Long-term changes in Phytoplankton Lifeforms and SST at the Lorn Pelagic Observatory, v.1

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Abstract

This note reports methods used in 2019 to obtain and process phytoplankton data from the Lorn Pelagic Observatory ($\sim$ 56.5°N, 5.5°W) for comparison with long-term changes in Sea-Surface Temperature (SST) according to ICOADS. The methods, and some associated issues are illustrated with data for Diatoms and the dinoflagellate genus *Ceratium*. Analyses were also made for 'Reliably Identified Phototrophic Armoured Dinoflagellates' (RIPAD) and (oligotrich) Ciliates. There has been a significant upwards trend in SST and a significant downwards trend in Diatoms cells/L, over the period from 1970 to 2015 (with no data 1982-1999) and these trends are correlated (table 3). There were no long-term temporal trends in the abundances of the other groups, but RPIDAL and Ciliates cells/L were significantly inversely correlated with SST. The work is a contribution to the ICEGRAPH project.

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1 Introduction

The *Lorn Pelagic Observatory* (LPO) is a (to some extent *post hoc*) programme of observation of the pelagic habitat in a region at the northern end of the Firth of Lorn, a large fjord in western Scotland, near the Scottish Marine Institute of SAMS and the town of Oban. The *pelagic habitat* comprises water and plankton; this note is concerned with certain subgroups within the phytoplankton and the relationship between their changes and those in *Sea-Surface Temperature* (SST). It is a contribution to the ICEGRAPH project, and illustrates methods, results and statistical challenges with data for the 'Diatom' life-form and the dinoflagellate genus *Ceratium*. Data have also been analysed for two additional groups: 'Reliably Identified Phototrophic Dinoflagellates' (RIPAD) and (oligotrich) Ciliates. The term *microplankton* is used to refer to pelagic protoctists in general: only the Diatom group is exclusively autotrophic; the dinoflagellates include myxotrophs; and the Ciliates are heterotrophs.
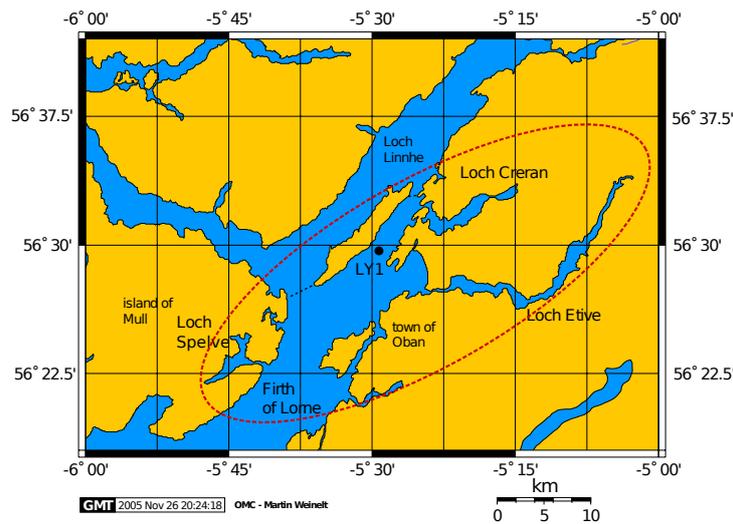

Figure 1: Map showing the Lorn Pelagic Observatory (LPO), which lies within the red ellipse. For the present work, only data from stations near LY1, and in Loch Creran, were used.

2 Methods

2.1 LPO stations

The northern part of the main basin of the Firth of Lorn shallows into a channel called the Lynn of Lorn, and water-sampling at the station LY1 at

the entrance to this channel, at 56.5°N, 5.5°W, commenced in 1970. Sampling in the small fjord or sea-loch Creran, which exchanges with the Lyn of Lorn, commenced in 1972. This phase concluded in 1984; a new phase of observation commenced in 2000. Studies were made in the course of research programmes with specific aims, including, early on, the documentation of conditions in these water-bodies. It was circa 2000 that the value of maintaining a time-series observatory began to be appreciated. Nevertheless, the pattern of observations (including the stations visited) depended on the research aim and funding of the research projects current in a given year.

2.2 Microplankton sampling and microscopic analysis

Water samples were taken with ship-lowered bottles (initially of the NIO type and more recently using the Niskin type) or Collins-type (or similar) bottles lowered from piers in Loch Creran. In a few cases a hose was used to take an integrated sample 0 - 10 m. Samples were preserved with approx 1% acidified Lugol's Iodine and examined by inverted microscopy. Microplankters were counted as cells (even when forming chains or colonies) after sedimentation in a 10 ml chamber (in some cases 50 ml). Larger, rarer organisms were counted across the whole chamber base; smaller, more abundant organisms were counted in strips or eyepiece fields of known size. Appropriate multiplication factors were later applied to convert to cells/L (table 1).

Identification was initially attempted to species, as these were described c. 1970 in standard texts (Lebour, 1925, 1930; Cupp, 1943). Where this was not possible, broader categories were used, such as 'small flagellate' and 'oral [i.e. oligotrich] ciliate'. As taxonomy evolved, it came to seem difficult to identify to species level from light-microscopy and in the context of completing sample counts within a few hours. Thus many organisms were in recent years identified only to genus (e.g. *Skeletonema*, or to subgenus levels (e.g. '*Pseudo-nitzschia delicatissima* group'), or to a size group within a genus (e.g. '*Chaetoceros* < 20μm'). Consistency was however maintained because each identified taxon or grouping was given a number for purposes of digitisation, and this number was conserved against taxonomic changes.

Methods are described in Tett (1973); Tett et al. (1981); Fehling et al. (2006); Tett (2013, 2014); Whyte et al. (2017).

2.3 Lifeforms

Abundances are reported here for four lifeforms or microplankton groupings (table 2). **Diatoms** were chosen as the group in LPO plankton that likely makes the greatest contribution to new primary production and that (despite taxonomic churn) can be considered reliably enumerated over the period from 1970 until the present.

Table 1: Main counting patterns and multiplication factors. The multiplication factor (MF) converted cells counted into cells/L. Optical details for Wild M40 microscope; other inverted microscopes were also used.

MF	
1	used for counts already converted into cells/L
100	10 mL sedimented, entire chamber base examined (with x10 phase objective, x15 eyepiece and 1.5x intermediate magnification)
490	10 mL sedimented into chamber of diameter 2.5 cm; central 1 cm examined (x 10 ph obj, x15 ep, 1.5 x)
5250	10 mL sedimented, 2 transects across entire chamber (with x20 ph obj, x10 transect eyepiece, 1.5x)
16550	10 mL sedimented, 2 transects across central 1 cm square (with x20 ph obj, x10 transect eyepiece, 1.5x)

Table 2: LifeForms used. LF columns give the numeric codes used (in `volf_Jul2019_AugLF.xlsx`) to assign taxa to a lifeform/group. rel = reliability, with maximum 5.

		LF1	LF2	rel
Diatoms	*All diatoms*: all taxa and groupings that belong to the Baccillariophyceae, including those characterised as *tychopelagic*;		1	5
Ceratium	All species belonging to the genus *Ceratium* of large dinoflagellates, including those sometimes assigned to genus *Tripos*	14		5
RIPAD	'Reliably Identified Phototrophic Armoured Dinoflagellates', including the *Alexandrium, Ceratium, Dinophysis, Gonyaulax* (and related genera), *Heterocapsa triquetra, Prorocentrum micans, Scrippsiella*, and similar chloroplast-containing armoured dinolfagellates above 15 μm in length		4	4
Ciliates	Pelagic oligotrich ciliates, including tintinnids and caseless oligotrichs, often not identified precisely, but probably including genera such as *Laboaea, Strombidium* etc in the 'oral ciliates' (as they were called) and *Heliocostomella, Tintinnopsis* etc amongst tintinnids.	105		3

'Dinoflagellates' are the usual partner group to Diatoms, but the representatives of the Dinophycae present in LPO samples comprise a mixture of heterotrophs and myxotrophs, including 'naked' forms and dinoflagellates less than 15 μm that may not have been reliably or consistently enumerated. Thus the group reported here is that called **RIPAD** ('Reliably Identified Phototrophic Armoured Dinoflagellates'). It exlcudes naked dinoflagellates, very small dinoflagellates, and armoured heterotrophs.[1] Because the RIPAD category is slightly fuzzy (see appendix A), RIPAD abundances were given a lower level of reliability than diatoms.

Ceratium comprised a group of large, distinctively shaped, chloroplast-containing dinoflagellates, consistently identified and enumerated. The main issue in this case was that for much of the year the group's abundance was too low for any members to appear reliably in sedimented subsamples of between 2 and 50 mL of seawater.

The group **Ciliates** included a variety of heterotrophic oligotrich ciliates, some inhabiting cases (tintinnids), others not. Both preserved well in Lugol's iodine, albeit the staining making it dificult to see characteristics allowing identification to genus. Neveretheless, the concentration of cilia at the oral end of the cell allowed reliably assignation of all 'oral ciliates' to this group even when generic or specific identifications were not made. The group's consistency score is however lower than that for RIPAD, because it is possible that ciliates were ignored in some years because attention was focussed on 'phytoplankton'.

2.4 Computational methods for phytoplankton

Digitisation

Digitisation began in 1981 to provide data that could be processed by a HP9825 desk-top programable computer. The analysis programs written for this machine and OS were subsequently adapted and improved (post 2005) using Matlab.[2] However, the 9825 data-tapes could no longer be read in 2005, and a re-digitisation was necessary, as well as initial digitisation of 21st-century data. In order to avoid future losses, the data were stored in files with a row-oriented (ascii-coded) text data base (ROTDB). These files are platform independent and can be read by a variety of software, including in the present case the BBEdit text editor and the MATLAB™ programming, analysis and visualisation system. Further details are given in appendix B.

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[1] 'Armoured' dinoflagellates exhibit a rigid cell wall made up of cellulose plates. 'Naked' dinoflagellates lack these and preserve less well.

[2] 'Matlab' is used to refer to the programming language as well as the software package MATLAB™.

Analysis of digitised data

Three Matlab programs were used. Two were started in 2006 – 2008 as recreations of the HP programs written in 1981-2, but have subsequently grown to include more functions. The current versions are `HPLP3Ga`, nicknamed 'ENV', and `HPLF2e`, nicknamed 'WAVES'. The third program was started in 2005 to implement the 'Plankton Index' (PI) tool (called PCI in Tett et al., 2008) and is currently in version `PI2C`.

Data were extracted from the ROTDB by ENV, and further processed with WAVES. Each resulting data-file was input to the PI tool to make the diagrams shown, and the statistics reported, here. Details of processing are given in appendix C.

ENV is documented by Tett (2019). In addition to loading data-base files, ENV used two auxiliary files:

- VOLF, containing species codes, typical cell volumes, and life-form assignments, in version `volf_Jul2019_AugLF.xlsx`;

- MULTFAC, containing a look-up table of multiplications and 'factor codes' (corresponding to particular patterns of observation of a sedimentation chamber base), in version `multfac_Oct2017.csv`.

Data were selected from all stations in the Lynn of Lorn and Loch Creran (including piers), and from water depths between 0 and 10 m. They were aggregated to the lifeforms in Table 2. For each life-form, ENV exported a text file containing columns for year, day-of-year, cells/L and biomass (mm^3 cell volume/L). The examples given here refer to the Diatom-Ceratium pair, which was used to explore issues and develop Matlab code.

WAVES was used to merge the data files for Diatoms and Ceratium, employing its *union* option. In effect, this used the diatom column as an indicator of samples taken, and entered a zero into the Ceratium column in any row where there was no record for this lifeform. WAVES output was a text-file with columns for 'year.day' (e.g. `1970.0164`), Diatom cells/L and Ceratium cells/L. The same strategy was used with the other lifeforms, constructing a Diatom-RIPAD and Diatom-Ciliate pair.[3]

The PI tool is a set of Matlab functions initially developed to calculate and plot a value of the *Plankton Index* (Tett et al., 2008), and subsequently expanded (Tett, 2016). For the present work, the set was updated to `PI2C` with the addition of a new function `annualplot`, which:

- makes contour plots of lifeform abundances (and SST) on year-month surfaces, exemplified in figure 2 ;

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[3] The contents of the Diatom column differed slightly amongst the three pairs, because there were a few samples without diatoms but containing at least one of the other lifeforms.

- inputs the year-month-value matrices to function `climateroll` to return vectors of annual mean values (of lifeform abundance or SST), using a 'rolling climate' interpolation to replace missing values;

- plots mean values against year (exemplified in figure 4) and calculate Kendall and Pearson correlations with time and between lifeform abundances and SST.

Log-transforms and 'no data'

The files output by the WAVES union option included values of zero on dates when only one member of the lifeform pair had been observed. This could have given rise to problems when abundances were log-transformed for plotting or during the calculation of annual means.

All three programs applied a logarithmic transform to microplankton abundances. There were several reasons for this, including the need to display changes at low abundances with as much precision as those at high abundance. The transformation was also needed for statistical reasons, so as to "stabilize the variance" and ensure a normal distribution of error (Barnes, 1952). However, because $\log_{10}(x = 0) = -\inf$, it was necessary to add a finite positive z to abundance x, giving the transformation $\log_{10}(x + z)$.

What should z have been? Barnes (1952) gave $z = 1$. This has the advantage of placing observed 0 values at 0 on the transformed scale, since $\log_{10}(1) = 0$. However, given that estimated abundances came from the multiplication $M(C + 1)$, where C is the raw count, M a multiplier to convert raw counts to numbers per Litre, and $M \cdot C = x$, there was a strong argument that z should equal M, or, perhaps $0.7M$ Tett (2013).[4]

The group Ceratium was the lowest in abundance (measured as cells/L) and thus corresponded to the largest volume sedimented and microscopically scanned. During the 1970s, *Ceratium* spp. were typically counted in a settled volume of about 2 ml, with $M = 490$. Post-2000, 10 mL were examined ($M = 100$) or in some 50 mL ($M = 20$). Because the computer programs required a single value, this was set as standard to the theoretical compromise of $z = 50$ cells/L. However, for comparability with methods used by other contributors to ICEGRAPH, the PI tool was also run with $z = 1$ cells/L.

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[4] Although not relevant to the ICEGRAPH study, the matter of an appropriate value for z arises when abundances are reported as biovolumes. They rarely exceed 10 mm^3/L, an thus taking $z = 1$ compresses the dynamic range of abundance to one order of magnitude. In these cases, z is best set to a value close to the lowest reported biovolume for a lifeform, perhaps 0.0001 mm^3/L. See example in figure 9.

2.5   ICOADS SST data

The "International Comprehensive Ocean-Atmosphere Data Set (ICOADS) offers surface marine data spanning the past three centuries, and simple gridded monthly summary products for 2° latitude x 2° longitude boxes back to 1800 (and 1° x 1° boxes since 1960) – these data and products are freely distributed worldwide."[5] Monthly values of Sea-Surface Temperature (SST) in the ICOADS box containing the LPO were extracted and supplied by Jacob Bedford (Plymouth University). They are understood to be synthesised from several data types, including CTD measurements made during research cruises or from moored or drifting buoys, and temperature measurements from the sea-water intakes in the engine rooms of commercial ships.

The ICOADS data used came from observations between 56.5° and 57.5° N, 5.5° and 6.5°W. LY1 lies at the bottom right of this 'square'. The spatial distribution of ICOADS observations within the square is unknown.

3   Results

The contour plots of lifeform abundances or SST on year-month surfaces, such as those shown in Figures 2 and 3, allow inspection of the data and its gaps. In the case of microplankton, there are no data between 1982 and 1999, and a dearth of Winter data between 2003 and 2010. There was a scarcity of SST data before 1975 and, seemingly, after 2013. Nevertheless, there were sufficient data to establish seasonality and multi-decadal changes in the contour diagrams and to calculate annual means for trend and correlation analyses.

Time-series of annual means are shown in figures 4 and 5 for the Diatom-Ceratium pair, in figure 6 for RIPAD and figure 7 for Cilates. The time-series of annual mean SST has been included in all these diagrams. Regression lines have been fitted where values correlate significantly with year.

The two sets of diagrams for the Diatom-Ceratium pair show the effect of the different values of z. However, these differences do not appear to have resulted in significant differences in the correlation statistics summarized in Table 3. These include significant correlations between SST and year (+ve) and Diatoms and year (−ve), and significant inverse correlations between Diatoms, RIPAD and Ciliates, on the one hand, and SST, on the other hand.

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[5] icoads.nasa.gov.

Table 3: Correlations amongst annual means of lifeforms, annual means of SST and year. All microplankton abundances (in cells/L) transformed $\log_{10}(x+z)$ with two values of z. τ is the Kendall rank correlation coefficient and ρ the Pearson product-moment correlation coefficient. p gives the probability of the observed correlation on a hypothesis of random association, although without correction for autocorrelation. The final column marks correlations significant at $p \leq 0.05$.

| C1	+	C2	$z = 1$		$z = 50$		
			τ (p)	ρ (p)	τ (p)	ρ (p)	
SST	+	year	$+0.44(0.000)$	$+0.65(0.000)$	$+0.44(0.000)$	$+0.65(0.000)$	↑
Diatoms	+	year	$-0.30(0.03)$	$-0.49(0.01)$	$-0.30(0.03)$	$-0.50(0.01)$	↓
Ceratium	+	year	$+0.09(0.52)$	$+0.29(0.15)$	$+0.18(0.19)$	$+0.09(0.66)$	
Diatoms	+	SST	$-0.43(0.004)$	$-0.59(0.003)$	$-0.43(0.004)$	$-0.59(0.003)$	↓
Ceratium	+	SST	$+0.19(0.20)$	$+0.43(0.04)$	$+0.06(0.69)$	$+0.08(0.72)$	
Diatoms	+	year	$-0.32(0.02)$	$-0.51(0.01)$	$-0.31(0.02)$	$-0.51(0.01)$	↓
RIPAD	+	year	$-0.02(0.88)$	$-0.31(0.12)$	$-0.04(0.76)$	$-0.33(0.09)$	
Diatoms	+	SST	$-0.40(0.01)$	$-0.58(0.004)$	$-0.41(0.01)$	$-0.58(0.004)$	↓
RIPAD	+	SST	$-0.41(0.01)$	$-0.51(0.01)$	$-0.45(0.002)$	$-0.54(0.01)$	↓
Diatoms	+	year	$-0.32(0.02)$	$-0.50(0.01)$	$-0.31(0.02)$	$-0.50(0.01)$	↓
Ciliates	+	year	$+0.13(0.34)$	$-0.06(0.76)$	$+0.12(0.38)$	$-0.09(0.66)$	
Diatoms	+	SST	$-0.38(0.01)$	$-0.57(0.004)$	$-0.39(0.01)$	$-0.58(0.004)$	↓
Ciliates	+	SST	$-0.40(0.01)$	$-0.54(0.01)$	$-0.43(0.004)$	$-0.56(0.01)$	↓
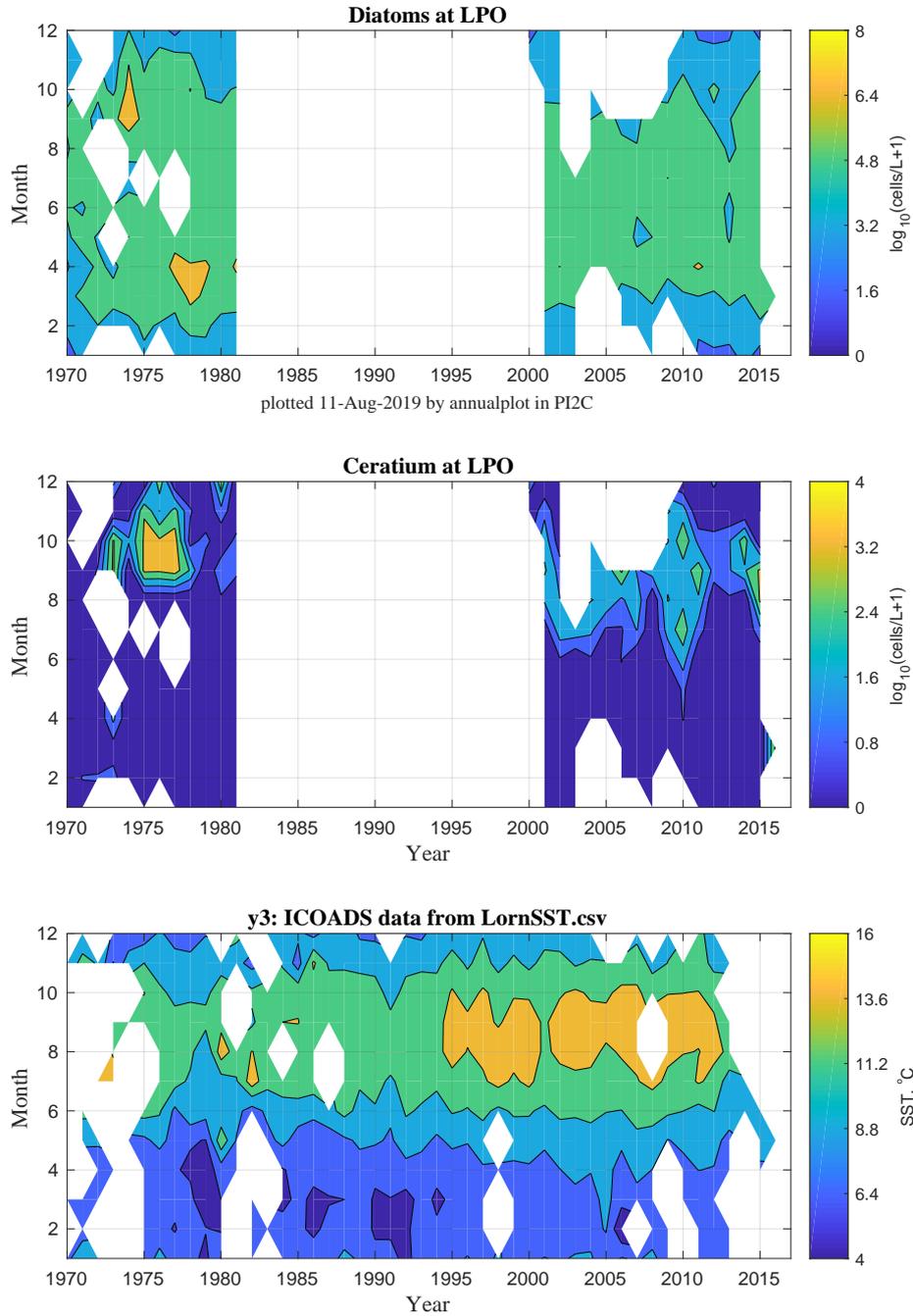
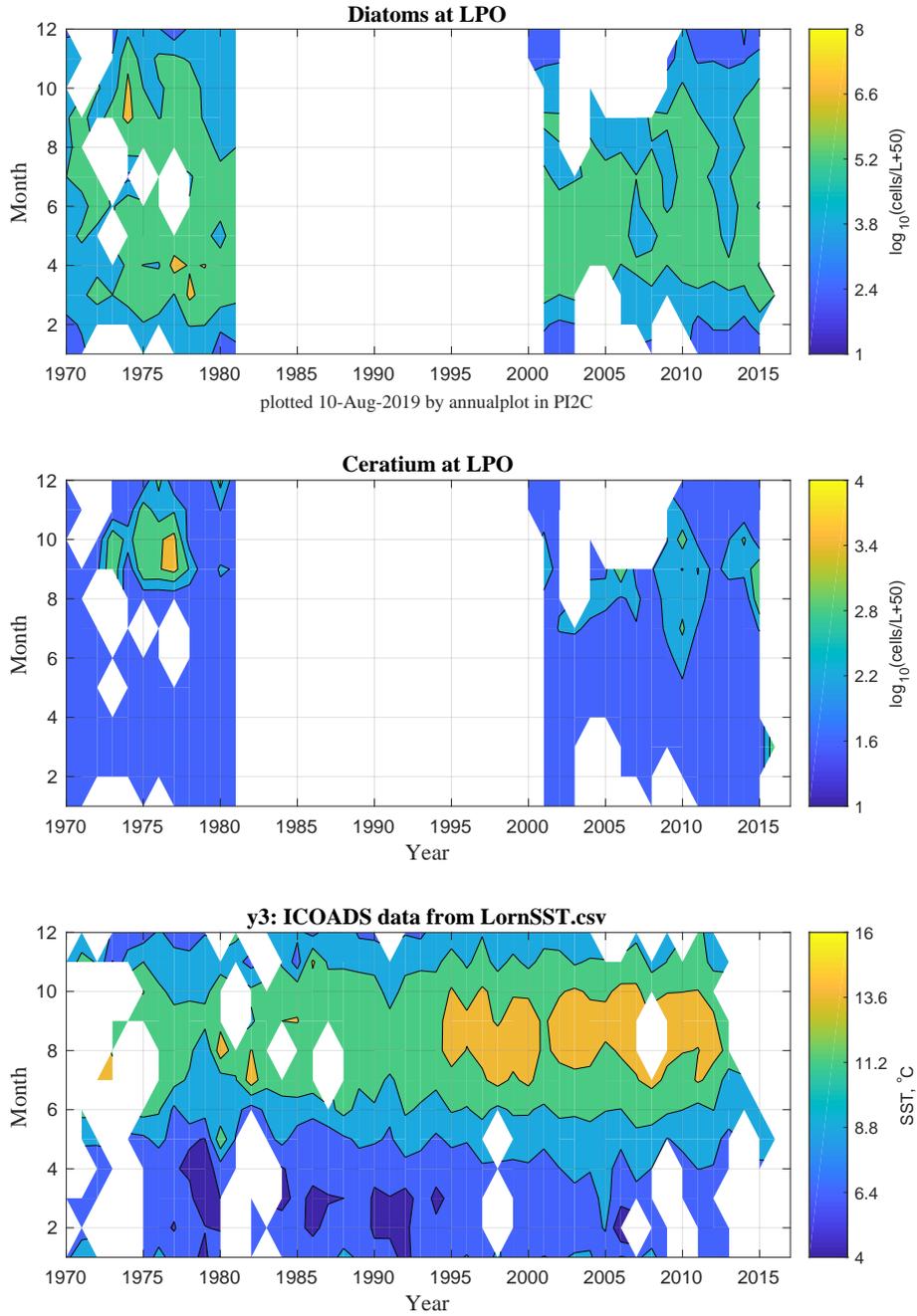Figure 2: Contours for Diatoms, Ceratium and SST with $z = 1$

Figure 3: Contours for Diatoms, Ceratium and SST with $z = 50$

Figure 4: Annual means for Diatoms, Ceratium and SST with $z = 1$. Red lines show number of months with data in each year of the time-series.
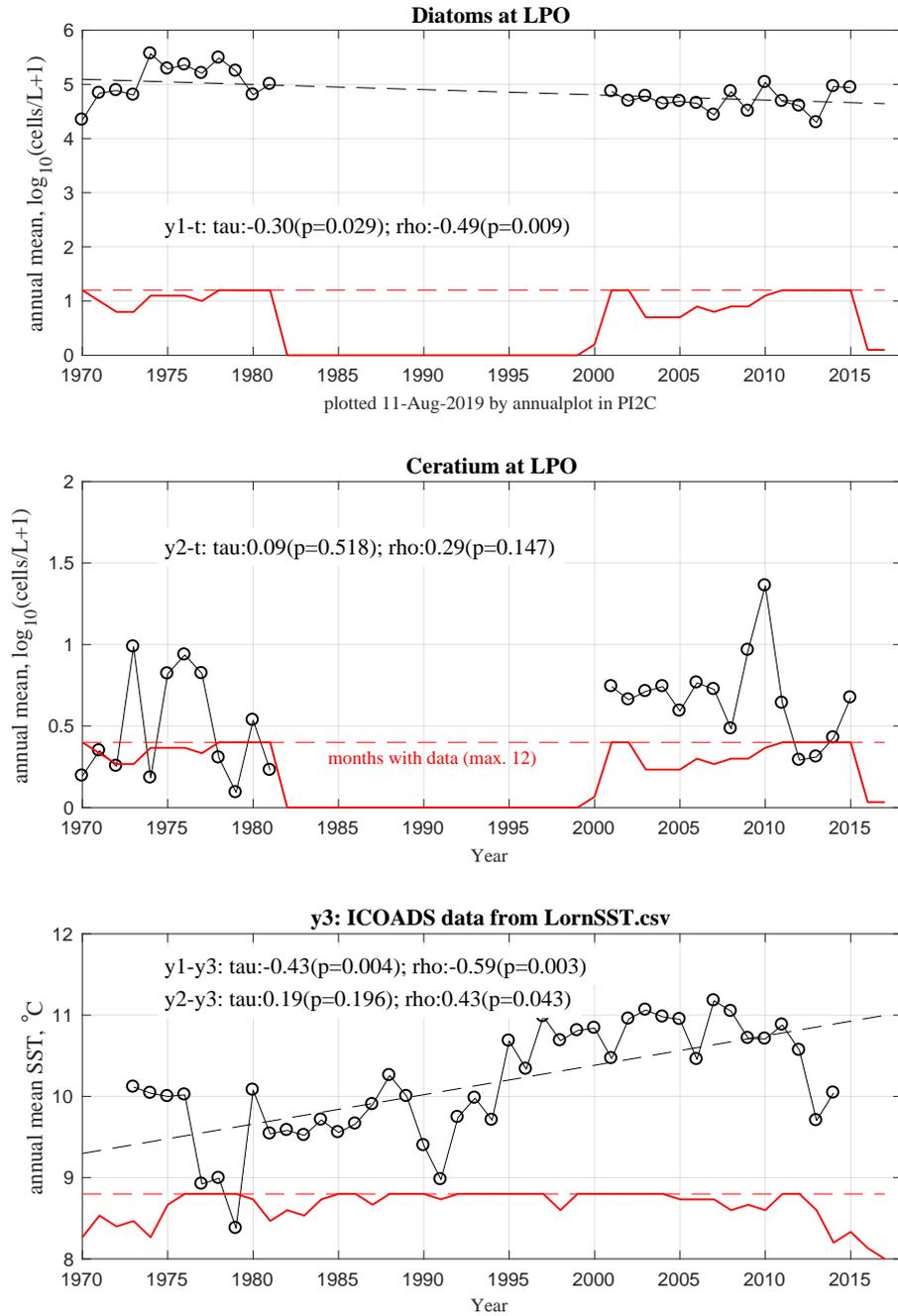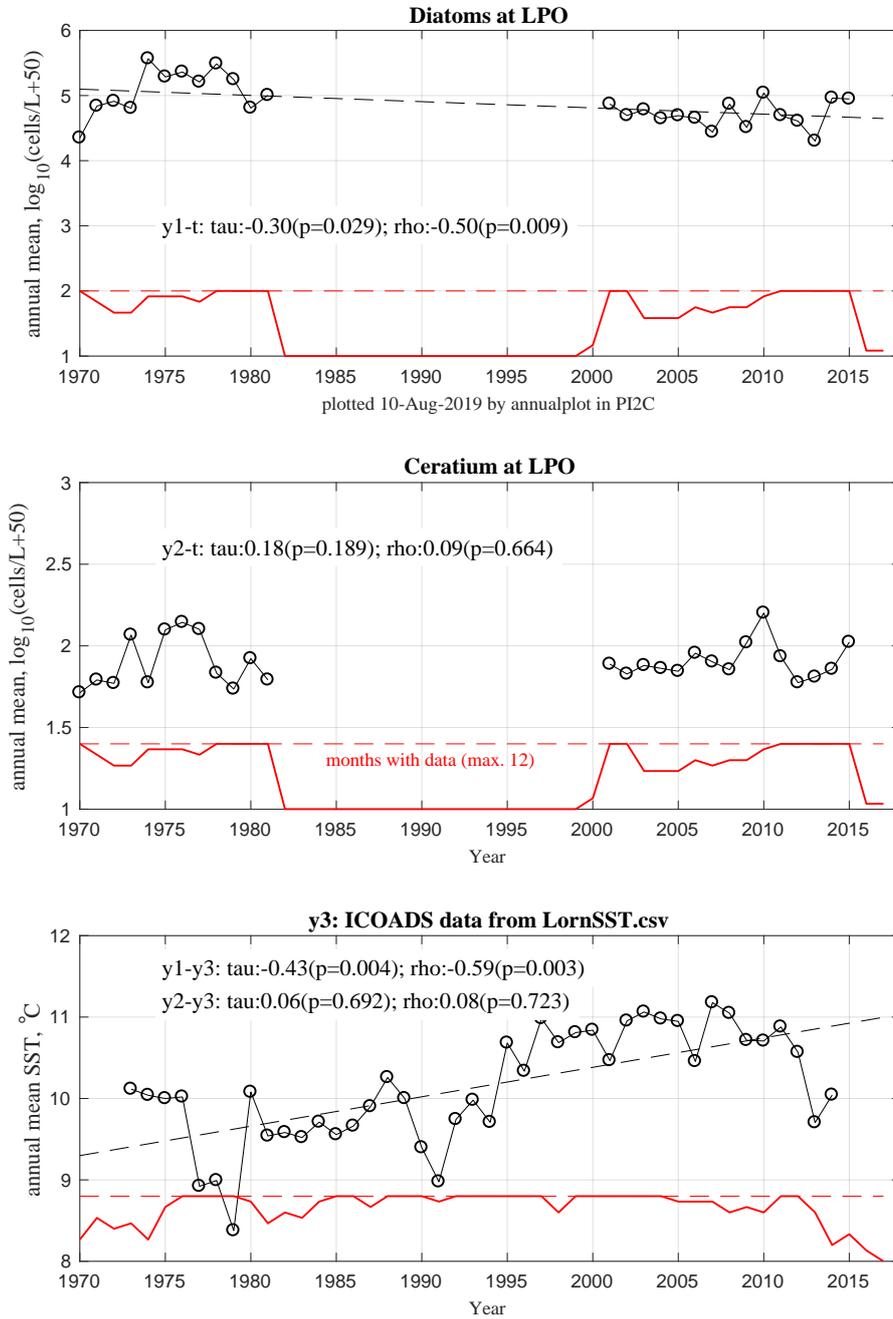
Figure 5: Annual means for Diatoms, Ceratium and SST with $z = 50$. Red lines show number of months with data in each year of the time-series.
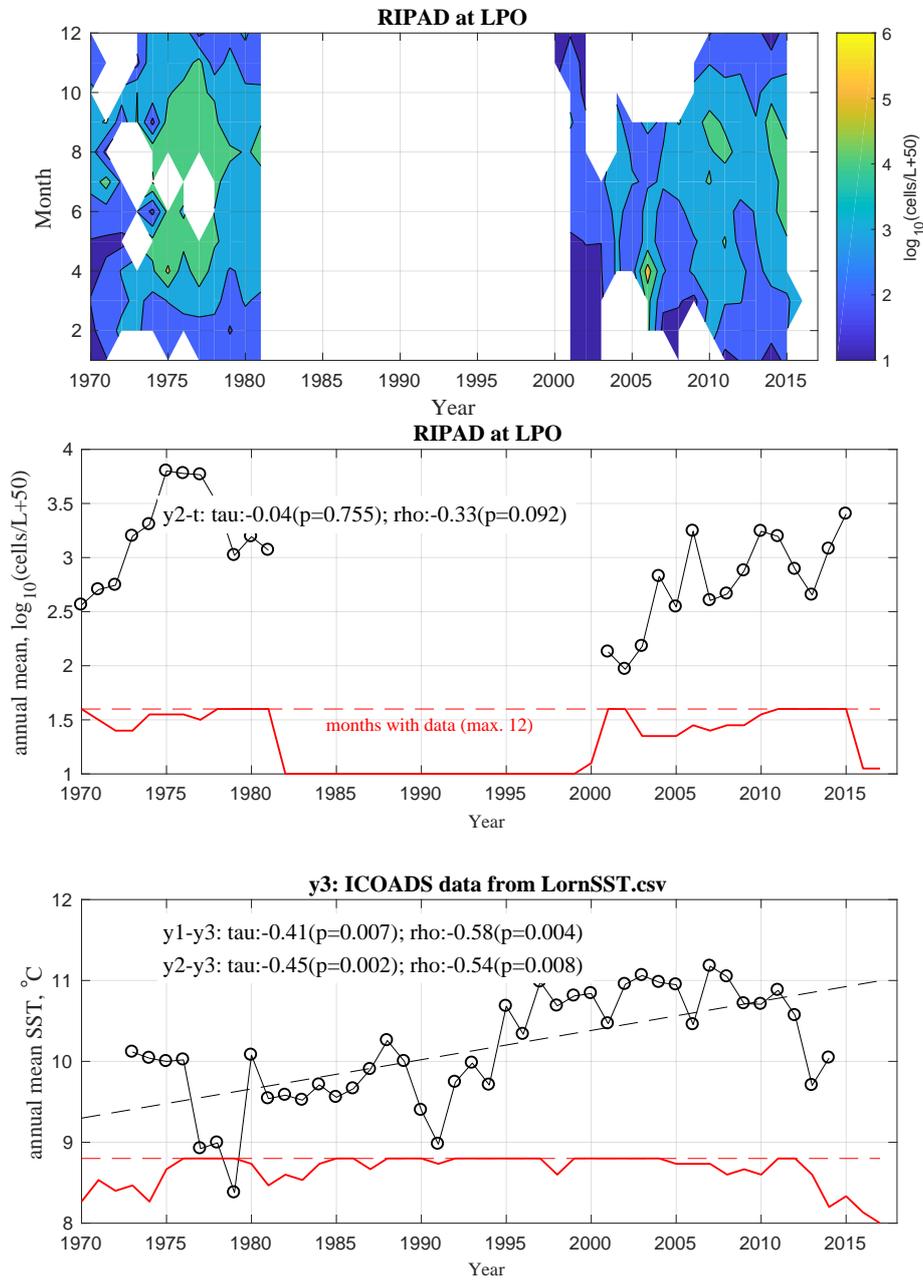
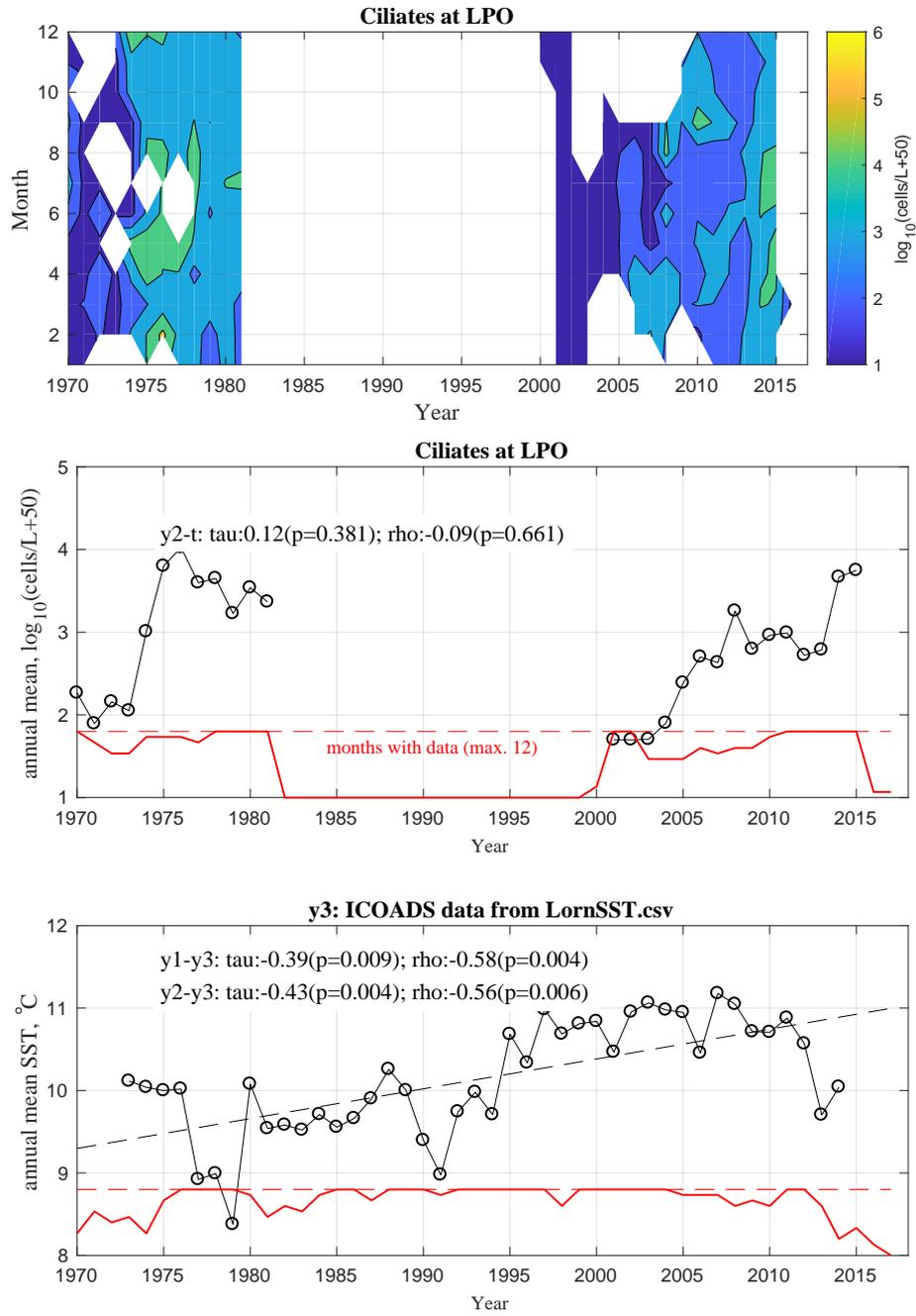Figure 6: Contours and annual means for RIPAD with $z = 50$

Figure 7: Contours and annual means for Ciliates with $z = 50$

4 Discussion

4.1 The value of z

In the case of Diatoms, the value used for z in the transformation $\log_{10}(x + z)$ had little effect on either the contour plot (fig. 2, 3), or the annual average time-series (fig. 4, 5). This was because the 'raw' Diatom time-series contained few zero values. In the case of Ceratium, however, where the 'raw' data included many zeroes, there are obvious differences between the diagrams with $z = 1$ (figures 2, 4) and those with $z = 50$ (figures 3, 5). Nevertheless, conclusions reached from the correlation analysis (table 3) were unaffected by the choice of z. This is to be expected in the case of Kendall's τ: it is a rank correlation coefficient, and thus unaffected by any monotonic scaling of the variables.

4.2 Artefacts and shifting biases

LPO sampling was not planned as a multi-decadal time-series, and the results presented here for microplankton might thus suffer from artefacts due to to changes in: sampling position and depth; analysis method; analysts; and taxonomy.

- Station: shifts in the balance of effort between LL, Creran open water, and pier sampling, have been neglected in the analyses presented here, and need further examination.

- Analysts: estimates of phytoplankton abundance can change because of changes in observers, who might differ in what they count and in how they identify it. In the present case this report's author (PT) was responsible for all identification and counting between 1970 and 1975, and trained microscopists who shared the analyses until 1981. He was also responsible for microscopy or project supervision in some of the work after 2003, whereas the main SAMS work, was in other hands. [6] Further comparison of observers is needed.

- Method: volume examined: maximum volume examined has ranged from 2 to 50 mL and this has implications for the reliable estimation of low abundances.

- Taxonomy: distinct from observer issues are those of taxonomy: what identified organisms are called and what are the boundaries of accepted taxa. Although it appeared in 1970 that the taxonomy of Diatoms and Dinoflagellates was well-established, advances in electron microscopy and nucleotide sequencing has overturned this certainty.

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[6] Some SAMS analysis methods have been validated by inter-laboratory comparisons. Further details needed.

However, the consequences of taxonomic churn have largely been by-passes by the fortuitous decision in 1980 to assign numerical identifiers to taxa, indentifiers that were maintained despite name changes. See appendix A.

Finally, the LPO lies in one corner of the ICOADS square for which SST were available, and it is likely that the SST are biased towards more oceanic conditions. A separate study (Colvile, 2019) has compared measurements made with CTD at or near the LPO stations and the ICOADS monthly means, concluding that the ICOADS time-series did provide a rough proxy for near-shore SST, although over-estimating it during Summer.

4.3   Correlations

The use of Spearman's ρ to establish correlations can be criticised because of the likelihood of autocorrelation in the time-series and the possible non-normality of error distributions in microplankton data transformed with an incorrect value of z. However, the findings from the ρ correlations are supported by those from the more robust but less efficient Kendall τ. The key findings are these:

- SST and Diatoms show significant temporal trends, the former increasing and the abundance of the latter decreasing, over the years from 1970 to 2015; furthermore, the two trends correlate inversely.

- although RIPAD and Ciliate (abundances) did not correlate with year, they did inversely correlate with SST.

The inversely correlated multidecadal trends in SST and Diatoms could be interpreted as increasing temperatures causing decreased diatom abundance. Another explanation is however possible: the climate change partly instanced in marine warming is likely also associated with increased rainfall, runoff, and increased concentrations of terrestrial yellow-substance (or CDOM) in superficial layers of the LPO. This could result in lower water transparency (Tett, 2014) and thus less favourable conditions for diatom growth in the early spring and late autumn.

The (inverse) correlations between RIPAD, or Ciliates, and SST, which exist in the absence of a long-term trend in the two microplankter lifeforms, seems more strongly suggestive of a temperature effect, with fewer of these organisms during years that were warmer than the long-term average.

4.4   What next?

Some tasks remain, including:

- seek additional phytoplankton data, especially for samples taken during Winters 2003 - 2009;

- update ICOADS time-series, and consider augmenting with SAMS in-shore CTD data;[7]

- check spatial biases, perhaps by using ENV comparison (see appendix D) of 2000-2015 with 1970-1981 for: LY1 only, stations excluding LY1, Creran piers;

- check observer biases where comparisons can be made at the same station;

- examine time-series for auto-correlation and normal distribution of errors;

- consider whether abundances can reliably be estimated for a wider Dinoflagellate life-form/group including heterotrophs, and for other major lifeforms such as cryptomonads.

Finally, additional actions were identified in London during the June 2019 workshop, including investigating different length time series as the reference conditions for the PI, and correlating phytoplankton lifeform abundance changes with nutrients.

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[7] It might be that observations in 2014 and 2015 had not found their way into the ICOADS database when the version used was published. Colvile (2019) reports the availability of relevant data up to 2018, although with many gaps.

In addition, SAMS has a mooring in the Tiree passage, near $56.63°$N, $6.40°$N, measuring near-bed currents and temperature since 1981. Since 1987 measurements have also been made at $20 - 25$ m below the sea-surface. Conductivity sensors were added in 1993, giving salinity. See British Oceanographic Data Centre global identifier 5774. Although this position is outside the ICOADS square for the LPO, the mooring data might usefully be investigated as a proxy for SST in the LPO.

A Reliability of lifeform abundance estimates

Principles

The reliability of estimates of lifeform abundance depends on a number of factors (table 4), and these have been taken into account in estimating the 'reliability factors' in table 2. They would only cause error in the present work if the reliability changed with time.

Table 4: Dimensions of reliability for abundance estimates: the unit under consideration might be taxon or lifeform.

	dimension	example
RFI	failure to observe or include all organisms or lower level taxa that should be assigned to this unit, or to count some of them adequately	difficulty in recognizing and counting those naked dinoflagellates that preserve badly; failure to include some or all ciliates in 1970-71 or 2000-03 records
REI	erroneous inclusion of organisms or lower level taxa in the unit, or over-estimation thereof	double counting of organisms in certain taxa when these taxa are enumerated in different scans of the same sedimented sample
SIZE	assignment of organisms to incorrect category with respect to size, with implications for biomass estimates	*Chaetoceros* cells categorized as small, medium, large, rather than precisely as $\geq 20\mu$m and $< 20\mu$m
LFI	life-form inconsistency - issues with, or deviation from, the UK MasterList of taxa	what should be included in 'Dinoflagellate' lifeform - all, or only auto/myxo -trophs? this might change with time as more taxa get identified or start to appear in samples.
TAX	Taxonomic changes	diatom *Rhizolenia delicatula* renamed *Guinardia delicatula* (in Hasle and Syvertsen, 1997); dinoflagellate genus *Gonyaulax* split into *Gonayulax, Amylax* and *Lingulodinium* (in Steidinger and Tangen, 1997).

Taxonomic changes and their implications

When microscopic analysis of phytoplankton was commenced in 1970, it appeared that the taxonomy of Diatoms and Dinoflagellates was well-established at all levels from species to class. This proved not to be the case. New methods, including advances in electron microscopy and nucleotide sequencing, has overturned this certainty. Nevertheless, the effect of taxonomic changes on the reliability of abundance estimates has largely been avoided by the initial choice (forced by data storage and processing charatacteristics of the HP9825) to identify taxa by numeric codes in the ROTDB. For example:

- The diatom genus *Skeletonema* was believed, c. 1970, to contain a single, ubiquitous,species, *S. costatum*, but was subsequently shown to include numerous species (Zingone et al., 2005; Kooistra et al., 2008). Several of these, but not *S. costatum*, have been reported from European waters. Thus organisms originally assigned to *Skeletonema costatum* must now be called *Skeletonema* spp. However, the numeric code `001` assigned to this taxon remains unchanged.

- Dinoflagellate species that were understood in 1970 as belonging to the single dinoflagellate genus *Gonyaulax* have been divided amongst the related genera *Gonayulax* (e.g. *G. spinifera*, ROTDB code `141`), *Amylax* (*A. triacantha*, `142`) and *Lingulodinium* (*L. polyedrum*, `143`). Organisms assigned to *Gonyaulax* 'small' spp. (code `139`) included those sometimes tentatively identified as *Gonaylax tamarensis*, which is now part of the polyspecific genus *Alexandrium* and viewed as only distantly related to *Gonayulax* s.s. During the 1970s, when *G.tamarensis* was considered uncommon on the west coast of Scotland, and post 2000, when *Alexandrium* spp. is known to be sometimes abundant in Creran,[8], examples might also have been included in the category 'SABD' (see below). Difficulties arise in the case of organisms assigned to the original genus without a firm identification of species (which requires examination of the shape and arrangement of the cellulose plates covering the cell). The original name *Gonyaulax* has therefore been replaced (post 2003) by 'gonyaulacoids', code `138` when reported by PT, and as code `139` when reported from other SAMS studies. The potential problem of double counting might be an issue in this group.

Diatoms

The Diatom lifeform was for present purposes defined as including tychopelagic forms such as naviculoids or species of *Gyrosigma*. There were very few organisms that might have been incorrectly assigned to this lifeform, and very

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[8] *Alexandrium* is regularly monitored as a potential cause of shellfish toxicity, and reliably identified in these surveys/

few diatoms that might not have been recognized as such. A few chain forming diatoms were excluded from counts on grounds that they were re-suspended epiphytes, and it is possible that the very small centric diatom *Cyclotella nana* might have been missed. Apart from this, the abundance of the Diatom lifeform is thought to be reliable and consistent over the whole of the time-series, and is thus scored at 5.

Ceratium

Assignments to the lifeform called Ceratium are likely to have been highly reliable and consistent over the period from 1970 to the present. The organisms are large and characteristically shaped and thus both hard to miss and easy to identify to species. Even if the species identification was in error, there is nothing else that might have been misidentified as a species of genus *Ceratium* and no member of the genus at risk of being assigned to a different genus. There has been a recent suggestion (Gómez, 2013) that marine members of *Ceratium* should be assigned to the genus *Tripos*, but WoRMS does not make such assignments. Even if the name-change is accepted, the unit Ceratium remains consistent if it is deemed to include *Tripos*. Thus the reliability was scored 5.

RIPAD

A time-series of (total) Dinoflagellates was thought to be unreliable for several reasons. Some 'naked' dinoflagellates (those lacking obvious cellulose plates in their cell walls) preserved badly in Lugol. They were sometimes recognized (for example, because dinoflagellate-type flagella were seen) and enumerated as 'dinoflagellate blob', sometimes ignored. Small dinoflagellates ($< 15\mu$m) were not always recognized as such, and if counted at all, might have been categorised as 'small flagellates'. Starting in 1980 one set of small dinoflagellates was identified by PT as *Katodinium rotundatum*, which is now considered (in Steidinger and Tangen, 1997) to be *Heterocapsa rotundata*. But some of these organisms might have been the newly discovered *Azadinium spinosum* (Tillmann et al., 2009). The latter is a carrier of toxicity and has become of interest because of that, but it is possible that it and similar small armoured dinoflagellates were not well counted earlier.

Thus the group 'Reliably Identified Phototrophic Armoured Dinoflagellates' (RIPAD was devised, to include all those dinoflagellates that are thought to have been reliably and consistently enumerated during the time-series. One group of consistently counted armoured forms were not included: the peridinioids, including *Protoperidinium, Minuscula* and *Diplopsalis*. These are heterotrophs, and uncommon in LPO samples.

A final issue involves assignment to the grouping of 'Small Armoured

Brown Dinoflagellates' (SABD).[9] Organisms initially identified (Lebour, 1925) as *Peridinium triquetrum* and *P. trochoideum* were sometimes distinguished from other SABD. With the publication of Dodge (1982) these two were renamed as *Heterocapsa triquetra* and *Scrippsiella trochoidea*, and it began to seem that some other SADB might be *Gonyaulax tamarensis*, subsequently *Alexandrium* spp. (Steidinger and Tangen, 1997).[10] Although, with care to avoid double-counting in the case of SABD and gonyaulacoids, totals for RIPAD should have remained consistent, these ambiguities reduce the reliability score for RIPAD to 4.

Ciliates

As with the dinoflagellates, the taxonomy of ciliates seems to be in flux at all levels. 'Oligotrich' is both a fuzzy grouping (of organisms with the cilia most abundant at the oral end of the body) and, according to WoRMS, the class Oligotrichea (AphiaID 732974) that includes the cased ciliates called tintinnids (in order Choreotrichida, AphiaID 341353) and uncased ciliates in the order Oligotrichida (AphiaID 95083). But the distinction (between cased choreotrichs and uncased oligotrichida) is not absolute, and in practice the organisms assigned to this group have been reliably recognized as typically medium sized (15 - 50 μm across their oral region), shaped like a beaker or an ice-cream cone, and with a tuft of cilia at one end. In some cases the pattern of cilia was used to identify a genus, but this has not been done consistently or reliably. The cases of tintinnids are both characteristic of the group and in many cases of species, and identification to genus has sometimes been made on this basis. However, de-cased tintinnids appear as oligortrichidan ciliates to causal inspection. Thus the reliable grouping is that of the caseless forms (usually noted as 'oral ciliates') plus the cased tintinnids. But while enumeration should have been reliable given a decision to count oligotrichs, I don't think I had made such a decision in 1970,[11] nor had Fehling in her study 2000 – 2003. Thus the reliability level of the Ciliate group has been set at 3.

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[9] 'Brown' meaning heavily stained with Lugol's Iodine, taken as an indicator of photosynthesis when chloroplasts could not be distinguished.

[10] Identifying armoured dinoflagellates with precision requires examination of the plate structure, which is time-consuming and also difficult with Lugol-preserved sedimented samples. It is also unclear if species identified from morphology in this way correspond to species as interbreeding groups, or as genetically and physiologically homogenous.

[11] It may be possible to check this statement by reviewing old records.

B Appendix: Digitisation

Microscopic analysis of a water sample can result in lists of up to a hundred taxa and their abundances. The LPO data analysed for the present report represented about 1800 samples, approaching 30 thousand taxon-abundance pairs. Although the digitisation of such data is itself a massive task, their conversion to an electronic form allows many analyses that would be too laborious and error-prone to perform manually. Transcription of LPO data into digital form was begun in 1980, with data entered into a HP9825 desk-top computer for storage in proprietary format on magnetic tape (Tett et al., 1981). However, while the tapes have been preserved, the computer is no longer available. A lesson learned from this was to store the data in a platform-independent fashion. This led to the generalised approach sketched in figure 8
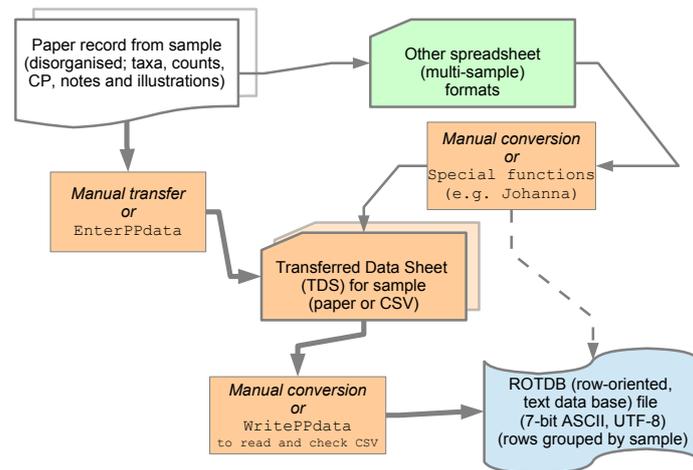

Figure 8: Digitisation of phytoplankton data. `EnterPPdata, WritePPdata. Johanna` refer to custom Matlab functions written to partly automate digitisation processes.

Key features are:

- a two-step process, in the interests of audit and quality control; in most cases, paper records were first converted to 'Transcribed Data Sheets', either paper or CSV spreadsheets for checking, before further transcription to ROTDB format;

- assignment of a numeric code to each identified and enumerated taxon,

allowing consistency despite changing taxonomy;[12]

- ROTDB: a row based, plain text, data base, in which each row is a completely contextualised entry for one taxon-abundance pair, and allowing files to be read by a variety of text editor or input to spreadsheets or programs such as those used for the present report and written in Matlab.

From 2005 onwards, the data-base was made by adding rows to a single text file; more recently, however, it has come to seem better to work with a series of files, each containing a discrete chunk of data. As an example, the file `Creran_phyto_2014.txt` contains all records of microscopic analyses by PT of samples taken in 2014 (mainly from loch Creran but including LY1). Here is the file's second header row and first data row, both broken into several lines:

```
%"replicate" "Year" "month" "day" "station" "depth" "name code"
        1     2014        3    13        351        1            1
% "factor code" "cells counted" "sal" "TDS"
        112               9        24.0  2016024
% "Count by" "year" "month" "day"
        1     2014        3     15
```

The "name code" references the taxon counted, and the "factor code" the counting pattern (in this case 12, which is subsequently associated with the multiplication factor 5250 per Litre in order to convert 9 'cells counted' to 47,250 cells/L). The data row contains only numbers and tab and EOL characters; the header row, starting with '%' (which is read by Matlab as the start of a comment row) also contains alphabetic and additional characters from the ASCII set. For convenience in checking and further processing, each set of data rows from a single sample is terminated with `-99` followed by 14 tab characters.

The data used for the present work were compiled from three somewhat different approaches, briefly described next. The titles are arbitrary.

SMBA1970: SMBA data from 1970-1981

Most phytoplankton data from this period had been entered into handwritten TDS by 1982, and c. 2005 the TDS were re-digitised in India, each to a page in an Excel workbook. These pages were subsequently manually converted to the ROTDB format (mainly by Catia Carreira), and the data now form part of the large file `Allphyto1970-13-Callum.txt`. Paul Tett was in

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[12] For example, the taxon originally called *Rhizosolenia stolterfothii* and now *Guinardia striata* remains numerically identified as species 053. This procedure differs from that used by the AphiaID system, in which each taxon is given a unique number. Nevertheless, the taxon-aggregation procedures used in ENV can in principle deal with either.

charge of the work, and analysed many of the samples. Other microscopists were Ken Jones and Jennifer Shaw. A 10 mL sedimentation chamber was standard, and microplankton were examined in a 1 cm^2 region at the centre of the chamber's base of 4.9 cm^2, or in transects across this region (Tett et al., 1981).

SAMS2000: SAMS student and project data from 2000 onwards

From 2000 onwards, SAMS recommenced observations in the LPO under the direction of Keith Davidson. Some of the work was part of PhD projects (including those by Johanna Fehling and Greg Moschanas); other studies were part of monitoring for harmful algal blooms or remote sensing purposes, with microscopist Elaine Mitchell. The data were, typically, transcribed into Excel spreadsheets. Some of these were manually converted into ROTDB format by Callum Whyte; others were manually converted to TDS.csv; and data in Fehling's spreadsheet covering 2000-2003 at LY1 were converted to TDS.csv by a specially written Matlab function `Johanna`. A 50 mL composite sedimentation chamber was standard.[13]

NS2003: Napier/SAMS-PT data from 2003 onwards

Starting in 2003, Paul Tett led a program at Napier University Edinburgh that included sampling in loch Creran (and sometimes at LY1) for PhD studies (Laurent et al., 2006; Whyte et al., 2017). PT resumed sampling (mainly at Barcaldine Pier and nearby sites) from 2010 after transferring to SAMS. Initially, data were converted to spreadsheet TDS and then to ROTDB by hand, and then more recently with the aid of two Matlab programs, `EnterPPdata` to make TDS and `WritePPdata` to convert to ROTDB. Sedimentation chambers of 10 mL were typically used, with the whole chamber base examined for less common taxa and transects examined for more abundant organisms.

The datafile: `Allphyto1970-13-Callum.txt`

As already mentioned, until 2013 the ROTDB was expanded by adding more rows of data to a single file. A late version of this was called `AllphytoOct2013.txt`, and comment lines (slightly edited here) gave its contents as:

```
% Creran & LY1 phytoplankton data
% this version of allphyto made 14 Nov 2010 includes (in order)
% (1) 1970-1981 Creran and LY1 - digitized from TDS  in India,
```

---

[13] Composite chamber: after sedimentation, the upper part of the chamber and its contents are slid to one side, so that illuminating light can be focussed through a thin water layer,

```
% transformed from Excel spreadsheets by Catia Carreira, July-August 2006
% (2) Creran 2006 - some NUE samples counted by Benoit Ginoux (22) in June 2006
% converted to flat data base by PT - August 2006
% (3) PT data from 2006 - 2007 starting with MSc visit in April 2007
% (4) PT data from 2009 and 2005-6, digitized and added Jan 2010
% (5) CW data from NUE 2008 - 2009, digitized by him, added  PT April 2010
% (6) PT data from NUE LY1 samples in (mainly) 2009, added 14 Nov 2010
% (7) PT data from Creran in 2012, added 6 May 2013 -
% (includes salinities in previously blank column)
% (8) PT data from Creran in 2011, added 30 Oct 2013
% (9) PT data from Creran in 2010, added 31 Oct 2013
```

Callum Whyte then added SAMS LY1 and C3 data for 2009 to 2013 to make
the file `Allphyto1970-13-Callum.txt`. After this, new ROTDB files were
made for each substantial chunk of additional data.

C   Appendix: Analytical computational methods in detail

Methods are reported here for the Diatoms and Ceratium pair of lifeforms. Similar procedures were followed with other pairs (Diatoms-RIPAD and Diatoms-Ciliates).

ENV and LifeForm assignment

The version of ENV used was `HPLP3Ga`, last updated on 6 June 2019. Tett (2019) gives incomplete instructions for the use of this program, which was originally written to plot climatological envelopes of the abundances of individual taxa or of lifeforms, as exemplified in figure 9. The following accessory files were loaded:

- the control script `hcf1_2019`;

- the file containing multiplication factors `multfac_Oct2017.csv`;

- the 'VOLF' file containing species codes, lifeform assignments, and typical cell volumes `volf_Jul2019_AugLF.xlsx`.

ENV reported that:

```
Data that have been loaded were originally in files on path:
/Users/sa02pt/Documents/MATLAB/Matlab2017fromMPB2011/ENV/Pdata/
22649 rows from Allphyto1970-13-Callum.txt
  775 rows from SAMSphyto2004.txt
  896 rows from SAMSphyto2005.txt
  806 rows from SAMSphyto2006.txt
 1116 rows from SAMSphyto2007.txt
  633 rows from Creran_phyto_2013.txt
  557 rows from Creran_phyto_2014.txt
  730 rows from Creran_phyto_2015.txt
 2028 rows from LY1_phyto_Johanna_2000.txt
```

ENV was run to select data rows with the following characteristics:

- all available years (digitisation incomplete);

- stations number 101 to 399, which comprises all those in the Lynn of Lorn (LY1 is 101) and in Loch Creran (301 through 311 with piers and shore stations at 326, 327, 351 and 352);

- samples from water depths between 0 and 10 m, including those taken with integrating hose;

- containing taxa assigned to the **Diatom** and **Ceratium** lifeforms (table 2) in the VOLF file; these two lifeforms were consistent across the years. They were selected by choosing LF2, no 1 (all diatoms) and LF1, no 14 (*Ceratium* spp.).

Each output from ENV was a text-file for one life-form, containing four columns:

- year;

- day-of-year (within year);

- cell abundance (cells/L), totalled over all the taxa or groups assigned to the lifeform in the VOLF file;

- biomass (mm^3 cell volume/L), the total over all taxa or groups of the product of cell abundance and mean cell volume (provided in the VOLF file).

Two files were output:

```
Ceratium9Aug19.txt
Diatoms9Aug19.txt
```

Two lines from the second are quoted:

```
% year  dayno    cell/L   biomass mm3/L
  1970       6   1.35e+03   2.98e-04
```

WAVES

The version of WAVES was `HPLF2e.` originally written to fit a Fourier series to time-series data, but used here only to merge two data files. In the present case these were the `diatoms` and `Ceratium` files. The challenge to merging is that the `Diatoms9Aug19.txt` and the `Ceratium9Aug19.txt` files contained only data from samples in which at least one taxa, belonging to the relevant lifeform, had been observed. Thus the Ceratium file had many fewer rows than the Diatom file, because there had been many samples from which *Ceratium* spp. were not recorded, while this was rarely the case for total diatoms.

WAVES had two options for merging files. First, the rows of the two files were aligned by year and year-day, any cases of multiple samples on the same day being averaged. Then rows were copied into a new file using one of the two methods:

intersection : only rows containing values in both abundance columns were copied;

union : all rows containing at least one value were copied, missing values being replaced by 0

In the present case, the *union* option was used. In effect, this used the diatom column as an indicator of samples taken, and filled out the *Ceratium* column with zeros . The first rows of the WAVES output in the file `DiatomsCeratium10Aug.txt` included:

```
%% data read from  Diatoms10Aug.txt
%% and from  Ceratium10Aug.txt
%% columns joined by union
%% de-transformed to original units
%% z for y1, y2, were:    50,    50
%% year.day diatoms Ceratium in cellno/L
1970.0164   1.45e+03   0.00e+00
1970.0712   2.55e+02   0.00e+00
```

Plankton Index tool

The PI tool is a set of Matlab functions initially developed to calculate and plot a value of the *Plankton Index* (Tett et al., 2008), but subsequently expanded to process and plot other indicators of plankton change. Most of the tool's capabilities (as developed until 2016) are described by Tett (2016). For the present work, the set was updated to `PI2C` with the addition of a new function `annualplot` invoked by a menu option (10):

```
------------- MAIN MENU FOR PI ----------------
End PI2C (0)
    Plot data time-series (1)
    or: make annual means and monthly contours (10)
        Make reference envelope (2)
        Change values of control parameters (5)
    Make state space plot of annual medians (6)
    Make statistics for data            (9)
Enter a number [0]:
```

(Note than menu options expand after successfully calling (2). Option (5) allows, amongst other parameters, the values of s to be changed and functions such as `annualplot` run in 'verbose' mode.)

    `annualplot` did the following:

- contour plots of abundances or SST on year-month surfaces (e.g. Figures 2, 3);

- calculation of annual means by the function `climateroll`

- estimation of correlations

- plots of time-series of annual means (e.g. Figures 4, 5).

Options are presented, as `annualplot` runs, to select a subset of years from the loaded time-series, and to include and load a SST file.

Contour plotting used the following lines of Matlab code:

```
subplot(Ncplots,1,1); % for Y1 ------------------------
CMmin = floor(log10(z1));
CMmax= ceil(max(y1)); % note, y1 already log-transformed
CMint = (CMmax - CMmin)/5;
CM = CMmin:CMint:CMmax; % contour intervals
[~,~]=contourf(yearA,monthA,yearmonthy1,CM,'k');
caxis([CMmin CMmax]); caxis manual;
CCM1 = colorbar('Ticks', CM);
CCM1.Label.String = ['log_{10}(' units '+' num2str(z2) ')' ];
grid on
```

In the call of the Matlab function `contourf`, `yearA` and `monthA` are matrices with rows for years (from 1970 to 2015 in the present case) and columns for months (from 1 to 12); each row in `yearA` is filled with the year, and each column in `monthA` is filled with the month. These matrices provide the framework on which the values in `yearmonthy1` are contoured at intervals set by `CM`.

Correlations were calculated using PT's `Kendl1` function (based on Press et al. (1989)) and the Matlab supplied `corrcoef` function.

```
k1t = Kendl1(Cyeary1, yearss);
k2t = Kendl1(Cyeary2, yearss);
% Pearson p-m correlation - 'complete' rows omit those with NaN
% corrcoef reurns a 2x2 matrix -
[rho1t, rp1t] = corrcoef(Cyeary1, yearss, 'Rows', 'complete');
r1t = rho1t(1,2); p1t = rp1t(1,2);
[rho2t, rp2t] = corrcoef(Cyeary2, yearss, 'Rows', 'complete');
r2t = rho2t(1,2); p2t = rp2t(1,2);
```

The new function `climateroll`, called by `annualplot`, takes in a 2D matrix (years (rows) x months (cols)) of values, and replaces missing values from a sliding 5 year climate before calculating a row mean that is returned by the function as, for example, the vectors `Cyeary1` and `Cyeary2` in the preceding code.

D Appendix: Example graphs from 'ENV'

ENV describes a 'climate' of seasonal change, by enclosing several years of data in an envelope. In `HPL3Ga` a sliding window provides the envelope and a running median. A second set of data can be overplotted, and compared with the median, to provides an alternative test for long-term change.
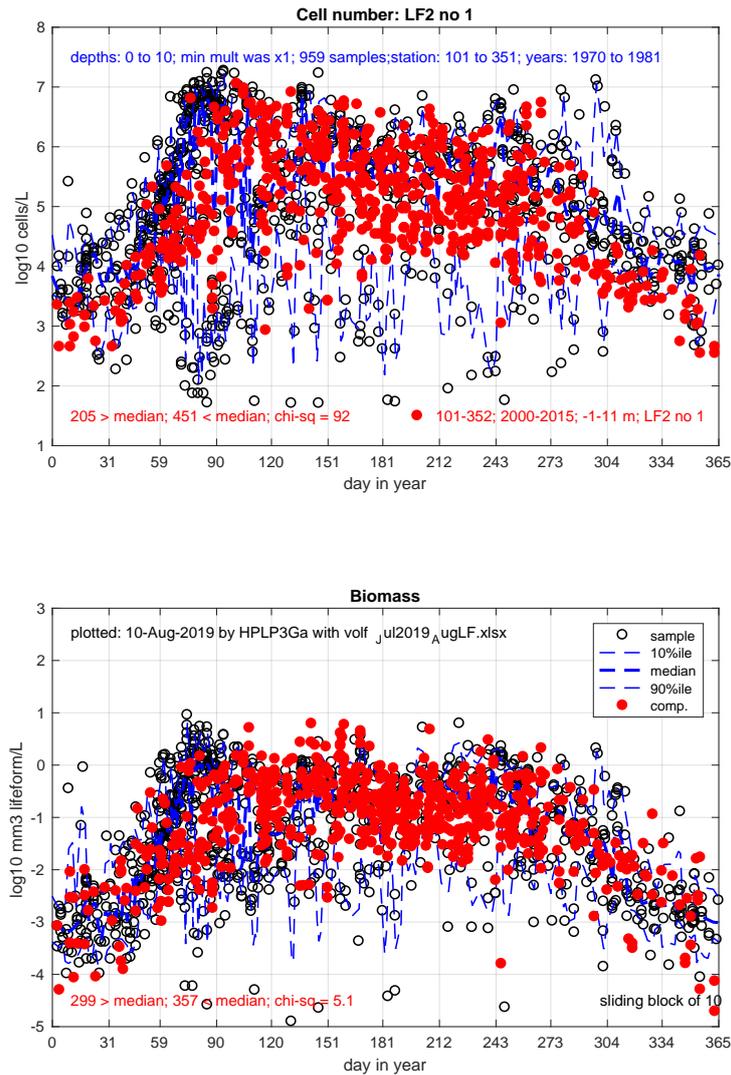

Figure 9: ENV output for Diatoms ($\log_{10}(x + z)$, showing abundance as cell numbers ($z = 50$ cells/L) and as biovolume ($z = 0.0001$ mm^3/L).

Figure 9 compares Diatom data for 2000 – 2015 (shown in red) with data for 1970 – 1981 (shown in blue), and a χ^2 test shows that significantly

more recent points plot below the median, whether as cells/L or biovolume in mm^3/L.

In addition, inspection of both parts of the diagram shows that, from 2000 onwards, diatoms increased later in the Spring and decreased earlier in the Autumn, than during 1970 – 1981. The same pattern is evident in both cell and biovolume data, showing that the long-term trend cannot be explained by change in species and cell size. The decrease in biomass has been confirmed by measurements of chlorophyll: see: Laurent et al. (2006); Whyte et al. (2017).

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