Constraining causes of fish mass mortality using ultra-high-resolution biomarker measurement

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ABSTRACT

Lamina by lamina measurement of biomarkers at a sub-millimetre resolution within the Achanarras Limestone Member has helped to resolve the changing environmental conditions associated with a fish mass mortality horizon. An anomalous proportion of C_{30} sterane (24-*n*-propylcholestane) marks the beginning of the horizon and likely corresponds to an influx of marine water. This appears to have been short lived and was likely analogous to a modern day storm tide. The subsequent laminae record an increased incidence of water column stratification and hypoxic bottom waters in the form of an elevated gammacerane index. The mass mortality horizon studied was from an upper interval of the Achanarras Limestone Member with a fossil fish assemblage comprising mostly *Dipterus*, an early Dipnoan (lungfish). However, lower intervals of the Achanarras Limestone Member have greater assemblage diversity, including species associated with marine conditions such as *Coccosteus*, and evidence higher proportions of C_{30} sterane indicating better connection to the marine environment. Therefore, it appears that ingressing seawater in and of itself was not responsible for creating a stressed environment. Rather, disconnection of the lake from marine waters stranded fish in a lake, that when perturbed

by storm tides, killed *en masse* by exposing fish to hypoxic conditions in a similar way to modern water bodies effected by storm tides generated during hurricanes.

1.0 INTRODUCTION

The fish bed section of the Middle Devonian-aged Achanarras Limestone Member, located in the Orcadian Basin in NE Scotland, preserves an abundant fish fauna many of which display excellent preservation. Trewin (1986) provided a detailed description of the fish genera present and their stratigraphic and faunal distribution. In summary; a wide range of life-habitats is evidenced by the genera encountered that range from *Dipterus* – an early dipnoan (lungfish) tolerant of dry-intervals that prevailed in shallow water, through to *Coccosteus* the fossils of which are most abundant in intervals deposited when lakewater levels were high, and which in many other settings are found in marine environments. Fossil fish are not evenly distributed throughout the Achanarras Limestone Member but instead found clustered within distinct mass mortality horizons, with a frequency exceeding one per ten year interval. Hamilton and Trewin (1988) suggested that mass mortality horizons within the Achanarras Limestone Member could have resulted from the overturning of a stratified lake and the consequent reduction of oxygen in surface water as it mixed with hypoxic bottom water. Decay of organic matter generated by algal blooms stimulated by the resupply of nutrients to surface waters could have further deoxygenated waters. Although such kill-mechanisms are documented from East African Rift Lakes (Beadle 1981), no further evidence has been presented to support this mechanism or indeed the responsible trigger.

The Achanarras lake itself was deposited in a basin bounded by NE-SW trending faults, with the nearest coastline (based upon the occurrence of demonstrably marine-successions) located to the South East (Fig 1). More locally, at least to the SW, SE and NW the Caithness flagstones pass into fluvial, alluvial and Aeolian facies, thus from a first consideration the lake would appear to have been truly “landlocked”. However, varying levels of land-sea interaction have been suggested for the beds. Microfaunal evidence for marine incursions into the Orcadian Basin (e.g. Marshall et al. 1996) has been reported for the Upper Middle-Devonian (Givetian) of Orkney, e.g. to the north of the of Orcadian lake. Additionally, certain members of the lakes fish fauna (e.g. *Coccosteus*) are also found within time-equivalent marine successions deposited in the ancient Rheic Ocean to the south of the Old Red Continent. However, these fish may have migrated to the lake and their presence in the fishbeds is therefore not conclusive evidence of an incursion of seawater (Trewin 1986). Thus there exists evidence of a connection to the marine environment, but it appears inconsistent, and to have only marginally influenced the lakes environment.

In addition to its fish fauna, the Achanarras Limestone Member is also noted for its seasonally laminated sediments. The Mid-Devonian 3.6 meter thick Achanarras Limestone Member comprises lamina couplets interpreted by previous authors as non-glacial varves reflecting intra-annual variation in sedimentation, where two adjacent laminae account for a years sedimentation (Rayner, 1963; Trewin, 1986). The varve couplets consist of carbonate / clastic pairs; carbonate laminae comprise a ferroan microdolomitic carbonate phase and clastic laminae are mainly siliciclastic (Andrews et al. 2010; Othman Wilson, 2012). A number of orders and scales of cyclicity are recognised within the Achanarras Limestone Member, but most relevant to this study are the very short order cycles corresponding to Schwabe cycles (~11 years) and shorter order cycles (3 - 8 years) that most likely represent the oscillation of shorter order climatic cells (Andrews et al., 2010). This accords with the view of many other workers that the cyclic patterns of sedimentation observed within the Caithness Flagstone group and particularly the Achanarras Limestone Member strongly reflect climatic forces rather than tectonic processes and rates of basin infill (Astin et al., 1990; Marshall et al. 1996; Trewin and Thirlwell 2002).

The purpose of this study is to look for organic geochemical anomalies that will help ascertain what was unusual about the Orcadian Lake's water column during the deposition of the fish mass mortality horizons and better understand its depositional environment. By making geochemical measurements on a lamina by lamina scale a high resolution chronology can be constructed to explore how environmental conditions changed and led to the deposition of a mass mortality horizon.

2.0 METHODS

Samples were collected from the Achanarras Quarry, Caithness (Fig.1). The specimen used for this study is from the uppermost fish-bearing section of the Achanarras sequence (facies 6 from Trewin 1986). Elemental (carbon and sulphur), stable isotope data ($\delta^{13}C$ for organic and inorganic carbon) and biomarker data were obtained for twenty four consecutive laminae. Limited data is also presented for lamina collected from the middle part of the Achanarras Limestone Member for comparative purposes.

Samples for geochemical analysis were obtained by micro-drilling discrete lamina from specially prepared 5 mm thick slabs using a MicroMill system (New Wave Research Ltd); slabs have flat polished surfaces, perpendicular to lamina and were lightly etched with hydrochloric acid. The semi-automated MicroMill, which can articulate submicron distances in three degrees of freedom (x,y,z), was used to sample laminae less than 1 mm thickness. For thick lamina, a drill mounted on a manually operated stand was used with a binocular microscope facilitating accurate positioning. For all methods no lubricating fluid was used and drills were operated at medium-speed to avoid generating excessive friction or long drilling times. Drill powder was removed from samples by gravity, air and mechanically by a fine brush. All powder was removed from a working surface prior to “drilling-out” the next laminae. Laminae were removed alternately, with in-fill drilling used to sample lamina remaining after the first pass of a sample. While working on a given slab etching and polishing were repeated to provide clean and clear working surfaces and prevent cross contamination between samples.

Extracts were obtained using a modified version of the mini-extraction methods presented in Bowden et al., (2008); a three stage extraction process was applied to ~100 mg of powder (using dichloromethane and methanol), after which extracts were combined and then concentrated by evaporating the solvent under an inert nitrogen atmosphere. Gas Chromatography Mass Spectrometry (GC-MS) analysis of the extract was performed using an Agilent 6890N GC fitted with a J&W DB-5 phase 50 m length column (0.25 mm id, 0.25 μm film thickness) connected to a 5975 MSD and a quadruple mass spectrometer operating in SIM mode (dwell time 0.1 s/ion and ionisation energy 70 eV). Fifteen ions were monitored; *m/z* 191, 205 and 412 to help interpret pentacyclic terpanes such as hopanes, *m/z* 113, 183 and 125 for isoprenoidal hydrocarbons including β,β-carotane and *m/z* 217, 218, 231 and 259 for four ring terapoids such as steranes, diasteranes and methylsteranes. Samples were injected manually using a split/splitless injector operating in splitless mode. Temperature program for the GC oven was 80 – 295 °C, holding at 80 °C for two minutes, rising at 10 °C min^{-1} for 8 min and then 3 °C min^{-1} and finally holding the maximum temperature for 10 min. Compounds were identified by comparing retention times to well-characterised materials that served as reference samples. All concentrations are reported relative to the internal standard. Illustrative chromatograms for the biomarkers used in the study are shown in Fig. 2 and Fig. 3.

Here we focus on β,β-carotane, 24-*n*-propylcholestane (C_{30} sterane) and gammacerane hydrocarbon biomarkers because these compounds demonstrate the clearest links to changes in palaeoenvironment during the interval concerned. From a technical perspective these compounds were easy to isolate and measure, because of their relative abundance. Instead of making use of β,β-carotane as ratio denominated by another biomarker, we report it as micrograms of β,β-carotane per g of sediment. Doing this permits biomarker concentration to be reported per laminae – e.g. per unit of time, which thus expresses biomarker data as a net burial rate. Concentrations of β,β-carotane are reported relative to an internal standard of D4-cholestane. The gammacerane index was calculated after the method presented in Peters et al. (2007), with peak assignments verified by use of the 412 and 205 *m/z* ion chromatograms. Similarly sterane parameters were verified by calculating parameters using the 217 and 218 *m/z* ion chromatograms. A comparison of the duplicate parameters obtained is presented in supplementary information 1. Errors for β,β-carotane measurement based on duplicate analysis of extracts are +/- 5.1 %.

For $\delta^{13}C$ carbonate (inorganic carbon) stable isotope analysis, 1-2 mg samples were dissolved overnight in phosphoric acid at 70 °C. The carbon dioxide that evolved was purged under positive pressure, and using helium as the transfer gas analysed on an AP2003 mass spectrometer. Repeat analyses of NBS-18 and internal calcite standards were generally better than ±0.2‰.

For $\delta^{13}C$ organic determination powders were acid digested (by sequentially exposing samples overnight to 10 % and then 25 % hydrochloric acid) to remove all inorganic carbon (carbonate). Samples rinsed with distilled water, dried and weighed into tin capsules. Samples were then analysed by continuous flow isotope ratio mass spectrometry (CF-IRMS) using a Thermo Finnigan Delta Plus XP Mass Spectrometer, coupled to a Costech Elemental Analyser (model ECS 4010). A minimum of 20 mg (equivalent to approximately 0.1 mg carbon) of sample, per lamination was combusted in a tin capsule for simultaneous determination of carbon isotope ratios. Three laboratory standards (prepared from gelatine and alanine standard solutions) were analysed for every 10 samples, allowing instrument drift to be corrected over the course of a 14 hour analytical sequence. Error on replicates is better than 0.2 ‰. Four aliquots (per run) of Tryptophan, an amino acid, were also analyzed simultaneously in order to calculate the carbon content of the samples. All stable isotope ratios are expressed in δ notation as parts per thousand (‰) relative to V-PDB and V-SMOW international standards.

3.0 RESULTS

The following sections describe data initially from the perspective of establishing an environmental baseline (dashed line representing an average value in Fig. 4), and then from the perspective of anomalous values associated with the mass mortality horizon (values exceeding a standard deviation).

3.1 Environmental Baseline

Total organic carbon is generally quite low with a biannual average of 0.23% (n= 24, σ = 0.06%). This is consistent with a lake environment in which sedimentation rates were known to be high (diluting organic carbon), and experienced occasional influxes of relatively coarse grained sediment that were likely fluvial in origin (Trewin 1986). Such inputs of sediment would have diluted organic carbon content and lowered TOC values – even if net rates of carbon burial were high. Conversely carbon/sulphur ratios appear low (there is a lot of sulphur with respect to carbon), especially for a lacustrine environment and indicate that a relatively high amount of sulphur was fixed within the ancient sediments as sulphide (Leventhal 1979; Berner and Raiswell 1986); far higher than would be expected for a freshwater lake or even a marine environment (e.g. they are less than 2.8). The $\delta^{13}C_{carb}$ data average -1.01‰ (n = 23, σ = 0.31‰) and the $\delta^{13}C_{org}$ values fall within the standard range reported for algal organic matter (-26 to -42‰, Leng and Marshall 2004). This might be expected, as a significant input of higher plant organic carbon would be unlikely for a Middle Devonian-aged sediment.

β,β-Carotane is notably prominent in all samples (see Fig. 2) and this is a feature observed in the solvent extractable organic matter obtained from numerous localities around the Orcadian Basin (Duncan and Hamilton 1988), where it is often the most easily resolved and abundant hydrocarbon-biomarker on gas chromatograms. β,β-Carotane (Fig.2) is derived from β,β-carotene by transformation of the unsaturated hydrocarbon precursor during early diagenesis (cf. Killops and Killops, 2005). Although β,β-carotene is ubiquitous, high concentrations of β,β-carotane in the geological record are not common and the very large proportions of β,β-carotane present in the Achanarras Limestone Member are notable because carotenoids typically degrade rapidly in most aquatic depositional settings (Jiang and Fowler 1985). Therefore, the very high proportions of this compound present in the Achanarras Limestone Member and similar lacustrine rocks and sediments have been interpreted as a consequence of a higher than typical input from precursor biological materials and a high net primary productivity (Killops and Killops 2005). Likely sources for this carotenoid-enriched organic matter include halophilic archaeobacteria which thrive in hypersaline environments (Kushwaha et al. 1974; Rønnekleiv and Liaaen-Jensen 1996) and contain very high proportions of carotenoids, including β,β-carotene. The concentration of β,β-carotane is high in all samples although there are several instances of values less than one standard deviation from the mean value.

The most distinctive feature of the sterane biomarkers is the low abundance of regular C_{27} steranes (Fig. 3). This likely indicates a low proportion of cholesterol in precursor organic matter, and hence limited contributions from animals/zoo plankton, which are the main sources of cholesterol in modern lake sediments (Huang and Meinschein 1979; Kodner et al., 2008). The 24-*n*-propylcholestane (C_{30} sterane) sterane-homologue is less commonly reported in solvent extracts obtained from Orcadian Basin sedimentary rocks (Duncan and Hamilton 1988) although it can be seen to be present in all of the samples during this study, but in varying proportions (Fig. 3). Regular C_{30} steranes (24-*n*-propylcholestanes), likely derive from C_{30} 24-*n*-

propylcholesterols which have been found to be present in a few largely marine chrysophyte algae (Rohmer et al., 1980; Moldowan 1984; Volkman 2002). Most important of these is probably the brown tide alga *Aureococcus anophagefferens* (Giner et al., 2003). Baseline values for the relative proportion of C_{30} sterane for the studied interval are low, both in comparison to stratigraphically lower intervals of the Achanarras Limestone Member (values shown as crosses on graph) where the most diverse fish fauna are preserved and also relative to the mass mortality horizon itself.

Gammacerane is a pentacyclic triterpanoid hydrocarbon that can be measured on the *m/z* 191 chromatogram (Fig.2) and is present in all samples. The varied proportion found in samples is indicated by the Gammacerane Index (GI) which is plotted in Fig. 4h. Gammacerane can be used as an indicator for water column stratification (Sinninghe Damsté et al. 1995; Stephens and Carroll, 1999). This is because its main biological precursor is tetrahymanol (Ten Haven et al. 1989) a compound that is synthesised by bacterivorous ciliates (Sinninghe Damsté et al. 1995) inhabiting anoxic waters. (Tetrahymanol is only produced by these organisms in the absence of dietary sterols, a situation that occurs in the anoxic part of a stratified water column where the growth of sterol-synthesising eukaryotic algae is inhibited). No precise definition has been offered as to what constitutes a ‘high’ GI value, however quoted GI values greater than 0.1 – 0.2 are generally described as being ‘high’ (e.g. Chen et al. 1996), thus background values indicate prevailing water column stratification.

3.2 Anomalous values associated with the mass mortality horizon

A sharp spike in the C/S ratio two standard deviations high, corresponding to the highest value in a 10 year interval, occurs during the mass mortality horizon (Fig. 4a – C/S ratio). This is accompanied by a TOC spike (Fig. 4b) two standard deviations above the average (also a 10 year maximum) and a reduced burial of sulphur relative to carbon (Fig. 4a), probably indicating less saline waters or a water column less able to support pyrite formation via reduction of sulphate. Immediately following the mass mortality horizon there is a large excursion of the $\delta^{13}C_{org}$ parameter (Fig. 4d) to its highest value in an 11 year period of – 28.65‰ (average = -30.74‰, n = 24, σ = 0.93‰). This is still consistent with algal organic matter being the dominant contributor to the lakes productivity. The concentration of β,β-carotane (Fig. 4e) is at a 4 year low during the mass mortality horizon and then immediately rises to a five year high in the following year. However, this is one of four big switches (where a parameter changes from a maximum to minimum value) in this parameter over the 12 year period concerned, and is only significant because it coincidences with the mass mortality horizon, rather than for its absolute magnitude (Fig. 4e). The beginning of the mass mortality horizon itself is characterised by a high C_{30}/C_{28} sterane ratio (nearly two standard deviations high) indicating an enhanced burial of biomarkers derived from marine phytoplankton (Fig. f). The gammacerane index spikes to a 6 year high at the mass mortality horizon and a 12 year high in the year following the mass mortality horizon indicating a relative increase in the prevalence of water column stratification (Fig. 4g).

Thus the interval associated with the mass mortality horizon evidences decreased sulphur burial relative to carbon, higher TOC values and greater proportions of biomarkers derived from marine phytoplankton and is associated with water column stratification and hypoxic bottom waters.

Changes in the net burial of β,β-carotane, that might indicate a decrease in water column salinity, are coincident with the mass mortality horizon but not uniquely associated with the horizon.

4.0 DISCUSSION

Geologically high concentrations of β,β-carotane (but not anomalous in the context of the section considered in this study) are reported from across the basin, particularly for localities located in palaeogeographic positions that are far from possible tributaries, indicating the prevalence of a saline or hypersaline habitat at the centre of the lake (Duncan and Hamilton 1988). From this perspective the water budget for the lake would seem to have been closed or at least heavily restricted, and at a first consideration this contradicts the relatively high rate of discharge proposed by other workers (Marshal et al., 2007), who found that riverine discharge from the lake was relatively high. The different perspectives can be reconciled by considering the seasonality of the lake; the dry seasons created a hypersaline habitat, whilst the wet seasons potentially saw large fluxes of water move through the lake. The relative duration of the two seasons would influence the net production of β,β-carotane, with less produced during a year in which the wet season predominated and riverine discharge enhanced. The changes in β,β-carotane concentration that occur several times in the studied interval suggest that the variation associated with the mass mortality horizon is not unusual and doesn't help constrain the anomalous factors at play in the genesis of the mass mortality horizon. These values represent only the routine cycling of the lake between wet and dry conditions.

Other biomarker evidence better constrains the anomalous factors that may have contributed to the formation of the mass mortality horizon. The very high C_{30}/C_{28} sterane ratio exhibits a peak value at the beginning of the mass mortality horizon, but C_{30} steranes are present in all lamina analysed albeit in trace quantities. A literal interpretation of this parameter, similar to that used for biomarkers found in oil, would suggest that the sedimentary organic matter found within the Achanarras Limestone Member predominantly derived from marine sources (Peters et al., 2007). However, as noted earlier, other geological evidence for such a strongly marine interpretation is lacking excepting the fossils of certain fish genera (such as *Coccosteus*), that are also found in similarly-aged marine successions at other localities (Trewin 1986). The proportion of 24-*n*-propylcholestane (C_{30} steranes) varies but infrequently exceeds a single standard deviation. This can be explained by the periodic recharging of the lake with sources of C_{30} steranes, (either phytodetritus or living organisms) during incursions of seawater, albethey some distance from the depocentre of the lake. The most likely modern analogue for such an incursion of seawater would be a storm-tide that carried non-hypersaline, marine waters into the lake or its downstream reaches.

Studies of modern day fish-kills resulting from large storm tides indicate the complexity of elucidating a definitive kill mechanism and its consequences, and generally show that the same storm will variably impact different populations in different places (Mallin et al., 2002; Schaefer et al., 2006). Van Vrancken and O’Connell (2010) described little long term change in the fish population of the downstream reaches of a small coastal tributary in Louisiana subsequent to Hurricanes Katrina and Rita, despite widespread fish-kills being evident. Conversely, at a different locality, but still within Louisiana, Perret et al., (2010) reported long term changes in fish populations that were still evident two years later. For both cases direct poisoning of fish by intrusion of saltwater itself is not considered to be the major kill-mechanism. Instead, both rapid and often localised but essentially temporary hypoxia or anoxia, and widespread longer term reductions in oxygen concentration have been proposed to be the major kill-mechanisms (Mallin et al., 2002; Buck 2005). Perret et al. (2010) also considered the release of hydrogen sulphide alongside depletions in oxygen concentration as a kill-mechanism.

The strongest evidence for atypically hypoxic conditions coincident with the mass mortality horizion is the significantly elevated values of gammacerane index (greater than 1 standard deviation) preceding the peak values in sterane parameters (both the % C28 sterane and C_{30}/C_{28} sterane ratio). Mechanistically the link between a higher gammacerane index and "more hypoxia" or "more anoxia" is not straight forward. Foremost, it is unlikely to represent a further reduction in the oxygen concentration of a dysoxic or hypoxic water body at a single geographical point. A further reduction in oxygen concentration in a body of water that is already anoxic over a substantial depth will have little impact on net gammacerane production (see prior discussion – a stratified and anoxic water column is a cause of gammacerane production and not an input to a process governing its rate of production). Instead of representing localised changes, the changing gammacerane index likely represents the consequence of changing environmental conditions at the lakes margins, where waters that were previously oxygenated have become anoxic, thus increasing the area of the lake capable of supporting gammacerane production.

The main mechanisms proposed by previous workers (Mallin et al., 2002; Buck 2005; Perret et al 2010) for generating hypoxic conditions during storm tides are: a) the physical mixing of deep hypoxic and sulphidic bottom water with surface waters that can cause an immediate drop in oxygen content; b) increased oxygen demand during heterotrophic activity subsequent to an algal bloom triggered by an influx of nutrients – essentially a longer term phenomena; c) the entraining of anoxic but carbon-rich sediment and pore fluids within ingressing seawater and the subsequent poisoning of surface water. Mechanism a) is a rapid process that occurs during a storm, and had this been the case for the mass mortality horizon the peak gammacerane index value might be expected to have been contemporaneous with the C_{30} sterane maxima. The maximum gammacerane index value that coincides with the C_{30} sterane parameter maxima occurs a season latter, indicating that the environmental change was probably not instantaneous. Therefore mechanism a) is a less likely explanation for the mass mortality horizon. Mechanism c), would be expected to have left evidence in the form silt and detrital organic matter, but this is not a distinctive feature of the mass mortality horizon (although it does occur during other intervals of the Achanarras Limestone Member). Evidence for mechanism b) is thus strongest because the greatly elevated gammacerane index, that is indicative of increased hypoxia, immediately follows a peak sterane parameter value indicative of an increased contribution of phytoplankton-derived sterols (e.g. an algal bloom).

The golden alga *Prymnesium parvum* is known to produce toxins that are responsible for fish kills (Landsberg 2010). However, in the present day this alga is largely freshwater and has not been reported as a source or C_{30} 24-*n*-propylcholesterols. Brown tide algae such as *Aureococcus anophagefferens*, that are tolerant of marine salinities (Doblin et al. 2004) and are reported as sources of C_{30} 24-*n*-propylcholesterols (Giner and Boyer 1998), are generally held not to be damaging to adult fish – except when decaying algal blooms create hypoxic conditions by depleting oxygen.

The data presented in this study are from the topmost section of the Achanarras Limestone Member that has the least diverse fish assemblage (it comprises almost entirely *Dipterus*) and the lowest abundance of fish fossils (Trewin 1986). While it is tempting to try and link storm tides, seawater-incursions, reduced biodiversity and fish kills, values of the C_{30}/C_{28} sterane parameter are greater in the lower sections of the Achanarras Limestone Member where fish assemblages are more diverse and contain fish with the strongest marine associations. Thus the limited fish assemblage found at the top of the Achanarras Limestone Member is most likely a product of limited but highly disruptive as opposed to continuous connection to a marine environment, and indeed previous work has suggested that regular and intermittent flooding is healthy for fish stocks because it provides juvenile fish refuges on the floodplain (Mallin et al., 2002).

5.0 CONCLUSION

The Achanarras Limestone Member was deposited in an environment that was periodically perturbed by incursions of marine water. The extent and frequency of this perturbation is recorded in the biomarker content of individual lamina, but at the top of the fish-bearing section of the Achanarras Limestone Member incursions were short lived and lasted less than a year and were probably analogous to a modern day storm tide. When considered as a time series, a clear chronological ordering of events can be seen within biomarker data, in which an influx of seawater was followed by a period of enhanced eutrophification. Instances of storm-induced hypoxia and anoxia, as deduced from biomarker data, are most strongly associated with intermittent perturbation by incursions of seawater, and acted as a powerful environmental selection filter favoring air breathing fish such as *Dipterus*.

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FIGURE CAPTIONS

Fig. 1. Map of Scotland to showing the location of the Achanarras Quarry, Caithness, the type locality for the of the Achanarras Limestone Member where samples were obtained (denoted by letter A). Distribution of Devonian deposits in Scotland is also shown.

Fig.2 191 and 125 *m/z* ion chromatograms illustrating the abundance of fossil-carotanes, hopanes and tricyclic terpanes. The y-axis of different ion-chromatograms for each sample share the same relative scale (ion counts/a.u.). γ = γ-carotane; $\beta\beta$ = β,β-carotane; C_{20} = C_{20} 13β(H),14α(H) tricyclic terpane; C_{21} = C_{21} 13β(H),14α(H) tricyclic terpane etc.; C_{29} $\alpha\beta$ hopane =C_{29} 17α(H),21β(H) 30-norhopane; C_{31} $\alpha\beta$ S hopane = C_{31} 17α(H),21β(H) (22S) hopane etc; G = gammacerane. Data are shown for the sample at the beginning of the MMH highlighted in figure 4, and for 1 year after the MMH.

Fig. 3. 218 *m/z* Ion chromatogram illustrating the relative abundances of regular steranes. C_{27} $\alpha\beta\beta$ R = C_{27} 5α,11β,14β (H) 20R cholestane; C_{27} $\alpha\beta\beta$ S = C_{27} 5α,11β,14β (H) 20S cholestane etc. Region of the chromatogram containing the 5α,11β,14β (H) 20S & 20R 24-*n*-propylcholestanes (C_{30} steranes) is shown as an inset with the y-axis at ×10 (the y-axis is ion count/a.u.).

Fig. 4. Data from the 24 consecutive laminae ordinated by time assuming that 2 lamina = 1 year. (a) C/S ratio of elemental carbon to sulphur, (b) TOC (total organic carbon), (c) $\delta^{13}C_{carb}$, (d) $\delta^{13}C_{org}$, (e) β,β-carotane (concentration per g of sediment), (f) C_{30}/C_{28} sterane (ratio of C_{30}/C_{28} steranes), (g) %C28 sterane (percentage C_{28} sterane), (h) GI (gammacerane index = gammacerane/ C_{31} 14α,17β (H) 22S & 22 R hopanes). The MMH is shown as a grey rectangle based on the uncertainty in determining exact positions for fish beds provided in Trewin (1986). Data from a lower section of the Fish-bearing horizon of the Achanarras Limestone Member (facies 5 from Trewin 1986) are plotted as crosses at the end of the axis. Average values and standard deviations are marked in dashed lines - middle, heavier dashed line denotes arithmetic average, lighter dashed lines denote standard deviation.

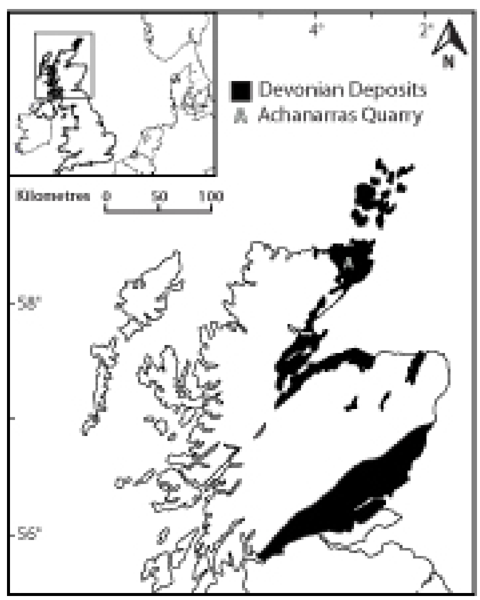
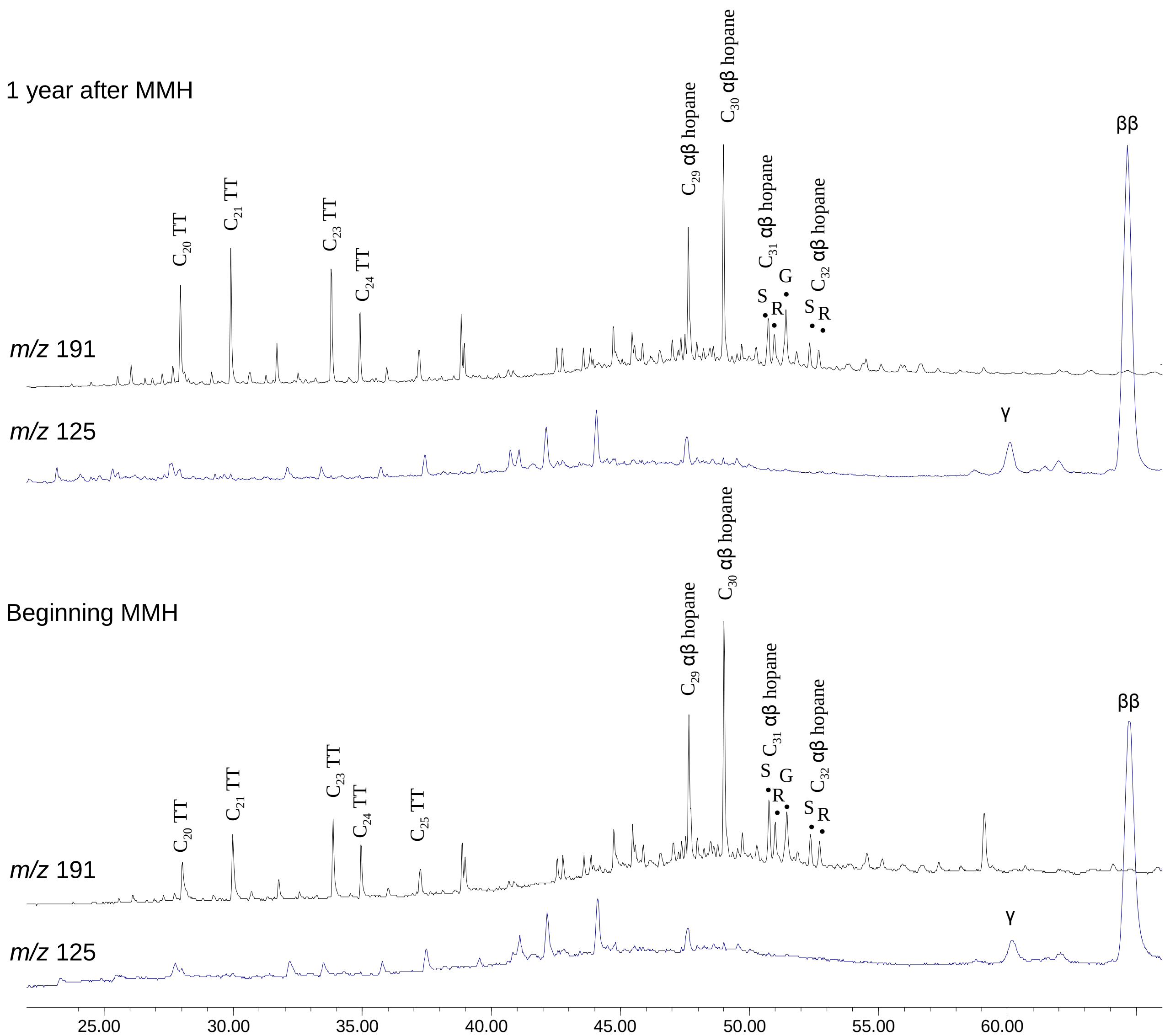

4°
2°
N
Devonian Deposits
A Achanarras Quarry
Kilometres 0 50 100
58°
56°

Fig 1



1 year after MMH
m/z 191
m/z 125
C20 TT
C21 TT
C23 TT
C24 TT
C29 αβ hopane
C30 αβ hopane
C31 αβ hopane
C32 αβ hopane
S
R
G
ββ
γ
Beginning MMH
C25 TT
25.00
30.00
35.00
40.00
45.00
50.00
55.00
60.00

Fig 2

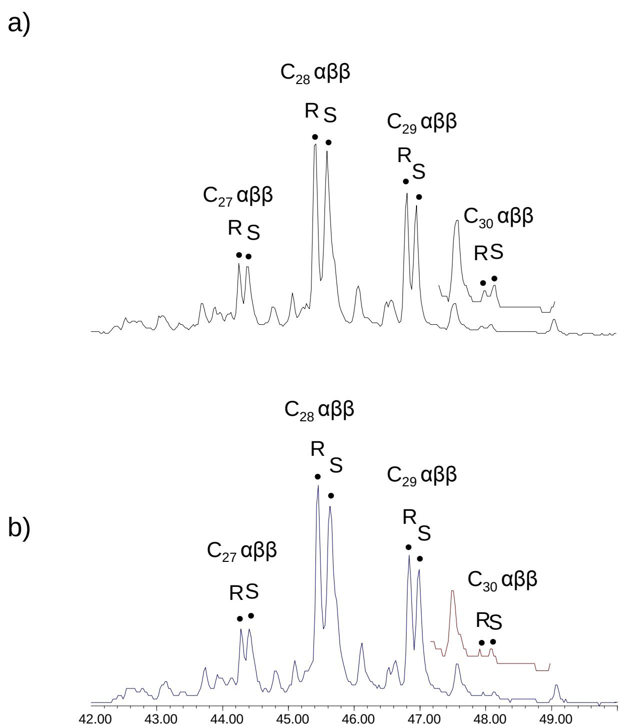

a)
C28 αββ
R S
C29 αββ
R S
C27 αββ
R S
C30 αββ
RS
b)
C28 αββ
R S
C29 αββ
R S
C27 αββ
RS
C30 αββ
RS
42.00
43.00
44.00
45.00
46.00
47.00
48.00
49.00

Fig 3

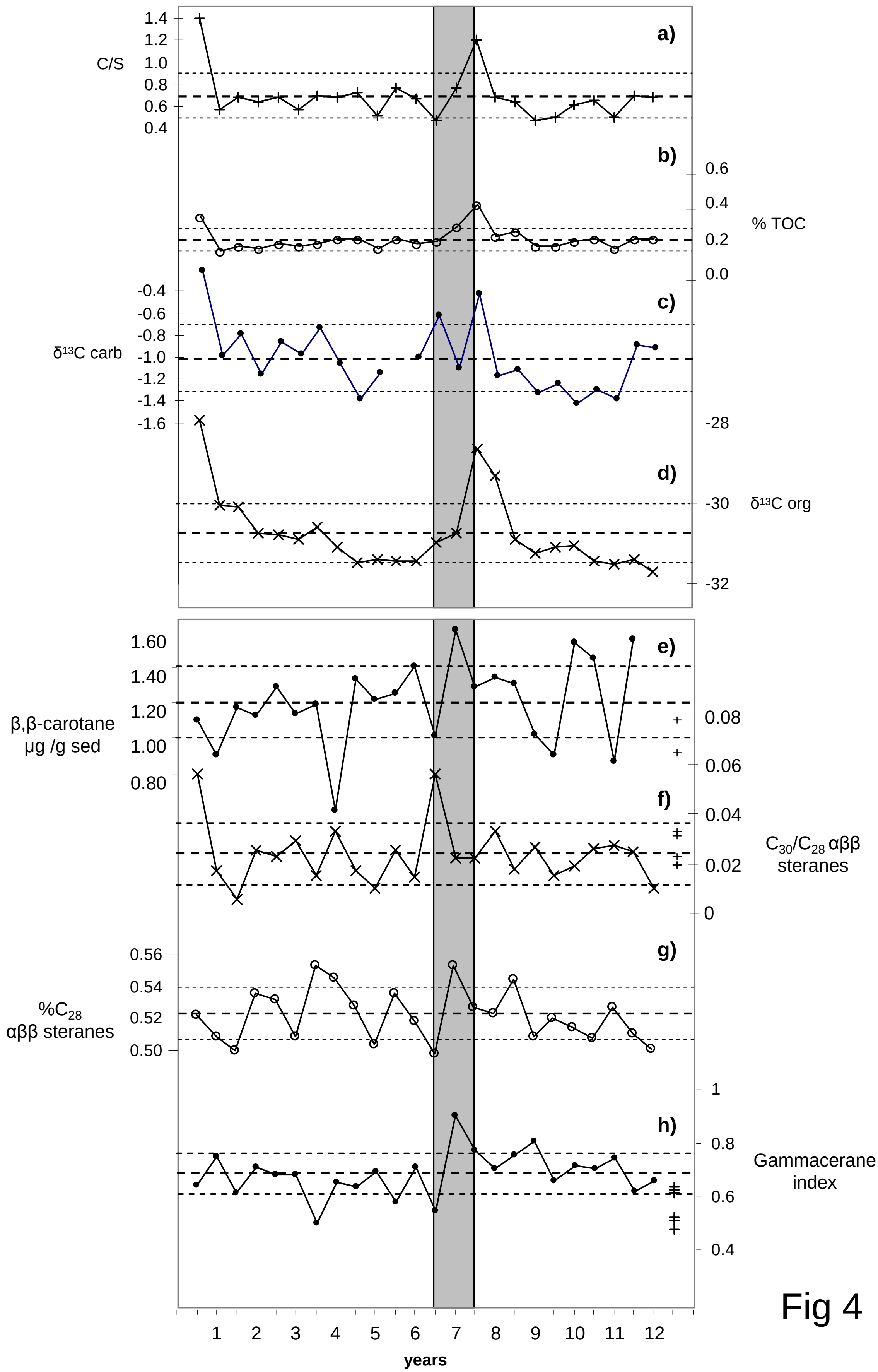

a)
b)
c)
d)
e)
f)
g)
h)
C/S
% TOC
δ13C carb
δ13C org
β,β-carotane
µg /g sed
C30/C28 αββ
steranes
%C28
αββ steranes
Gammacerane
index
years

Fig 4