Tracking changes to a microplankton community in a North Atlantic sea loch using the microplankton index PI(mp)

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Abstract

Microplankton plays a vital part in marine ecosystems and its importance has been recognised by the inclusion of microplankton community composition in regulatory frameworks such as the European Water Framework Directive and the Marine Strategy Framework Directive as an indicator of ecological status. Quantitative techniques are therefore required to assess the environmental status of the microplankton in a water body. Here we demonstrate the use of a method known as the Microplankton Index PI(mp) to evaluate changes in the microplankton community of the West coast Scottish Sea Loch Creran. Microplankton in this fjord has been studied since the 1970's providing a data set spanning four decades. Our analysis compares an arbitrarily chosen reference period between 1979 and 1981 with a period between 2011 and 2013 and demonstrates that between these two periods community structure has changed considerably with a substantial drop in the numbers of observed diatoms accompanied by a rise in the number of autotrophic/mixotrophic dinoflagellates as well as an increase in the potentially toxin producing genus *Pseudo-nitzschia* and that these are related to changes in both the intensity and timing of local patterns of precipitation. The PI(mp) is shown to be a useful and robust method to visualise and quantify changes in the underlying structure of the microplankton community and is a powerful addition to the toolbox of techniques needed to determine the health of our seas.

Keywords

Scotland, Microplankton Community Index, Loch Creran, Marine Strategy Framework Directive, Indices, Ecological status.

1 Introduction

Microplankton is an integral part of marine ecosystems. Here the term is defined after Dussart (1965) as being comprised of many types of pelagic micro-organisms between 20 and 200 µm in the longest dimension including protozoa and micro-algae but excluding micrometazoans and forms the basis, either directly or indirectly, of most marine food webs. It plays a major role in global biogeochemical processes (Calbet and Landry 2004, Domingues *et al*., 2008) and is responsible for around 50% of the photosynthetic activity on the planet (Field *et al*., 1998). Its ability, given sufficient nutrients (Howarth, 1988, Howarth and Marino, 2006, Ryther and Dunstan, 1971) to grow rapidly can, in some locations, make its elevated biomass a good indicator of eutrophication. Our use of the term is a result of increasing evidence of the nutritional flexibility of many pelagic protists, including, for example, the recognition that many kinds of micro-algae are mixotrophic (Stoecker, 1999) rather than purely autotrophic. Furthermore, referring to microplankton allows us to avoid the error of using phytoplankton to include all dinoflagellates (including heterotrophs) whilst excluding the functionally photosynthetic ciliate *Myrionecta rubra* (Crawford, 1989).

The importance of phytoplankton (defined as micro-algae plus cyanobacteria) is recognised by its inclusion in such regulatory frameworks as The European Water Framework Directive (WFD; 2000/60/EC), OSPAR's Strategy to Combat Eutrophication (OSPAR 2003) and the Marine Strategy Framework Directive (MSFD; 2008/56/EC) in which phytoplankton community composition (as well

as total abundance or biomass) is considered as one of the indicators to be used when determining the ecological (WFD) and environmental (MSFD) status of a water body (Ferreira *et al*., 2011). We argue that it is important to take account also of the contribution of pelagic micro-heterotrophs to community composition and function, and thus, when relevant data exist, to examine the state of microplankton rather than that of phytoplankton *sensu stricto*.

Ecological (WFD) or environmental (MSFD) status reflects both a measure of how well an ecosystem is functioning and the state of its structure, properties that Mageau *et al.,* (1995) name as 'vigour' and 'organisation'. In this paper we deal with organisation. This aspect of status can be determined by comparing the system, as it is found now, with some previous reference condition (Laurent *et al*., 2006). Several different tools have been developed that attempt to determine the state of phytoplankton communities in coastal waters and estuaries (Borja *et al.,* 2012). A number focus on Chlorophyll-*a* (Chl-*a*) concentration alone, for example the Trophic State Index (TRIX), (Vollenweider *et al*., 1998). The Environmental Protection Agency's National Coastal Assessment (APA NCA) (USEPA, 2008b) and the HELCOM Eutrophication Assessment Tool (HEAT), (Anderson and Laamanen 2009) also use Chl-*a,* and compare concentrations from an annual index period during the summer with a set of historical reference conditions. The Transitional Water Quality Index (TWQI), (Giordani *et al*., 2009), includes features that transform average Chl-*a* concentrations, from different representative sites, into quality values and then multiply these with a weighting factor to account for their contribution to the final index. Other indices include additional metrics, for example the Assessment of

Estuarine Trophic Status (ASSETS), (Bricker *et al*., 2003), which combines measurements of the 90th percentile of Chl-*a*, dissolved oxygen, nutrients, macro-algae and the spatial coverage and frequency of harmful algal blooms (HABs).

It is also a requirement of the MSFD that member states assess the structure and composition of the phytoplankton community (Borja *et al.,* 2010, Ferreira *et al.,* 2011). However, gauging the status of a phytoplankton community *in situ* is fraught with difficulties as inter-seasonal and inter-annual variability can be as much due to stochastic processes as to seasonal succession (Gowen *et al*., 2012). Although changes in community composition are included in some of the above indices, these are restricted to observe changes in the abundance of toxic or nuisance species. In some cases the relative abundances of different size categories are used (Bricker *et al*., 2003). However, differences in the definitions of species and the size thresholds used can result in very different final assessments while, depending on the approach, the level of taxonomic expertise required is often high. True evaluation of ecosystem health therefore requires more taxonomic resolution.

The Microplankton Community Index (PI(mp)) was developed originally as the Plankton Community Index (PCI) by Tett *et al*., (2008) and is renamed here to avoid confusion with the existing Plankton Colour Index used by the Sir Alister Hardy Foundation for Ocean Science (SAHFOS) to categorise the amount of chlorophyll filtered by their continuous plankton recorders. The PI(mp) was

designed to illustrate the state of the pelagic ecosystem and quantify the composition of the microplankton community, as it is now, compared to a given, possibly arbitrary, starting state or a set of reference conditions. It therefore affords a way of evaluating changes to a community over time and provides a method to examine the structure of the community.

Our study evaluates the ability of the PI(mp) to detect changes in Loch Creran (Figure 1), a small fjord situated on the west coast of Scotland and often regarded as a typical Scottish sea loch (Edwards and Sharples 1986), and expands on Tett *et al*., (2008), through an evaluation of changes to the potentially toxic genus *Pseudo-nitzschia,* a comparison of the state of the life-forms: ciliates, heterotrophic dinoflagellates and small flagellates (comprised of a variety of flagellated unicells, less than 10 µm, including cryptomonads, prymnesiophytes and the small dinoflagellate *Katodinium rotundatum*) with reference conditions and a fuller interpretation of the possible drivers behind the observed changes in the microplankton community.

The loch is a site for both shellfish and, since 1993, finfish aquaculture and has been extensively studied since the early 1970's (Solórzano and Grantham, 1975, Solórzano and Ehrlich, 1979, Tett *et al.,* 1981, Tett *et al*., 1986). Throughout this time a record of the species and their abundance has been maintained, resulting in a good historical data set that can be used to examine changes linked to climate or caused by nutrients. The aim of this paper is to evaluate changes in the structure of the microplankton community in loch Creran between c. 1980 and c. 2013, using the PI(mp), to visualize and explain those changes, and to use these results to assess the utility of the PI(mp).

2 Methods

2.1 Introduction to the PI(mp)

Margalef was perhaps the first to suggest that different species of microplankton could be categorised in terms of their functionality into "life-forms" (Margalef 1978). Concentrating on the supply of nutrients and the effects of decaying turbulence he conceived "life-forms" as an aggregation of adaptations of different organisms to these selective pressures.

Key to the PI(mp) are two main concepts. The first, that an ecosystem can be treated as a system which can be defined at different points in time by a set of system "state variables". The second is that these variables can be represented by the relative abundances, related as numbers or biovolume, of a small set of life-forms, such as, pelagic diatoms, autotrophic/heterotrophic dinoflagellates and ciliates (Tett *et al.,* 2008). Depending on the availability of data, combining these concepts could lead to plots in multi-dimensional state space. For convenience, however, we use sets of 2-D plots.

The abundance of microplankters changes on many time-scales. One of these is that of seasonal succession, which we see as part of the organisation of these communities of pelagic organisms. To take account of this, and to distinguish changes in the microplankton community from the noise generated by inter-annual variability, the abundance of one life-form is plotted against that of another into a two dimensional phase space. As seasonal succession,

affecting community organization continues, the relative abundances of different life-forms change, throughout the year and between years and this generates a cloud of points. An envelope can then be drawn around these points to represent the expected reference composition of the microplankton community (Figure 2). Ideally these reference conditions would be representative of a healthy ecosystem (i.e. Good Environmental Status (GES) under MSFD) or pristine conditions (type-specific reference conditions under WFD) but in practice any time period can be chosen allowing a comparison to be made between conditions then and now. To compare the present state of the community, new observations can then be plotted into this phase space. Providing the new points plot somewhere inside the envelope it can be assumed nosubstantial change has taken place. If, however they lie outside the envelope it indicates that a change has occurred in the state of the community.

In this paper the “historical” reference conditions have been determined from the observations made between 1979 and 1981, a period prior to the introduction of a fish farm to the loch. While the length of the period chosen to determine the reference conditions can vary, the inclusion of too many years will increase the size of the reference envelope and thus tend to reduce the responsiveness of the PI(mp) to change. As the PI(mp) relies on the identification and enumeration of the microplankton species in a community the existence of a record of microplankton species spanning several decades makes Loch Creran an ideal site to carry out an evaluation of the PI(mp) method.

2.2 Water sampling

During the 1970's and early 1980's water samples were collected from several sites in Loch Creran at 0, 4, 10, 20 and 40 m depths with a NIO 1.5 litre water bottle deployed from the RV *Calanus,* RV *Seol Mara* and the RV *Beaver* (Tett *et al*., 1975, Tett and Wallis 1978). Between1979 and 1982 additional samples were collected weekly at Barcaldine Pier and South Shian oyster hatchery at a depth of 1m (Figure 1), (Tett et al., 1981). Microplankton was preserved with 1% final concentration by volume, acidified Lugols solution.

From 2011 to 2013 water samples were collected weekly at depths of 3 and 10 metres by Niskin bottle deployed from the RV *Calanus* and the RV *Seol Mara*, between March and October from site C3 (Figure 1) in Loch Creran. The samples were preserved with 1% final concentration by volume, acidified Lugols solution.

Between 2011 and 2013 supplemental samples were also collected throughout the year at variable intervals from fortnightly to monthly from Barcaldine pier on the southern shore of the loch. These samples were preserved as described above.

2.3 Nutrient analysis

Nutrients were analysed using methods based on those described by Strickland and Parsons (1972). During the 1970's samples were analysed by a Technicon analyser or in discrete samples (Solorzano and Grantham, 1975). During the 2000's aliquots were removed from the water samples with a 60 ml syringe fitted with a Sartorius 25mm polycarbonate syringe filter holder and fitted with a Whatman 25mm GF/C circular glass microfibre filter. The water was then

injected into a small acid washed bottle and stored in a freezer at -18 ^{0}C until analysis. The filter holders were disassembled, acid washed and then rinsed in de-ionised water after each use. Once defrosted the samples were analysed on a QuickChem 8500 LACHAT flow injection auto analyser as described in Davidson *et al.* (2013), to determine the concentrations of nitrates, phosphates and silicates present in the loch. 2.4 Chlorophyll

. During the 1970's aliquots were filtered using Whatman GF/C glass fibre filters within 6 hours of sampling. The filters were then extracted into neutralised 90% acetone. Chlorophyll concentrations were estimated from fluorescence measurements, before and after acidification, calibrated against spectrophotometrically determined solutions of pure chlorophyll supplied by the Sigma Chemical company and based on the methods described in Holm-Hansen *et al*. (1965), UNESCO (1966) and Stricklands and Parsons (1968) During 2011, 2012 and 2013 500 ml of the collected seawater samples were filtered in duplicate on a 25 mm glass fibre filter (type A/E Pall Corporation, Portsmouth, UK) and stored in a freezer at -20 ^{0}C in ependorff tubes. Prior to analysis filters were thawed in 15 ml centrifuge tubes and pigments were extracted overnight in the dark at 4 ^{0}C with 8 ml of 90% acetone (VWR). Filters were sonicated for 1 minute and then centrifuged. During the 1970's Chl-*a* was measured with a Turner model 111 fluorometer. In order to reduce fluorescence due to chlorophyll-b and c, a 5-60 excitation filter was used with an emission filter combination of 70 (nearest the photomultiplier) and 16 (nearest the sample). During the 2000's Chl-*a* was measured with a Turner TD-700 fluorometer (Davidson et al., 2007).

2.5 Analysis of Wind, Precipitation and Water Temperature

Daily mean wind and precipitation data was downloaded from the British Atmospheric data centre (BADC) for the Dungrianach weather station (src_id 13972) located at Strath of Appin in the Loch Creran catchment area. Dates were converted into day of the year and the wind speeds for each day of the year were then averaged to create climatology's for the periods 1979-81 and 2011-13. For reasons discussed below (see discussion) water temperatures were not considered during this study.

2.6 Microscopic analysis and Life-form Climatologies

During the 1970's and early 1980's water samples were settled for a minimum of 12 hours in 10 ml sedimentation chambers then examined at a magnification of 225x for larger cells (in $1/5^{th}$ of chamber base) and at a magnification of 300x for smaller cells (in $1/20^{th}$ of chamber base) using the phase contrast objectives of a Wild M40 inverted microscope.

Between 2011 and 2013 samples were examined using the Utermöhl sedimentation method outlined in Lund *et al*., (1958), aliquots of the sampled water were placed into 50ml *Hydro-Bios* settling tubes and allowed to settle for a minimum of 20 hours. Full chamber counts at 200x magnification were then carried out using Carl Zeiss Axiovert inverted microscopes. The samples were

examined using both phase contrast and bright-field illumination. Where necessary cover slips were removed and dissecting needles were used to manipulate cells to aid in identification.

Aliquots from supplemental samples were placed in 10ml settling tubes and allowed to settle for a minimum of 12 hours. Full chamber counts were carried out at magnification of 225x for larger cells (Full chamber base) and at a magnification of 300x for smaller cells (in 1/50th of chamber base) using the phase contrast objectives of a Wild M40 inverted microscope. To calculate the cell biovolume, geometric models were used as described in Hillebrand *et al.,* (1999).

During the 1970's 10ml aliquots were settled for 12 hours whereas during the 2000's 50ml aliquots were settled for 20 hours. The settling chambers are the same diameter and the difference in volume is a function of their height. The different settling times were chosen to allow the contents of a chamber of either volume sufficient time to fall to the bottom of the chamber. The different sedimentation volumes used between the 1980's and the 2000's, combined with changes to the proportion of sedimentation chamber base examined altered the minimum number of microplankton cells that could be detected in a sample. The limit of detection (LOD) during the 2000's was 20 cells/L. This contrasts with a LOD of 490 cells/L for samples examined during the 1980's. These differences have to be taken into account during the calculation of the PI(mp) (see section 2.9 below).

Climatologies were created, to illustrate the abundance of pelagic diatoms, autotrophic/mixotrophic and heterotrophic dinoflagellates and ciliates detected in Loch Creran (Figure 3), by plotting the log_{10} transformed numbers detected during each day of each year between 1979 and 1981. An envelope was fitted to these points, given the right conditions phytoplankton can grow exponentially, attaining high abundance in a very short time then just as quickly declining, the envelope was fitted to the 5^{th} and 95^{th} percentile to eliminate those outlying values that would result in an overly large envelope

A median was also plotted mapping the annual distribution of each life-form through the year. The data for the comparison period 2011-2013 was then plotted over this envelope.

2.7 Calibration of Historic and Present data

Microplankton taxonomy is an ever changing field and changes are regularly made to scientific equipment. As a comparison was being made between samples collected in the 1970's and in recent years it was necessary to ensure that the analysis and enumeration techniques used remained comparable. While the use of life-forms reduces the degree of taxonomic accuracy required, to minimize the possibility of a species being misidentified or changes in the type of microscope being used, biasing the counts, regular calibration exercises were conducted involving the different researchers examining the samples. Further, as water samples were collected at differing times, both from Barcaldine Pier and from the research vessels; RV Calanus and RV Seol Mara

it was felt prudent to compare the results obtained from samples collected at the pier with those collected in the main basin. Figure S7 (supplemental material), shows that while there was greater variability in chlorophyll concentrations measured in the main basin, where samples were integrated over the top 10 metres, they agreed well with those samples taken at a depth of 1-2 metres from the pier.

Additionally during the 1980's water samples were filtered through Whatman GF/C filters with a pore size of 1.2 µm whereas during the 2000's the samples were filtered through Pall A/E GF filters with a slightly smaller pore size of 1.0 µm. While the smaller pore sized filter papers used in the 2000's could potentially have retained more phytoplankton thereby increasing the concentration of Chl-*a* observed, work by Morán *et al.,* (1999) comparing different filter types found no noteworthy differences between the ability of Whatman GF/F filters, with a pore size of 0.7 µm, and Whatman GF/C filters with a pore size of 1.2 µm to retain Chl- a. It was assumed that the use of Pall A/E GF filters with an intermediate pore size did not affect the results.

Chl- *a* extraction methods also differed between the two periods. Samples in the 1980's were extracted within 6 hours of collection whereas samples collected in the 2000's were filtered and the filters stored at -20^{o}C before analysis. Wasmund *et al.,* (2006), comparing chl *a* extraction methods found that Chl *a* concentrations obtained from samples that had been stored at -20^{o}C were lower than those obtained from samples that were analysed within a few

hours of collection. There was also recognition that the fluorometric methods used in this paper to determine Chl-*a* are problematic (Gowen and Tett 1983, Stitch and Brinker 2005). They were included in this study as the methods employed during the 1970's were the same as those employed during the 2000's and it was felt that the observed changes in pheopigments (Figure S1) were of interest. Additionally a study in Loch Creran by Gowen, Tett and Wood (1983) suggested that those species containing Chlorophyll-*b* (members of the *Euglenophyceae*, *Chlorophyceae* amd *Prasinophyceae*) did not substantially contribute to the phytoplankton biomass in the loch, particularly during the spring bloom when much of the change took place.

2.8 Life-forms

Determining which species should be categorized as a particular life-form can be challenging and controversial. Rather than being taxonomically related, a life-form will represent those groups of species that have similar roles in the functioning of the ecosystem. Some distinctions such as that between photosynthesis and primary production (phytoplankton) and consumption and recycling (zooplankton) may seem clear; however, given the complexity within an ecosystem it can be difficult to choose which further distinctions are needed to categorize its main functional relationships (Tett 2014).

For the purposes of this paper four life-forms were chosen, “Pelagic diatoms” consisted of pelagic centric and pennate diatoms but excluded tychopelagic species. “Autotrophic/mixotrophic dinoflagellates” comprised of those armoured and naked dinoflagellates that contain chloroplasts. “Heterotrophic dinoflagellates” represented by those dinoflagellates without chloroplasts. “Ciliates” comprised of all oligotrich ciliates, including tintinnids and “Small flagellates” a category comprised of Cryptophyceae, Euglenophyceae, Haptophyta and Raphidophyceae between 5 µm and 20 µm. As the mixotrophic ciliate, *Myrionecta rubra* was rarely distinguished from other ciliates during the sampling that was carried out in the 1970’s and 80’s it has been excluded from this group. Additionally the genus *Pseudo-nitzschia* was included to allow an evaluation of the state of potentially toxin producing diatoms in the loch.

2.9 Calculation of the PI(mp)

To calculate the PI(mp) cell counts (cells/l) for the 1970’s and the 2000’s were restricted to samples collected in the top ten metres of the water column. The reference data was defined as that collected from 1979 – 1981, with the index being used to compare this to recently collected data. The data (cells/l) were first converted by log10(X + z) transformation, where X = life-form abundance and $z = 0.5X_{min}$. X_{min} = minimum abundance recorded for a particular life-form. The addition of z avoids errors when values are based on non-continuous data with some zero values (Welham *et al.,* 2014). It also helps to ameliorate the consequences that result from using different sedimentation volumes. For example a species count made on a 50ml sedimented sample will have a limit

of detection (LOD) of 20 (cells/l) while those on a 10 ml sedimented sample will have a LOD of 100 (cells/l). In some cases during the 1970's only a portion of the base of the settling chamber was counted giving a LOD of 490 (cells/l). For this reason, when calculating the PI(mp), z was set at 245. For a more detailed description of the methodologies used in the PI(mp) see Tett (2014).

The abundances of the two life-forms were then combined to form a vector which was plotted into a two dimensional state space. To create the envelope a convex hull algorithm was programmed into MATLAB using the "convhull" function. Due to the large amount of variation in microplankton communities, plotting all the values, including extreme outliers, results in an excessively large reference envelope and reduces the sensitivity of the PI(mp). This is because relatively few, high values greatly expand the convex hull generated envelope (Tett *et al*., 2008), thereby reducing the methods sensitivity to change. Excluding a large proportion (p) of points however, while increasing the sensitivity, would give results that were less likely to be statistically significant. On reflection a value excluding 10% of the points (p=0.9) gave the best outcome however the script does offer the user the choice to exclude points or not and over the proportion of points to exclude. Plotting weekly values can, for similar reasons, also result in a large reference envelope (see Figure S4 for an example of a plot using weekly values). Monthly means were therefore used in this analysis.

The PI(mp) is given by:

$$\mathrm{PI(mp)} = \frac{number\ of\ new\ points\ (n) between\ inner\ and\ outer\ envelopes}{total\ number\ of\ new\ points\ (N)}$$

A value of one indicates that there has been no change while a value of zero would indicate a complete change. The significance of the PI(mp) can be calculated by using a binomial series to determine the probability of finding that number of new points outside the reference envelope (Tett *et al.,* 2008). The expectation was that, if there had been no true change, the majority of the new points would have plotted inside the reference envelope i.e. PI(mp) = p, with pN points lying inside the envelope, where N refers to the total number of new points. The probability of the result is estimated by examining all the possible outcomes, using the Matlab function *nchoosek* to calculate the probabilities of 0, 1, 2… (1-p)N, points falling outside the envelope.

As described above, the PI(mp) allows a comparison to be made between the present state of various life-form pairs in a microplankton community with a given reference period. However, by determining the PI(mp) for individual years, compared with a reference period, it can also be used to produce a time series, mapping any changes that have occurred in a microplankton community.

3 Results

3.1 Climatologies of pelagic diatoms, ciliates and dinoflagellates in Loch Creran

The Climatologies of pelagic diatoms, ciliates and dinoflagellates, Figure 3, show major change (Table 1) between the periods 1979-1981 and 2011-2013. The data illustrates that from 1979-81 pelagic diatoms in Loch Creran generally followed a seasonal pattern with a large spring bloom in late March, predominantly comprised of *Skeletonema* spp., which declined during summer and was then followed by a smaller, mixed assemblage bloom in the autumn (Tett *et al*., 1981). Figure 3 demonstrates that the 2011-2013 spring bloom occurred later in the season. In general, relative to historical data, the numbers of pelagic diatoms decreased throughout the year.

In contrast to diatoms the numbers of autotrophic/mixotrophic dinoflagellates observed in the loch during the same period increased with time (Figure 3). This increase was particularly marked during August and September but was also evident during March. At the same time heterotrophic dinoflagellates decreased, although it should be noted that due to a lack of data the period used to create the climatology of heterotrophic dinoflagellates was increased, incorporating observations made between 1970 and 1981. There was no change in the annual number of ciliates however, there was an increase in the numbers of ciliates observed during the spring.

3.2 PI(mp)

There have been marked changes (see Figures 4-7) in the relative abundances of the life-form pairs both in terms of cell numbers and biovolume and that the community, at least in terms of diatoms, dinoflagellates and ciliates, has

undergone a major disturbance in comparison with the 1979-81 reference period.

In comparison with the 1979-81 reference period, there were important changes in time in the relative numbers of diatoms and auto/mixotrophic dinoflagellates observed in the loch. Including much greater variability in the relative abundances observed throughout the period 2011-13. In terms of biovolume the results were again noteworthy. The changes in biovolume mirror the changes in cell numbers.

There were major changes in the heterotrophic part of the community (see Figures 5-6). Cell numbers changed substantially (Table 1). Relative to the reference period, the numbers of ciliates dropped both in real terms and in relation to heterotrophic dinoflagellates. These were accompanied by similar changes to the biovolume. Additionally greater variability in the biovolumes was observed.

There was no data available for small flagellates during 2013 so in this case the comparison is based on the years 2011 and 2012 (see Figure 6). Again there were changes (Table 2) in the community structure. In comparison with the reference period the number of both ciliates and small flagellates in the loch increased. However while the biovolume of ciliates decreased slightly, that of the small flagellates fell.

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481 There have been important changes (Table 2) between the abundance of
482 pelagic diatoms (excluding *Pseudo-nitzschia* spp.) and the numbers of *Pseudo-*
483 *nitzschia* spp. in the loch (Figure 7)..
484 Figure S5 (supplemental material) illustrates the same data in colour where
485 different coloured circles represent different times of the year. The numbers of
486 *Pseudo-nitzschia* observed during the spring bloom, consisting mostly of the *P.*
487 *delicatissima* group have dropped in number relative to the reference
488 conditions. However during the summer, when blooms predominantly consist of
489 the *P. seriata* group the numbers of *Pseudo-nitzschia* relative to all other
490 diatoms has increased.
491
492
493
494 While each life-form pair can be analysed separately, revealing changes to
495 different components of the community, an average PI(mp) can also be
496 calculated to give an over view of the state of the community (see “Table 2).
497 Considering the life-form pair diatoms and auto/mixotrophic dinoflagellates, the
498 PI(mp) has ranged from 0.25 in 2011 to 0.58 in 2012 and 0.0 in 2013. A value
499 of 1.0 would indicate no change in the community structure. Although the
500 PI(mp) rose to 0.58 in 2012 It is clear that, in comparison with the reference
501 period, this part of the community has undergone major change. Similarly for
502 the second life-form pair; ciliates and heterotrophic dinoflagellates, the PI(mp)
503 has varied from 0.25 in 2011 to 0.0 in 2012 and 2013, again indicating that
504 change has occurred. It is also possible to calculate a mean PI(mp) for the

505 different life-form pairs and again we can see that there has been an important
506 change to the community.
507
508 ## 3.3 Nutrients
509 Nitrate concentrations measured between 2011 and 2013 were found to be
510 significantly higher than those measured during the reference period, Exact
511 Binomial Test, $p < 0.05$, (Figure 8). Phosphate concentrations, although
512 elevated during the winter, showed a significant decrease, particularly during
513 the spring, Exact Binomial Test, $p < 0.05$. Silicate levels were also elevated
514 during the winter and although the summer concentrations were noticeably
515 lower than those found during the reference period, this change was not
516 significant. It should be noted that Silicate concentrations for 1979 -1981 were
517 not available. Instead concentrations measured between 1971 – 1976 were
518 used for the comparison.
519
520 ## 3.4 Rainfall
521 A comparison of precipitation levels (Figure 9) recorded during the reference
522 period 1979-81 and 2011-13 showed that the pattern of rainfall in the Loch
523 Creran catchment had changed, with a significant increase in precipitation
524 between January and July during the period 2011-13, Kolmogorov Smirnov, $p <$
525 0.01. During this period January, February and March were characterised by
526 intermittent periods of higher than expected, intense rainfall followed by notable
527 increases between April and May.
528
529 ## 3.5 Chlorophyll concentrations

Chlorophyll concentrations (Figure S1) in samples collected from Loch Creran between 2011 and 2013 were significantly lower than those recorded in the loch between1979 and 1981, Exact Binomial Test, $p < 0.0001$.

4 Discussion

4.1 Reliability of the data

Carrying out a study that includes data collected over a period of several decades can be problematic. Changes to sampling methods, locations, equipment and taxonomic identification all have to be accounted for. In this study, one researcher, Paul Tett, was involved in the collection of water samples from Loch Creran for over four decades. This allowed a high degree of calibration between the various studies that were carried out. While the taxonomy of several species has changed over the past forty years, the use of life-forms makes this much less problematic than would be the case if individual species were used. The comparison of chlorophyll concentrations carried out between samples collected from Barcaldine Pier and the main basin of Loch Creran (Figure S5), illustrates that the use of these different locations has not biased the results. The work carried out by Wasmund *et al*. (2006) however, suggested that the change to the method of extracting Chl-*a* could reduce the amount of Chl-*a* detected in the samples collected during the 2000's by 20-25%. This is a large difference and may account for some of the reduction in Chl-*a* concentrations observed in the loch (see Figure S1).

Perhaps the biggest problem in analysis comes from the change in sedimentation volume. Between 1979 and 1981 only the central 1 cm^2 square of the sediment chamber base was examined, effectively reducing the observed volume to 2 ml. During 2011 and 2013, an observed volume of 10 - 50 ml was used. This change has reduced the limit of detection of cells in a sample, from 490 cells/l in the 1970's, to 20 cells/l between 2011 and 2013. In practise this increased ability to detect cells at lower densities in a sample can cause any new points plotted onto the PI(mp) diagrams to move downwards and to the left, making it appear as if there has been a decrease in cell abundance. To account for this the term z, set at 0.5(LOD) was added to the log transformed cell numbers. As well as compensating for any bias caused by changes to the LOD it prevented any numerical errors when trying to log transform observations where the cell numbers were zero. A further change in methodology, while not affecting the outcome of the present study, did reduce its scope. The sedimentation method used during the 1970's, unlike that used in the 2000's, did not allow for the manipulation of cells while under microscopic examination. This meant that counts of potentially toxin producing dinoflagellates such as those belonging to the genus *Alexandrium* could not be reliably identified in the samples. Although it would have been useful to determine if there had been any changes over time in this part of the community it had to be omitted from this study.

4.2 Changes to the phytoplankton community

It is clear that the microplankton community in Loch Creran underwent substantial changes in its structure between the chosen reference period of

1979 - 1981 and the comparison period 2011 - 2013. Seasonal succession in Scottish sea lochs generally follows an annual pattern of a spring bloom, typically dominated by diatoms that are succeeded by dinoflagellates and flagellates as they become starved of nutrients (Tett and Wallis 1978). Figure 3 shows that in comparison with the period 1979-81 while the magnitude of the spring bloom did not change, the onset of the bloom was delayed by three weeks and the numbers of diatoms dropped during January, February and March. During the same period the number of autotrophic/mixotrophic dinoflagellates increased. This move from diatoms to dinoflagellates in a system is often indicative of eutrophication (Paerl *et al*., 1997).

With the establishment of a fish farm in Loch Creran in the early 1990's a certain amount of nutrient enrichment, both from waste food and faeces, would be expected and indeed nitrogen concentrations in the loch were found to have increased, much of this occurring during the spring. Phosphorus levels, although high in the winter, however, showed a decrease, particularly between March and May. If eutrophication was the cause of the changes in the structure of the microplankton community then both nitrogen and phosphorus concentrations should have increased. A further argument against eutrophication can be found in the measurements of Chl--a made in the loch which were lower in 2011-13 than those made in 1979-81.

The ability of diatoms to grow is also very sensitive to the concentrations of silicon available to them (Martin-Jézéquel *et al*., 2000. Gilpin *et al.,* 2004, Davidson *et al.,* 2012). Silicon is of particular importance as it is needed to

construct the diatom frustule (Brzezinski 1985, Davidson and Gurney 1999). Therefore if concentrations of this nutrient have decreased this could explain the drop in diatom numbers that has been observed in the loch during the spring.

Silicon in Loch Creran comes from the rivers feeding into the loch and from tidal exchange. Jacobsen *et al.,* (1995) and Conley *et al*., (2002) found that globally the concentration of silicon in rivers has slowly declined; much of this drop connected to river modification and the subsequent sedimentation of silicon in reservoirs and increased eutrophication. However this is unlikely to be the case in Loch Creran as there have been no major structural changes to River Creran, the main source of fresh water into the loch.

Kunishi *et al.,* (1972) have shown that as water drains from a watershed a large proportion of the available phosphate is adsorbed onto sediments while Arheimer and Lidén (2000) have noted that phosphate concentration is negatively correlated to water runoff. The pattern observed in the spring, of elevated nitrogen and lowered phosphorus levels, could be indicative of an increase in freshwater flowing into the loch. The most likely source of this extra water would be rainfall. This raises the question; have rainfall levels risen in the Loch Creran catchment area relative to the reference period? It is apparent (Figure 9) that the pattern and intensity of the rainfall has changed. Compared to the period between 1979 and 1981 mean rainfall in the period 2011 and

2013 has increased by 89% and this has been accompanied by a large increase in the intensity of the rainfall.

Increased precipitation can affect the state of the loch in different ways. Greater freshwater flow into the loch can raise the flushing rate; altering the amount of water displaced through tidal exchange and with it the amount of microplankton that are washed out of the loch (Ross *et al*., 1994, Su *et al*., 2004, Lionard *et al*., 2008, Peierls *et al*., 2012). Tett (1986) observes that rainfall would need to be greater than three times the mean value, resulting in volume displacement rates of between 0.04 and 0.1 d^{-1}, for this to be a possibility. Although there were days when less rain fell during the first six months of 2011-13 than during the same period between 1979-81 (73 days out of 180), these were outnumbered by the number of days when rainfall was greater than the 1979-81 levels (107 days out of 180). On 56 of these days, rainfall exceeded the mean 1979-81 values by more than a factor of three (see Figure 9).

It would therefore seem reasonable to assume that washout events in the loch have increased during the spring months. However Gillibrand *et al*., (2013) who applied a box model of seasonal exchange to Loch Creran found that while increases in rainfall could certainly influence the internal water properties of the loch it would have little effect on flushing times, so some uncertainty remains in relation to the impact of rainfall.

Nevertheless, whether changes in the amount of flushing during spring account for the decrease in the magnitude of the spring bloom or not, it does not, on its own, explain the decrease in diatom and increase in dinoflagellate abundance.

Unfortunately there is very little information on zooplankton composition and abundance in Loch Creran and so it is not possible to determine if grazing rates have changed between the 1980's and the 2000's. However Tett and Wallis (1978) did not think that grazing by zooplankton had a major impact on phytoplankton abundance in the loch.

A recent study by Thomson *et al.,* (2015) who looked at patterns of phytoplankton abundance over 106 sites worldwide, found that in areas that they considered "wet", i.e. areas where they found that precipitation levels were increasing, wet springs were strongly linked to a decrease in the abundance of diatoms and an increase in the abundance of dinoflagellates.

Diatoms may be expected to have a competitive advantage in turbulent conditions, whereas dinoflagellates may be expected to favour relatively stable, nutrient limited conditions (Falkowski et al., 2004, Tozzi et al., 2004), characteristic of low flushing rates (Peierls et al., 2012). At a first glance pulses of higher flushing rates would favour diatoms. However, more freshwater flowing in to Loch Creran would increase water column stability by intensifying haline stratification which would favour dinoflagellates (Diaz *et al.,* 2011). Therefore, short periods of intense rainfall possibly leading to an increase in the amount of flushing which, are then followed by periods of enhanced water column stability, could account for the observed changes in the microplankton community.

Related to this, a possible mechanism for the drop in diatom numbers may be found in a study by Hansen *et al.,* (1995). As mentioned earlier the spring diatom bloom in Loch Creran is mostly comprised of *Skeletonema* spp.

Hansen and his colleagues looked at the coagulation dynamics of *Skeletonema*, a particularly “sticky” species. They found that it dominated the community in Isefjord, a Danish Fjord, by acting as a flocculating agent. Particularly in the presence of larger competitors it was adept at aggregating cells causing them to settle out of the photic zone. However *Skeletonema* would only have an advantage provided that it has an ample supply of seeding stock available. This seeding population may come from suspended cells present in the water column between blooms or re-suspended resting cells. An increase in the amount of stratification or in the number of cells washed out of the loch would have a detrimental effect on the ability of *Skeletonema* to resupply its seed stock.

An increase in the intensity of local rainfall may wash more humic acids “gelbstoff” into sea lochs. Gelbstoff concentrations in Loch Creran have been observed to be negatively correlated with surface salinity and positively correlated with ammonium and phosphorus concentrations near the seafloor (Solórzano and Ehrlich, 1979).These yellow tinted materials can lower the ambient sub-surface light flux and, by virtue of their colour, act as a filter absorbing light at the wavelengths required by diatoms. Autotrophic or mixotrophic dinoflagellates possess the capacity to compensate for lower light levels through their abilities both to move through the water column and to ingest prey. Solórzano and Ehrlich (1979) also suggest that the presence of humic acids may enhance the remineralisation of ammonium and again this may favour the growth of dinoflagellates relative to diatoms (Berg *et al*., 2003).

Water temperatures were not included in this study in part because stratification in Loch Creran is predominantly controlled by freshwater inflow (Tett and Wallis 1978). While thermal stratification does occur during the summer months the shallow depth of the loch often results in a well-mixed water column. Higher water temperatures could, potentially, increase heterotrophic processes within the system. In a sea loch such as Loch Creran, where the onset of the spring bloom is controlled by the amount of light available in the early part of the year, this can potentially lead to a mismatch between the abundance of grazers and phytoplankton present in the loch at any particular time. Sommer *et al*. (2007) carrying out mesocosm experiments noticed a significant increase in the numbers of ciliates and other protozoans when water temperatures were raised. This was also accompanied by an earlier onset of growth.

A comparison of temperatures during the 1970's and 2008/2009 was carried out by Whyte (2012) who found that water temperatures in the loch had increased. However as this comparison was only based on measurements made over two years it is difficult to draw conclusions as this may have been due to annual or short term variation rather than a long term trend.

It is unlikely that the changes to the phytoplankton community in loch Creran are due only to one of the mechanisms mentioned above. It is more probable that they are the result of a combination of some or all of these factors. However, central to each is that the shift in the local pattern of precipitation, in terms of timing and intensity, is the major driver behind the observed changes to the microplankton community in the loch.

4.3 PI(mp)

Tett et al., (2008) note that the health of an ecosystem has components that include "vigour", a measure of the energy flowing through the system, "resilience", the ability of a community to recover from disturbance and the organization of a community or "structure" and have developed the PI(mp) as a measure of the latter.

The PI(mp) has allowed a quantitative assessment of the state of the pelagic, micro-planktonic community in Loch Creran to be undertaken and has shown that its structure has changed relative to the chosen historical reference period. Not only has it indicated that changes have occurred but it has also provided information on the nature of those changes. For example, an increase in the biovolumes of autotrophic dinoflagellates relative to diatoms; an increase in ciliates (between January and June) relative to heterotrophic dinoflagellates and an increase in the range of sizes of phytoplankter present. It has also provided valuable information with regards to the potentially toxin producing genus *Pseudo-nitzschia*. Many of the species associated with the production of toxins belong to the *P. seriata* group which, in Scottish waters, tend to bloom during the summer and as can be seen in Figure S4 are increasing in Loch Creran.

It is also worth noting that levels of Chl--a measured in Loch Creran have actually fallen (Figure S1). Tett and Wallis (1978) recorded Chl-*a* distribution in the surface waters of the loch between 1974 and 1976 and found peak Chl-*a* values of 1.57 Log_{10}(µg/l). During 2011, 2012 and 2013 recorded levels of Chl-

a did not exceed 0.96 Log_{10} (µg/l). A monitoring regime focussed only on Chl-*a* concentrations, such as many of those mentioned above, may overlook important changes that are occurring in the structure of a community regardless of whether those changes are related to eutrophication or not .

The PI(mp) is also robust in terms of the quantity of monitoring data required. Assuming that the data used to create the reference envelope has been collected over two to three years and that the samples have been collected throughout the large part of each of those years, so encompassing most of the inter-annual and inter-seasonal variability present in the community, new measurements can be taken when the opportunity arises and don't necessarily require a strict sampling regime to give accurate results. By focussing on life-forms rather than specific species it reduces the level of taxonomic identification skills needed by samplers. Recent work by Brito *et al.,* (2015) has shown its usefulness can also be extended to estuarine waters.

5 Conclusion

It is evident that there have been changes in the overall numbers of microplankton in Loch Creran and that these changes are having an effect on the actual compositional structure of the microplankton community in the loch. It seems likely that these changes are driven by changes to the timing and the intensity of local rainfall patterns.

771 There are several different indices in use around the globe, each exhibiting
772 strengths and weaknesses. As the need to assess the ecological status of
773 water bodies continues to grow, it will be necessary to find some way to
774 integrate these tools and it is very unlikely that one solution will be suitable in all
775 occasions.

776 Abundance (or biovolume) based analyses such as those shown in Figure 3
777 are very useful as a straight forward measurement of community size but they
778 fail to capture important structural features of the microplankton community.
779 Structural information, a key requirement of the MSFD, is needed to determine
780 the ecological status of a body of water.

781 The PI(mp)'s ability to visualise and highlight areas undergoing change can
782 allow scarce resources to be better targeted enabling a more focussed,
783 monitoring and assessment programme to be undertaken in those areas
784 deemed most at risk. Undeniably the PI(mp) has room for improvement. For
785 now, there is a lack of studies that can effectively connect clearly observed
786 changes in the PI(mp) with different environmental "pressures". More research
787 is needed to make these links clearer. Nevertheless, given its ease of use, the
788 PI(mp) provides a useful tool for evaluating the state of microplankton
789 communities. Hopefully its development and use will continue.

790

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Authors Contributions

CW has contributed in generation of data, analysis and paper writing. KD has contributed in analysis and paper writing. PT has contributed in data analysis and preparation of MATLAB scripts. LG has contributed in paper writing. SM has contributed in data analysis. EM has contributed in data analysis, identification and enumeration of microplankton and paper writing. GM has contributed in paper writing.

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