some capacity to
40
41 develop resilience to such changes, or localised ambient fluctuations may indeed be superimposed on
42
43 or additive to the effects of a seemingly steady reduction in global oceanic pH, resulting in extreme
44
45 events with large impacts ¹⁰ . In addition, it has been proposed that the effects of an approximate
46
47 doubling in pCO₂ pressures in oceanic surface waters will be strongest in coastal hypoxic zones, as
48
49 pCO₂ during hypoxia will increase proportionally ¹ .
50
51

52
53 Lough Hyne, a semi-enclosed marine lough and nature reserve in southwest Ireland, experiences
54
55 localised seasonal hypoxia, including eventual anoxia, below an approximate depth of 25 m on an
56
57 annual basis, due in large part to its bathymetric profile. The Lough, in common with other seasonally
58
59 hypoxic and anoxic zones in temperate marine waters globally, experiences seasonally stable
60
stratification and suppressed ventilation of the hypolimnion from above, making conditions in the

1
2
3 subsurface waters ideal for development of hypoxia and anoxia in the summer and autumn months¹¹⁻
4
5¹³. Thus, it is of interest to measure the pH of Lough Hyne both during and outside conditions of
6
7 seasonal hypoxia. There is evidence that ocean acidification will amplify the effects of hypoxia in
8
9 coastal zones¹, therefore understanding the dynamics of pH within unequilibrated zones such as
10
11 Lough Hyne is of increasing urgency.

12
13
14 No continuous monitoring programme for the main biogeochemical parameters exists at Lough Hyne
15
16 at this time. Therefore, our aim in this paper has been to measure the variability in pH associated with
17
18 hypoxia and to compare this with available historical data. Thus, we present high-resolution time
19
20 series of direct pH measurements associated with seasonal stratification at Lough Hyne, collected
21
22 using autonomous sensors from 2011 to 2013. These measurements demonstrate some of the
23
24 gradients and main drivers of pH variation at Lough Hyne. This study also provides a baseline dataset
25
26 for future analysis of change at this location, as well as providing insight into the natural ranges and
27
28 localised variability in pH that can be measured in coastal waters.
29
30
31
32
33

34 **2 Experimental**

35 **2.1 Site and location description**

36
37
38 Lough Hyne Marine Reserve (LHMR), established as a marine reserve in 1981, is located on the
39
40 southwest coast of County Cork (Grid reference 51.502064°N, 9.305506°W), Ireland. It is connected
41
42 to the nearby Atlantic Ocean by a narrow (~ 25 m) and shallow (3-4 m at high tide) channel called the
43
44 Rapids (Figure 1). Lough Hyne is divided into two basins (North and South), separated by an island
45
46 (Castle Island). This morphology results in differing hydrodynamic characteristics within each basin:
47
48 the South Basin site, close to the Rapids, is a high-energy site with a low residence time, while the
49
50 North Basin is a low energy site with a much longer flushing time in comparison with the South Basin
51
52¹². The two basins are joined to the West by the Western Trough, in which the deepest depths of the
53
54 Lough occur (approximately 50 m). The depth, volumes, and bathymetry of Lough Hyne have been
55
56 previously described in detail¹³⁻¹⁶.
57
58
59
60

Figure 1: (a) A map of Lough Hyne Marine Reserve, including the 25 and 35 m bathymetric contours. These contours encircle the area of greatest depth; known as the Western Trough, which has a maximum depth of approximately 50 m. Sonde deployment locations from 2011 to 2013 are shown with deployment depths in parentheses. (b) A cross-section of the Western Trough at Lough Hyne, illustrating sensor positioning within the water column during seasonal hypoxia/anoxia.

2.2 pH electrode details, calibration and validation methods.

Direct *in situ* measurements of pH at locations shown in Figure 1 were conducted with YSI Fast-Response pH Probes (Probe 6589). This electrode probe design has an operational resolution of 0.01 pH units and an accuracy of ± 0.2 pH units, within the standard range of 0-14 units. Probes were regularly calibrated in pH 7 and pH 10 standard buffers (NIST traceable, supplied by Lennox Laboratory Supplies, John F. Kennedy Drive, Naas Road, Dublin 12, Co. Dublin, Ireland) before deployment and then re-calibrated on average every 2 weeks whilst deployed. Although use of low-salinity buffers does not achieve the accuracy possible with buffers of seawater salinity, errors are smaller than the natural variations described and do not affect the conclusions of this study. Probes were protected from biofouling by coating the probe housing with adhesive copper tape and installation of a mechanical wiper that was set to brush the glass membrane twice before each measurement was obtained.

2.3 Continuous data acquisition

Environmental conditions at LHMR during seasonal stratification were measured over the time periods detailed in **Table 1** using multiparameter environmental sondes (YSI 6600 V2-2, YSI Hydrodata, Letchworth UK). Sondes were equipped with a suite of sensors capable of measuring the parameters listed in **Table 1**.

Table 1: Details of measured parameters at Lough Hyne between 2011 and 2013; SB = South Basin, NB = North Basin, WT = Western Trough. Y: parameter measured, N: Not measured

Start Date (YYYY-MM-DD)	End Date (YYYY-MM-DD)	Code	Depth (m)	Sample Number	pH	Salinity (ppt)	Dissolved Oxygen (mg L ⁻¹)	Temperature (°C)	Turbidity (NTU)	Chlorophyll a (µg L ⁻¹)	Oxidation / Reduction Potential (mV)
2011-04-09	2011-04-24	SB	10	1441	Y	Y	Y	Y	Y	N	N
2011-07-02	2011-07-31	NB	10	2781	Y	Y	Y	Y	Y	N	N
2011-05-10	2011-07-01	SB	10	4945	Y	Y	Y	Y	Y	N	N
2011-08-02	2011-09-26	WT	15	5199	Y	Y	Y	Y	Y	N	N
2011-08-02	2011-09-26	WT	35	5199	Y	Y	Y	Y	Y	N	N
2012-08-13	2012-12-09	WT	15	10844	Y	Y	Y	Y	Y	Y	N
2012-08-03	2012-12-09	WT	35	10844	Y	Y	Y	Y	Y	Y	Y
2013-03-24	2013-04-09	WT	35	1583	Y	Y	Y	Y	Y	Y	N

To examine variability in pH and other chemical parameters associated with seasonal hypoxia and anoxia, two sondes were deployed in the Western Trough (Grid Reference 51.5006°N, 9.3043°W at

1
2
3 depths of 15 m and 35 m in the location shown in Figure 1. Depths chosen were based on historical
4 measurements indicating that seasonal anoxia occurred below 25 m water depth during the summer
5 season ^{14, 16, 17}, thus 15 m and 35 m depths were expected to correspond to conditions above and
6 below the depth of the seasonal thermocline respectively. Sondes were also anchored in the North and
7 South Basins of Lough Hyne at a depth of 10 m during 2011 at the dates indicated in Table 1. Prior to
8 deployment, each sonde utilised was calibrated according to manufacturer's recommendations and all
9 sondes were set to collect data synchronously before deployment. A sampling interval of 15 min was
10 set for all instruments and data were periodically downloaded onsite. Collected data were analysed
11 using EcoWatch software for Windows (YSI Hydrodata, UK, version 3.18.00) and AQUARIUS 360°
12 software (Aquatics Informatics Inc, BC Canada, version 3.0 R2), and statistically analysed with R
13 statistical software ¹⁸.

24 25 26 27 28 29 **2.4 Water column profiles, Western Trough**

30 31 **2.4.1 Electrode measurements of pH**

32 Profiles were obtained of the water column in the Western Trough (Grid Reference 51.5006°N,
33 9.3043°W) with a multiparameter sonde simultaneously recording depth, salinity, turbidity,
34 temperature, dissolved oxygen (DO) and pH. The rate of descent for profile measurements was
35 approximately 5 m min⁻¹, and profiles were terminated at a depth of 40 – 42 m below the surface to
36 prevent the possibility of contact between the sensors and the seabed. To reduce sensor stabilisation
37 times, sensors utilised for profile measurements were first immersed in fresh seawater at ambient
38 temperatures collected from the surface of Lough Hyne and allowed to stabilise for at least 2 h prior
39 to conducting any profile measurements.

40 41 42 43 44 45 46 47 48 49 50 51 52 53 **2.4.2 Measurement of pH using 1952 methods**

54 To allow comparison between pH values from 1952 and contemporary values, pH profiles were also
55 carried out in the Western Trough of Lough Hyne in 2013 using the 1952 methods ¹⁴. These were
56 based on indicator dyes, (cresol red and thymol blue) compared against standards consisting of
57 Palitzsch's borate-boric acid buffers. The chemical methods were repeated as described in Bassindale
58
59
60

1
2
3 *et al.* (1957), and pH was measured at 18 °C, however, samples were retrieved from the water column
4
5 at the same location using a Niskin sampler, rather than the homemade sampling apparatus described
6
7
8 in their paper.
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

3 Results and Discussion

3.1 Natural variability of pH and DO in surface waters at LHMR

Simultaneous measurement of both DO concentrations and pH at Lough Hyne indicated that both variables were highly correlated during 2011 and 2012, albeit with large temporal and inter-site variability. The mean DO concentrations and pH at 10 m depth across both the North and South Basin sites were 9.37 mg L^{-1} ($n= 7918$, $sd = 0.56 \text{ mg L}^{-1}$) and 8.23 pH units ($n= 7918$, $sd = 0.08$) respectively. Considerable variation was found between measurement sites, with the South Basin having the highest mean pH and DO as summarised in Table 2. However both sites were well oxygenated and average pH values were above those values reported for coastal waters at this latitude

19 .

Table 2: Summary of DO concentration and pH at a depth of 10 m in the North and South Basins of Lough Hyne Marine Reserve during 2011.

Location and date	Mean DO conc. (mg L^{-1})	Mean pH	St. Dev DO	St. Dev pH
North Basin July 2011	9.70	8.30	0.659	0.02
South Basin May-June 2011	9.10	8.18	0.384	0.05
South Basin April 2011	9.92	8.34	0.286	0.02

Although both parameters were highly variable, positive linear relationships existed between pH and measured DO concentrations across both individual sites, and in the complete dataset from 10 m depth for 2011 (residual standard error: 0.0508 on 7916 degrees of freedom, multiple R^2 : 0.6122, p-value: < 0.001) (Figure 2). A positive linear relationship exists across the entire dataset, however, both intra- and inter-site variability can be observed in this relationship, which is especially pronounced between the measurement of pH in April 2011, and those measurements collected between May-July 2011.

Figure 2: The relationship between DO concentrations and pH (depth =10 m) in the North and South Basins at Lough Hyne.

The covariance of pH and DO, and independence from temperature, as measured at a depth of 10 m is shown in Figure 3, where the correlation between temperature, DO and pH is illustrated using Pearson's product moment correlation coefficient. The coupling of both diurnal and longer-term fluctuations in DO and pH can be observed over the 30 day deployment (Pearson's correlation coefficient between pH and DO = 0.818, 95% confidence interval 0.80 - 0.829, $t = 76.02$, $df = 2856$, p-value $< 2.2e^{-16}$; correlation between pH and temperature = 0.45, 95% confidence interval: 0.423 -

0.481, $t = 27.115$, $df = 2856$, $p\text{-value} < 2.2e^{-16}$; correlation between temperature and oxygen = 0.01, $t = 0.7747$, $df = 2856$, $p\text{-value} = 0.4386$, 95% confidence interval: -0.02 - 0.05).

Figure 3: Covariance of DO concentrations, temperature and pH at a depth of 10 m in the North Basin of Lough Hyne during July 2011.

3.2 Seasonal hypoxia and pH change in the Western Trough, Lough Hyne

Profile measurements are commonly taken with respect to depth to determine the presence of stratification in aquatic systems²⁰. Seasonal hypoxia, and eventual anoxia, in the water column below 25 m depth in the Western Trough of Lough Hyne was first demonstrated and measured by Bassindale *et al.* (1957) in 1952 by taking water column profiles at this location. In addition to DO and temperature measurements, Bassindale *et al.* (1957) also carried out the first studies of pH associated with seasonal hypoxia in the Western Trough, and it is these early profile measurements that provide an indication of the changes that have occurred in this location over the intervening 6 decades.

For comparison with the data of Bassindale *et al.*, profiles were periodically undertaken at the same location as the original study during 2012 (June until October). These profiles indicated that rather than dissipating suddenly, the thermocline depth (as indicated by the greatest rate of change in temperature with respect to depth) deepens gradually in the water column, increasing from an approximate depth of 25 m on the 28th June 2012 to 37 m on the 6th October 2012. Temperature, salinity, DO and pH measurements from these profiles demonstrate the gradients in these parameters associated with the presence of seasonal hypoxia at this location (Figure 4).

Figure 4: Profile measurements obtained during 2012 of pH, DO concentration, temperature and salinity from the Western Trough, Lough Hyne. Data from the profiles of Bassindale et al., (1952), have been included for comparison of pH (note that the y-axis (Depth) is inverted).

Table 3 summarises data from these profiles, where it can be seen that, although the mean and median are similar, the largest difference between the 1952 and 2012 data is in the maximum value recorded (8.27 pH units for 1952 against 8.12 pH units in August 2012). Figure 5 illustrates the relative comparison of the profiles of pH taken in July 1952 and through the summer of 2012.

Table 3: Comparison of profiles of pH in the Western Trough, Lough Hyne from 2012 and 1952.

Date	June 2012	Aug. 2012	Sept. 2012	Oct. 2012	July 1952
Min	7.57	7.53	7.51	7.53	7.64
1 st Qu.	7.78	7.56	7.65	7.97	7.70
Median	8.03	8.01	7.98	8.05	8.06
Mean	7.94	7.89	7.88	7.96	7.99
3 rd Qu.	8.06	8.11	8.05	8.05	8.25
Max	8.11	8.12	8.07	8.08	8.27

Figure 5: Boxplots of pH profiles at the Western Trough, Lough Hyne from 2012 and 1952 to a depth of approximately 42 m below the surface.

It is important to note that 2012 data shown were collected with a glass pH electrode, while those of 1952 were measured using indicator methods. The higher interquartile range can be seen in the 1952 data, while the median is comparable across the profiles measured from both years. Profiles produced using the 1952 measurements gave values comparable to those of the glass electrode obtained, however due to the time consuming nature of the methods of Bassindale *et al.*, and the difficulties in controlling for temperature and salinity variation, this was only achieved for June 2012. The differences in methods and the magnitude of the measurement errors associated with each may influence these results, and drawing any direct conclusions from comparison of these results is difficult.

3.3 Continuous measurement of pH at 35 m depth in the Western Trough during anoxia/hypoxia

While profiles provide a snapshot of conditions at a particular location and instant in time, deployment of continuous monitoring equipment can provide much greater temporal resolution of changes in water chemistry. For this reason, continuous monitoring of water column parameters including pH and DO concentrations at 15 and 35 m depth was conducted in both 2011 and 2012 during turnover and ventilation of seasonal anoxia, allowing relationships between these parameters to be established.

Measurements taken during seasonal stratification indicated that the anoxic waters in the hypolimnion at the Western Trough had a range of 7.5 – 7.6 pH units (measurements taken at a depth of 35 m, temperature range ~ 10 – 11 °C) when DO concentrations were below the limit of detection, and pH decreased linearly with time, culminating in the lowest recorded pH values (~ 7.44) recorded prior to ventilation of the water mass surrounding the 35 m sensor with fresh seawater in autumn. This

1
2
3 ventilation occurred at different dates in 2011 (September 12th – September 18th) (Figure 6) and 2012
4 (October 3rd – October 8th) (Figure 7). Comparison of ΔpH and ΔDO (defined as the difference
5 between the mean value for seven days before detection of DO values $> 0 \text{ mg L}^{-1}$ by the 35 m sensor
6 and the mean value for seven days after stabilisation of DO values) associated with the shift from
7 anoxic to normoxic water in 2011 and 2012 at 35 m in the Western Trough indicated a maximum
8 difference of 0.54 pH units for these two states for 2012 and 0.64 pH units for 2011.
9
10
11
12
13
14
15
16
17

18 *Figure 6: Continuous pH measurements (sampling interval = 15 min) recorded during transition of*
19 *the hypoxic waters at 35 m to those of the normoxic waters during September 2011. Vertical red lines*
20 *indicate a change > 0.1 pH units between consecutive measurements.*
21
22

23 However, in 2012 complete ventilation of the hypolimnion at LHMR had not occurred by the end of
24 the measurement period (01st November), and profile measurements indicated that a volume of anoxic
25 water remained trapped in the Western Trough at depths > 40 m after the sensor at 35 m had been
26 flushed with fresh seawater. This resulted in a slow reduction in measured DO concentrations and pH
27 values at the 35 m sensor, as the water column above the anoxic water appeared to revert back to
28 hypoxic conditions (Figure 7). This reduction was coupled with pronounced diurnal fluctuations in pH
29 superimposed on a trend of negative linear decrease in absolute values, until halted by another
30 ventilation event occurring after October 28th 2012, during which time strong diurnal fluctuations in
31 measured values were recorded.
32
33
34
35
36
37
38
39
40
41
42

43 *Figure 7: Continuous pH data (sampling interval = 15 min) recorded during transition of the hypoxic*
44 *waters at 35 m to those of the normoxic waters during October 2012. The boxplot comparison (inset)*
45 *shows the range of variation between the pH values recorded in 2011 and 2012 during the transition*
46 *from hypoxic to normoxic conditions.*
47
48
49

50 Incomplete flushing of the water column below 35 m is reflected in comparison of the pH datasets for
51 2011 and 2012 (Figure 8), where a clear distinction between the two conditions can be observed in the
52 dataset for both years, however the reduction in pH for normoxic waters associated with incomplete
53 ventilation of the water column in the 2012 is evident.
54
55
56
57
58
59
60

Figure 8: Probability density of pH measurements recorded in both anoxic and normoxic conditions
at LHMR during 2011 and 2012 at a depth of 35 m.

3.4 Relationships between pH and DO concentration during transition from hypoxic to anoxic conditions at Lough Hyne

A strong linear positive relationship existed for both pH and DO concentrations for a DO concentration range of 2 – 8 mg L⁻¹ for the combined 2011 and 2012 data (Residual standard error: 0.05491 on 7070 degrees of freedom, multiple R²: 0.9231, p-value: < 0.001). However, the fit of the linear model between DO and pH was significantly improved if values containing DO values below the limit of detection (approaching 0 mg L⁻¹) of the YSI ROX™ optical DO probe were excluded (Figure 9).

Figure 9: Relationship between DO concentrations and pH during transition from hypoxia to normoxic conditions at Lough Hyne Marine Reserve during 2011 and 2012. A line of best fit through the data for both years represents LOWESS smoothing.

This pH change is likely solely related to temperature change when DO concentrations are below the limit of detection of the probes used at this location, and at this depth and location there is a steady increase in temperature during anoxic conditions. This in turn results in a negative linear relationship between pH and temperature, producing an approximate reduction in pH values of 0.06 pH units per month.

A mean of 7.58 pH units (time period (YYYY-MM-DD): 2011-08-02: 2011-09-03, n = 3060, SD = 0.015, range = 0.08 pH units) was measured during anoxic conditions in 2011, illustrating the stable conditions in the anoxic waters once this water mass is fully formed. During the same period, an increase in temperature of 0.3 °C was measured. The drivers of this increase in temperature and resulting decrease in pH at this depth are unclear, although either increasing microbial activity with time or gradual thermal exchange between the epilimnion and hypolimnion may perhaps have a role in causing the observed trends.

During ventilation of the anoxic waters at 35 m and transition to normoxic waters during autumn 2011 and 2012, a maximum daily range of 0.4 pH units was measured. The absolute difference in pH between the two states was much greater in 2011 than in 2012 and deepening of the thermocline past the 35 m depth monitoring station occurred later in 2012. Interestingly, evidence of thermocline deepening at the 35 m depth sensor was first detected in pH and temperature

1
2
3 measurements, while fluctuations in DO concentrations occurred over a day later, suggesting that the
4
5 oxycline is separated from the closely coupled chemocline and thermocline.
6

7
8 Lough Hyne, in common with marine reserves worldwide, acts as a vital refuge, habitat and
9
10 nursery for marine organisms, including commercially important species ²¹ . Understanding real
11
12 threats to these areas is therefore of vital importance in providing at least some possibility for timely
13
14 intervention and mitigation of likely negative impacts if the integrity of reserves such as Lough Hyne
15
16 are to be maintained. Ocean acidification and associated pH change are amongst a number of threats
17
18 to the reserve, and compete with changes in nutrient status and introduction of alien species for
19
20 designation as the most pressing threat to the biota of the Lough. The present study has demonstrated
21
22 through the use of direct pH measurements at Lough Hyne, that various scales of pH change occur,
23
24 both associated with well-oxygenated surface waters and associated with seasonal hypoxia. Surface
25
26 waters demonstrate a covariance with DO rather than temperature measurements, indicating that
27
28 biological activity and primary production are perhaps the main drivers of localised changes in pH in
29
30 surface waters at Lough Hyne. Such relationships have recently been demonstrated for other systems
31
32 (for example kelp forests and aquaculture systems ^{22, 23}). Measurements at all locations within Lough
33
34 Hyne demonstrated a positive linear relationship, whereby pH measured *in situ* decreased with
35
36 decreasing DO concentrations. These localised fluctuations can range over 0.4 pH units in extreme
37
38 cases during a summer season, but are typically constrained between pH 8.1 and 8.3.
39
40
41

42
43 Using electrode measurements for comparison with profile measurements from 2012 with a
44
45 published historical pH profile indicate that, although the shape of the profile associated with pH
46
47 change during seasonal anoxia in the Western Trough has remained largely stable over 60 years, a
48
49 reduction of > 0.1 pH units from 0 - 25 m depth may have occurred at this location. Records from the
50
51 CO₂ monitoring site at Mauna Loa, Hawaii, show that the CO₂ concentrations were around 310 ppm
52
53 in the mid-1950s but now exceed 400 ppm, therefore there has been an increase of greater than 90
54
55 ppm in the intervening 60 years. McGrath *et al.*, have found that there has been a resultant
56
57 acidification of the subsurface waters in the Rockall Trough by ~ 0.020 pH units per decade ⁹ . Taking
58
59 this into account, and assuming a constant rate of change before 1991, the resultant change in pH at
60
Lough Hyne over the 60 years from 1952 to 2012 would be expected to be about 0.12 pH units.

1
2
3 Comparison of the maximum pH values taken during profiles in 1952 and 2012 indicates that
4 differences in the maximum values recorded in the surface waters are actually between 0.15 and 0.20
5
6
7 pH units, rather greater than predicted. Nevertheless, as has been demonstrated with continuous
8
9 monitoring of the surface waters, such changes are within the ranges of localised pH variability during
10
11 the growth season, and thus inter-annual variability and localised events may account for such
12
13 differences.
14

15
16 Localised topographic and bathymetric profiles of semi-enclosed marine systems can result in
17
18 natural zones of low oxygen, such as those of the fjord systems of Norway or enclosed seas such as
19
20 the Black Sea. These natural systems are separate from those of recent “dead zones” induced by
21
22 changes in ocean circulation or coastal eutrophication. Lough Hyne belongs to the former category
23
24 and there is no reason to suspect that the Western Trough has not experienced seasonal hypoxic or
25
26 anoxic conditions on an annual basis since becoming a marine Lough some 4000 years ago. The
27
28 volume of anoxic waters at Lough Hyne has been demonstrated to comprise 15% of the total Lough
29
30 volume, when the anoxic zone is at its fullest extent during the summer months¹¹. If the chemical
31
32 characteristics of anoxic waters at Lough Hyne are similar to those of anoxic zones worldwide, then
33
34 this water volume is rich on dissolved H₂S and ammonia. The results of this paper have shown that
35
36 this water is certainly reduced in pH by approximately 0.6 pH units in comparison with the overlying
37
38 mixed water mass. Therefore, release of this reduced pH water into the overlying waters during
39
40 seasonal thermocline deepening may influence the pH of the waters of the Lough as a whole unless
41
42 sufficient buffering capacity exists to negate the influences of this release. The exposure time of
43
44 organisms to this water and the extent to which this influences species distribution within the Lough
45
46 requires further study.
47
48
49

50
51 The final question to answer concerns the effect of pH fluctuations on the flora and fauna of
52
53 Lough Hyne. Interestingly, Lough Hyne is a highly diverse system with many microhabitats that have
54
55 previously provided a successful cultivation area for many otherwise rare species. If Lough Hyne has
56
57 annually experienced wide pH ranges on both temporal and spatial scales, as measured in the current
58
59 work, since becoming a marine system, then perhaps such variability has had a role in maintaining
60
biodiversity at this location. Nonetheless, the extent to which this variability is a driver of ecosystem

1
2
3 diversity and the point at which pH variability becomes a limiting factor in the survival of sensitive
4 species is a key question for future research.
5
6

7 8 **4 Conclusions** 9

10 This study has demonstrated the scales of variability of pH in relation to other key
11 environmental variables such as DO and temperature in a semi-enclosed marine system that
12 experiences seasonal hypoxia. Measurement of surface waters (10 m depth) at Lough Hyne have
13 demonstrated that these waters are well oxygenated but show both temporal and spatial variability in
14 measured pH, while generally maintaining a linear relationship between measured DO concentrations
15 and pH. We have confirmed that the chemocline, as evidenced by pH measurements, is closely
16 associated with the oxy- and thermoclines at depth (35 m) in the Western Trough at Lough Hyne.
17 Comparison of water column profile measurements in the Western Trough of Lough Hyne in 2012
18 with historical measurements from the same location in 1952 provide some indication that the
19 maximum pH measured in profiles is now lower than previously, however any changes are within the
20 measurement error of both indicator and probe methods available and cannot be utilised to draw firm
21 conclusions. Nevertheless, gradients in pH associated with transition from anoxic to normoxic
22 conditions have been measured at as much as 0.6 pH units, and demonstrate a strong positive linear
23 relationship with DO concentrations in the range of 2 – 8 mg L⁻¹ in both 2011 and 2012. These natural
24 fluctuations in pH can be greater than those changes presently predicted by developing ocean
25 acidification, and organisms present in this enclosed system may already be experiencing extremes of
26 pH that wider oceanic ecosystems will only experience as ocean acidification develops. Thus, we
27 would suggest that Lough Hyne provides an ideal location to study the effects of future ocean
28 acidification, and that a continuous monitoring programme to understand the effects of climate change
29 on near-shore ecosystems should be implemented without delay.
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52

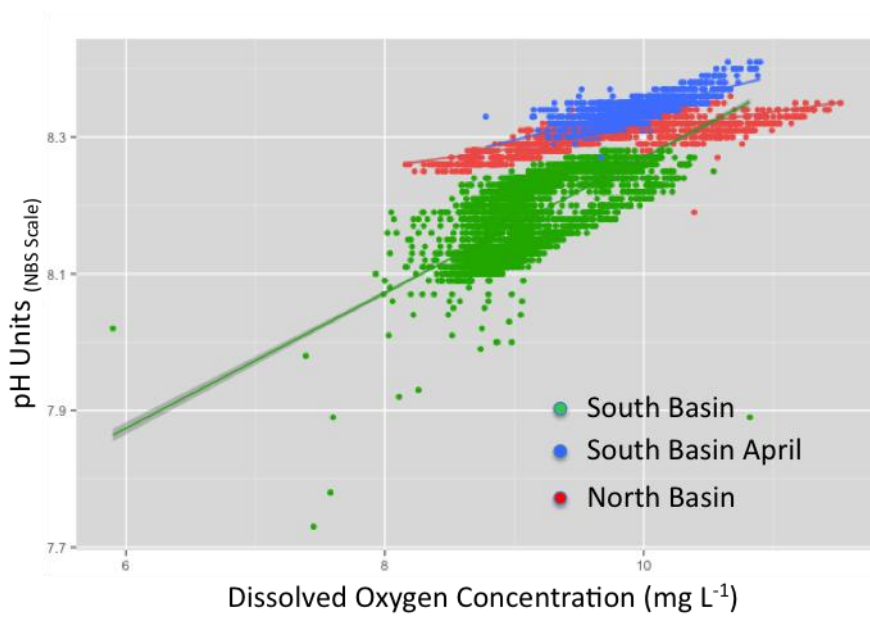
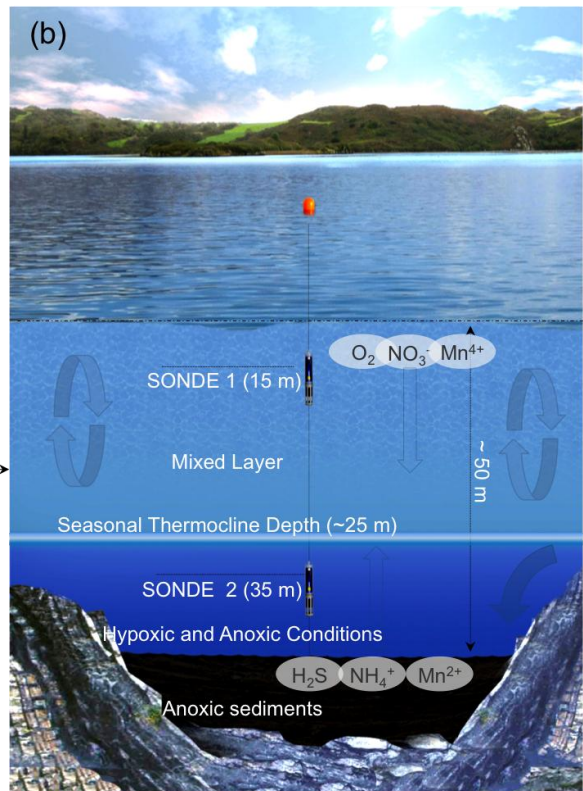
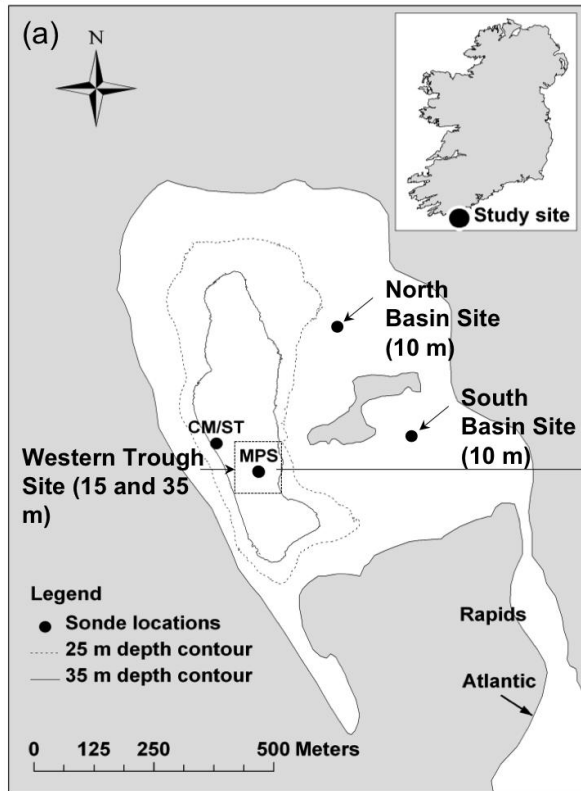
53 54 55 **Acknowledgments** 56

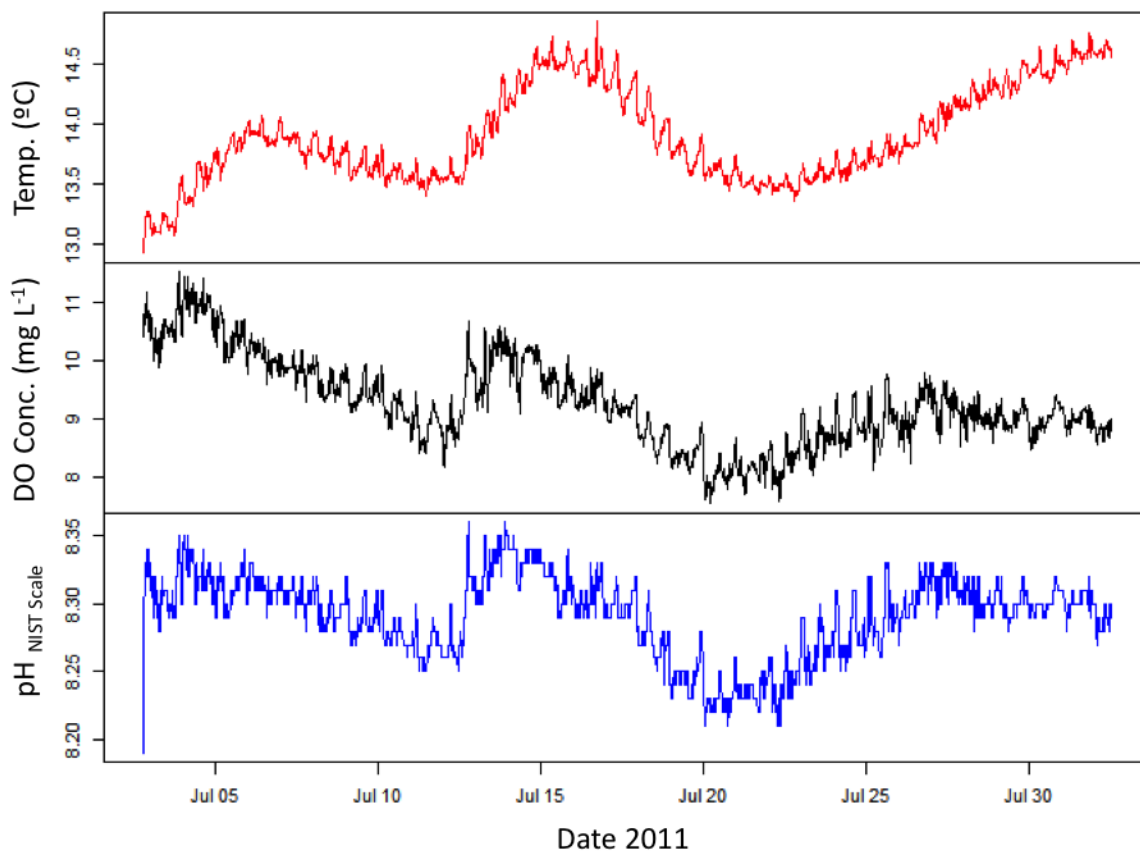
57 We wish to thank Patrick Graham and Declan O' Donnell from the National Parks and
58 Wildlife Service of Ireland for permission to conduct this work in Lough Hyne Marine Reserve. The
59 authors would also like to thank the anonymous reviewers for their valuable comments and
60 suggestions to improve the manuscript. T. S. acknowledges the support of the Beaufort Marine

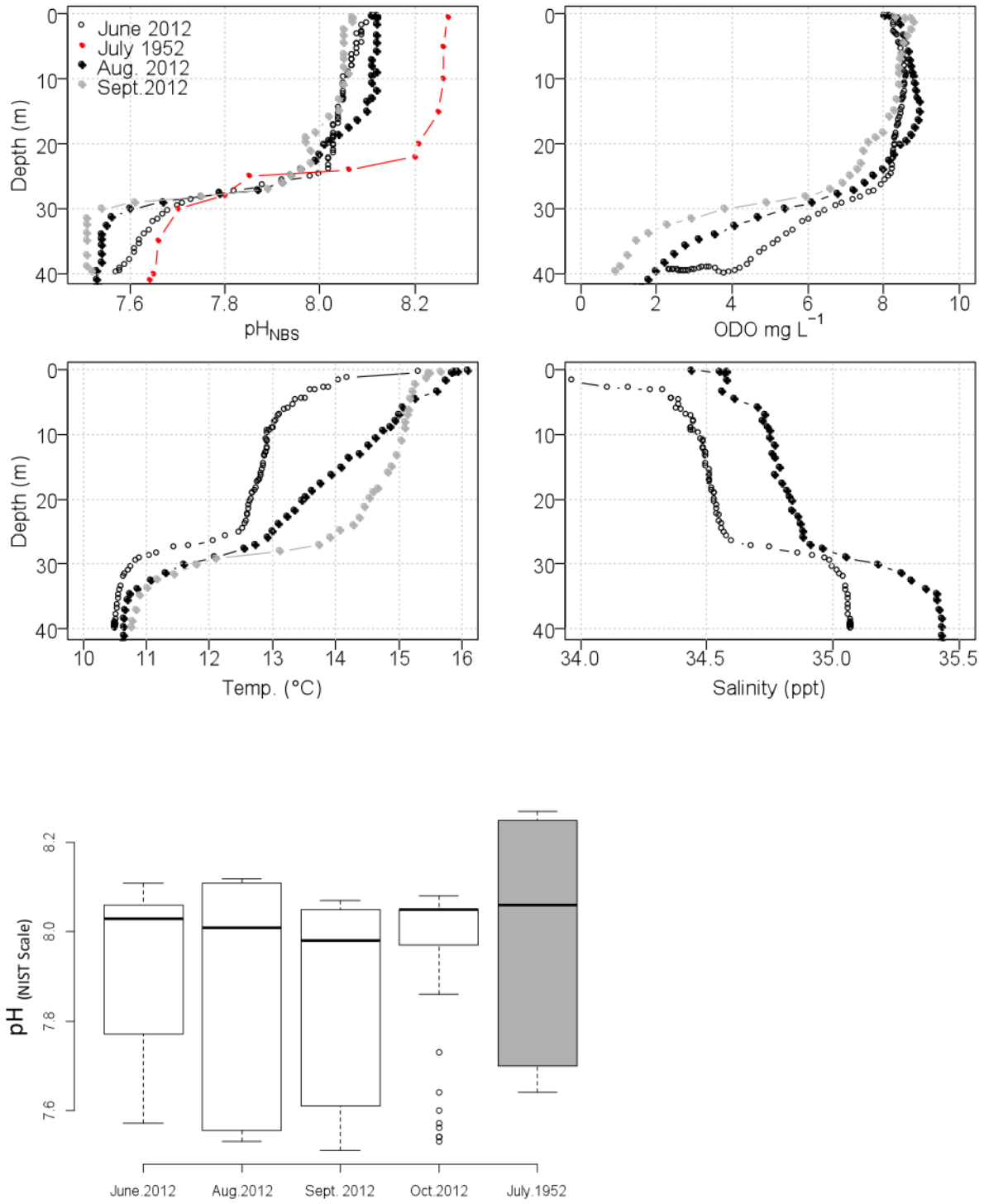
1
2
3 Research Awards, carried out under the Sea Change Strategy and the Strategy for Science Technology
4 and Innovation (2006-2013), with the support of the Marine Institute, funded under the Marine
5 Research Sub-Programme of the National Development Plan 2007–2013. No financial relations exist
6 between the authors and the commercial identities mentioned in this study.
7
8
9

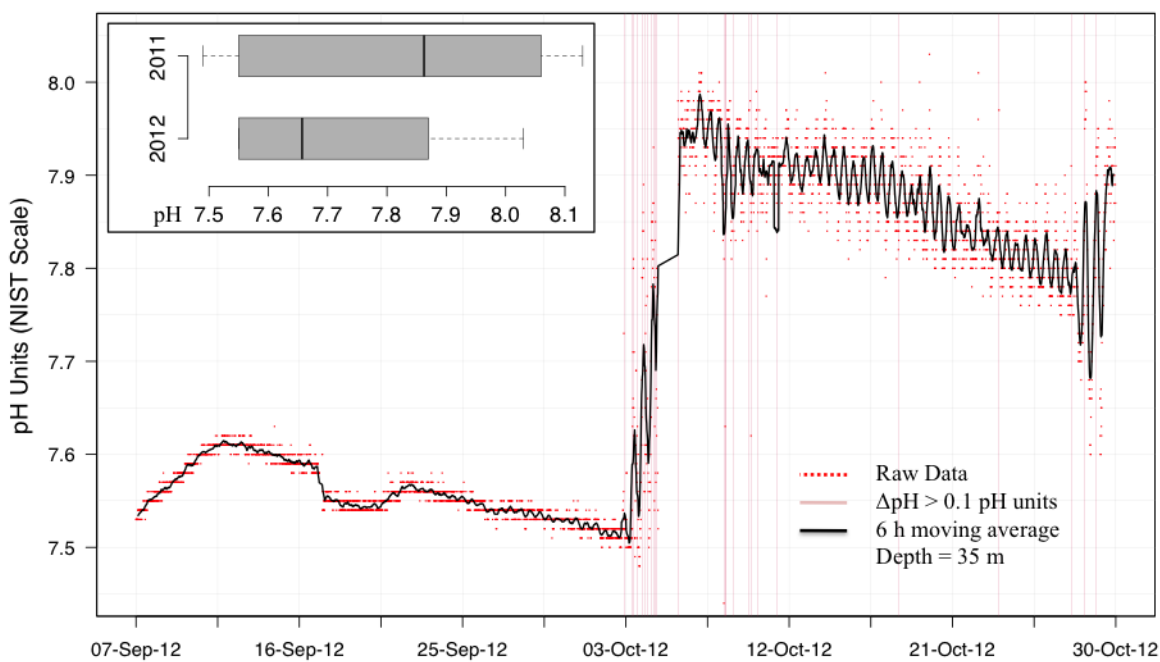
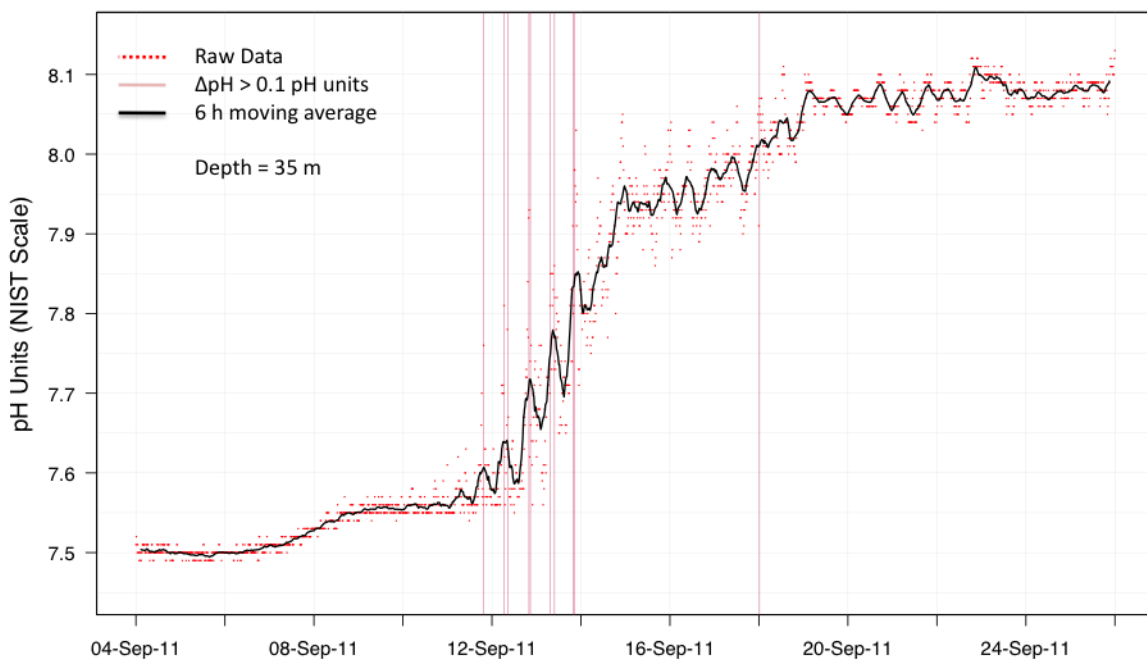
10 5 References

- 11
12 1 F. Melzner, J. Thomsen, W. Koeve, A. Oschlies, M. A. Gutowska, H. W. Bange, H. P. Hansen and A.
13 Körtzinger, *Mar. Biol.*, 2012, **160**, 1-14.
14 2 R. A. Feely, C. L. Sabine, R. H. Byrne, F. J. Millero, A. G. Dickson, R. Wanninkhof, A. Murata, L. A. Miller
15 and D. Greeley, *Global Biogeochem. Cycles*, 2012, **26**, 1-15.
16 3 K. Caldeira and M. E. Wickett, *Nature*, 2003, **425**, 365-365.
17 4 R. A. Feely, S. C. Doney and S. R. Cooley, *Oceanography*, 2009, **22**, 36-47.
18 5 J. T. Wootton, C. A. Pfister and J. D. Forester, *Proceedings of the National Academy of Sciences*, 2008, **105**,
19 18848-18853.
20 6 J. M. Santana- Casiano, M. González- Dávila, M. Rueda, O. Llinás and E. González- Dávila, *Global*
21 *Biogeochem. Cycles*, 2007, **21**, 1-16.
22 7 N. R. Bates and A. J. Peters, *Mar. Chem.*, 2007, **107**, 547-558.
23 8 J. Olafsson, S. Olafsdottir, A. Benoit-Cattin, M. Danielsen, T. Arnarson and T. Takahashi, *Biogeosciences*,
24 2009, **6**, 2661-2668.
25 9 T. McGrath, C. Kivimäe, T. Tanhua, R. R. Cave and E. McGovern, *Deep-Sea Research Part I-Oceanographic*
26 *Research Papers*, 2012, **68**, 79-91.
27 10 G. E. Hofmann, J. E. Smith, K. S. Johnson, U. Send, L. A. Levin, F. Micheli, A. Paytan, N. N. Price, B.
28 Peterson and Y. Takeshita, *PLoS One*, 2011, **6**, e28983-e28983.
29 11 T. Sullivan, S. Broszeit, K. O'Sullivan, R. McAllen, J. Davenport and F. Regan, *Estuar. Coast. Shelf Sci.*,
30 2013, **123**, 26-33.
31 12 M. Jessopp, R. McAllen, J. O'Halloran and T. and Kelly, *Nutrient and Ecosystem Dynamics in Ireland's*
32 *Only Marine Nature Reserve (NEIDIN): Final Report for the STRIVE-funded project: 2007-FS-B-4-M5*, 66,
33 Environmental Protection Agency, Ireland, 2011.
34 13 R. McAllen, J. Davenport, K. Bredendieck and D. Dunne, *J. Exp. Mar. Biol. Ecol.*, 2009, **368**, 67-74.
35 14 R. Bassindale, E. Davenport, F. J. Ebling, J. A. Kitching, M. A. Sleight and J. F. Sloane, *J. Ecol.*, 1957, **45**,
36 879-900.
37 15 F. J. Ebling, J. A. Kitching, R. D. Purchon and R. Bassindale, *J. Anim. Ecol.*, 1948, **17**, 223-244.
38 16 J. Kitching, F. Ebling, J. Gamble, R. Hoare, A. McLeod and T. Norton, *J. Anim. Ecol.*, 1976, **45**, 731-758.
39 17 V. M. Thain, J. Jones and J. Kitching, *The Irish Naturalists' Journal*, 1981, **20**, 292-295.
40 18 R Development Core Team, *R: A language and environment for statistical computing*, 2012.
41 19 S. N. Longphuirt, D. Stengel, C. O' Dowd and E. McGovern, *Ocean Acidification: An Emerging Threat to*
42 *our Marine Environment*, Marine Foresight Series No. 6, 2010.
43 20 J. S. Read, D. P. Hamilton, I. D. Jones, K. Muraoka, L. A. Winslow, R. Kroiss, C. H. Wu and E. Gaiser,
44 *Environmental Modelling & Software*, 2011, **26**, 1325-1336.
45 21 M. Jessopp and R. McAllen, *Hydrobiologia*, 2008, **606**, 81-92.
46 22 C. Zang, S. Huang, M. Wu, S. Du, M. Scholz, F. Gao, C. Lin, Y. Guo and Y. Dong, *Water, Air, & Soil*
47 *Pollution*, 2011, **219**, 157-174.
48 23 C. Frieder, S. Nam, T. Martz and L. Levin, *Biogeosciences Discussions*, 2012, **9**, 3917-3930.
49
50
51
52
53
54
55
56
57
58
59
60









Analytical Methods Accepted Manuscript

