Freshwater diatom transfer to clothing: spatial and temporal influences on trace evidence in forensic reconstructions

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Abstract

Environmental indicators are increasingly sought and analysed in a range of forensic reconstructions. Although the majority of casework and research studies are concerned with the criminal investigation of terrestrial habitats (soils, sediments, plants etc.), freshwater environments are also frequently encountered as crime scenes. As such, microalgae, particularly diatoms, may provide useful circumstantial trace evidence following their transfer to a victim or perpetrator. Diatom analysis is a relatively underused technique in forensic ecology, although an increased empirical research focus is beginning to recognise the evidential value of a transferred assemblage. This study aimed to examine three of the spatial and temporal variables known to influence the extent of an initial transfer of trace particulates, within the context of freshwater diatoms to clothing. A series of experiments were designed to consider the impact of recipient surface characteristics (clothing type), source environment conditions (seasonality), and morphological (type of diatom) variability, on the total number (no. per cm^2) and species richness (total no. *sp.*) of an evidential diatom sample recovered from clothing. Nine commonly used clothing materials were immersed in a freshwater river at three times of year – the early and late spring and in the winter. Diatoms were recovered using a H_2O_2 extraction technique and examined microscopically. The results demonstrated that diatom transfer to clothing varies significantly, with a greater abundance and a higher species richness transferred to coarse woven surfaces including acrylic, linen, and viscose. Significantly fewer diatoms were transferred to clothing in the winter, in line with seasonal fluctuations in the source environment diatom community. Furthermore, variation in the relative abundance of particular diatom species was identified between clothing types, provisionally suggesting that morphological characteristics may also support or limit the transfer of material. These findings highlight that, although clothing may offer a valuable repository of freshwater diatom trace evidence, the interpretation of evidential material should be approached within an exclusionary framework. Thus, empirical data has been generated to develop evidence bases within forensic

ecology, demonstrating some of the spatial and temporal factors which may contribute to or limit the transfer of evidence.

Key words: trace evidence, diatom analysis, freshwater crime scenes, transfer, clothing, forensic ecology

1. Introduction

Calls for empirical research to support the inferences made in forensic casework are increasingly being articulated by academics and practitioners within the forensic sciences and international policymakers [1-5]. In addition to situating the interpretation of evidence and ensuring the reliability of new and existing forensic techniques, efforts towards understanding the dynamics of different trace evidence indicators has consistently been highlighted as a priority for scientific study [6-8]. In her most recent report, the Forensic Science Regulator identified that the need for empirical data from '*structured studies on the transfer and persistence of trace evidence and the significant factors affecting such transfer*' remains one of the highest priorities for forensic research in the UK [8, pp. 36]. This paper presents the results from one such study examining the transfer of freshwater diatoms to various clothing materials which may be recovered as evidence during criminal investigation.

The value of environmental trace materials in forensic reconstructions is increasingly being recognised in casework and the accompanying empirical research bases [9-11]. The exclusionary assessment of trace materials transferred between crime scenes and persons or items of forensic interest, often includes environmental indicators as diverse as soil mineral grains [12, 13], algae [14, 15], pollen [16, 17], and microbial communities [18, 19]. Although most published studies in forensic ecology focus upon terrestrial crime scene environments, the value of aquatic trace evidence, particularly diatoms, is increasingly being articulated [15, 20-22].

Diatoms (*Bacillariophyceae*) are a group of unicellular microscopic algae characterised by a chemically resistant silica (SiO_2) cell wall. They are naturally abundant in freshwater and marine environments and frequently found in terrestrial habitats including soils, exposed rock surfaces, and damp environments [23]. Approximately 12,000 diatom species have been identified at present, although some experts estimate that an additional 190,000 taxa are yet to be discovered and documented [24]. Individual taxa have discrete tolerances for local environmental parameters including light, temperature, pH, silica and nutrient availability. Due to this variability, it is possible to infer the characteristics of a waterbody from which an environmental or forensic sample originated, based on the diatom assemblage (group of species occurring together [56]) present within that sample [22]. As such, species-level identification of diatoms in forensic casework should always be recommended. Furthermore, as diatoms are microscopic (2-200μm), and relatively little is known about their forensic value within the general population, it is likely that the transfer of diatoms from an aquatic crime scene to criminal perpetrator would go unrecognised [15]. Because of these attributes, diatoms have

frequently been used in forensic pathology to infer the cause and location of death by drowning [25-27], and to empirically estimate the time since death or submersion [28-29].

Diatoms can also be used as trace indicators to ascertain whether a victim or suspect has been in contact with a particular body of water [22]. Diatom analysis used in this manner is relatively recent, with the potential to offer valuable environmental and circumstantial intelligence during crime reconstructions. A limited number of empirical research studies have previously considered diatom transfer from freshwater and terrestrial environments onto clothing and footwear materials, and the methods appropriate for their collection and analysis [15, 20-22]. Despite this initial focus, additional research is required to investigate the transfer and persistence dynamics of diatoms in experimental scenarios pertinent to forensic casework. Such research is imperative to inform the investigative approach taken during the collection and analysis of diatoms recovered from evidential items, and to support the exclusionary interpretation of those findings in a court of law [10, 11].

Several variables are known to influence the process and extent of evidential transfer including recipient surface characteristics, the type and extent of an initial contact, prevailing environmental conditions, and the properties of the evidential particulate or substance [30]. Although previous research has examined the transfer dynamics of environmental indicators including soil and different pollen grain types on footwear, clothing, shovels, documents, and in different environmental contexts [19, 31-35], there are relatively few corresponding studies within the diatom trace evidence literature. An initial study by Scott et al [15], found that diatoms transferred to new/used 100% cotton clothing surfaces following different periods of contact (3 minutes to 24 hours) with freshwater and soil environments. Furthermore, Levin et al [21] examined freshwater diatom transfer to five different footwear materials (soles and uppers) following three immersion intervals (30 seconds to 30 minutes). Though both studies identified the diatoms present by morphology or by genus, neither considered the potential influence of particulate variability on diatom transfer to clothing.

This study builds upon these initial findings through the assessment of freshwater diatom transfer to nine common clothing materials. Clothing was chosen due to its frequent presence at a range of crime scenes [15], variability in the subsurface characteristics and micro-textures between different fabrics (Figure 1) [54], and because previous research has only considered diatom transfer to 100% cotton materials [15, 20]. To reflect similar studies within forensic palynology and to consider the forensic implications of diatom seasonality in freshwater environments, the impact of environmental variability and diatom species characteristics on the transfer of evidence is also considered [33, 36]. Though the organic components of the diatom cell may also support (or limit) the adhesion of evidence to clothing, this study primarily considers the impact of the morphology and ornamentation of the silica cell wall at both the general and species-specific level.

Specifically, this research aimed to assess:

1. Whether the concentration and species richness (the number of species per ml/ cm^2 [55]) of a diatom assemblage transferred to clothing varied significantly between cotton, denim, linen, nylon, polyester, acrylic, viscose, PVC, and lycra substrates
2. Whether a more abundant and species-rich diatom sample is transferred to the different clothing samples during the spring (March, May), when diatom populations are generally considered to 'bloom,' in comparison to the winter (November) when their population declines [36]
3. Whether particular diatom taxa are more susceptible to transfer to the different clothing fabrics based upon their general morphology (size, shape, integrity) and species-specific features
4. The ability of the H_2O_2 extraction method outlined in Scott et al [15] to recover diatoms transferred to clothing materials other than 100% cotton.

2. Materials & methods

2.1. Sample materials

To reflect the diversity of recipient surfaces often encountered during criminal investigations [54], diatom transfer to nine common clothing materials was tested (Figure 1). A range of natural and synthetic fabrics, with different surface textures and weave characteristics, were chosen for comparison. All clothing material sub-samples used were new (unused but washed) and were removed from the whole garment prior to initiating diatom transfer.

2.2. Sample collection

The experimental approach taken throughout this research was designed to replicate the circumstances which may accompany a forensic event around freshwater. Initially, a $5cm^2$ swatch of each material was attached to the bottom of a pair of waterproof trousers, which were then worn whilst walking through a five metre transect of the River Beane (Hertfordshire, UK) (National Grid Ref: TL313148) for a period of 3 minutes [15] (Figure 2). To initiate and support diatom transfer, all material swatches were fully immersed in the water during activity. Following wear, each individual clothing sample was detached from the trousers, the excess water removed, and the sample immediately double bagged, and stored in a dark cold store (5°C).

A reference control sample was collected for comparison to the transfer samples. The same five metre transect of the river was walked through and the suspended material collected (in line with the river flow) in a cleaned and sterilised 500ml bottle. This was done to ensure that the control sample was as representative as possible, as the diatoms present in a forensic sample could inevitably include species from different microhabitats (e.g. planktonic, eipilithic, epiphytic [23]) following a person's movement through the river. Environmental measurements including pH, temperature, dissolved oxygen, conductivity, and depth were also recorded at the site during each sample month (Figure 2).

To account for seasonal variation in the abundance and species richness of the diatoms present within the site, sampling was conducted at three different times of year: the early and late spring (May 2014, March 2015), and in winter (November 2015). The first sample run (May 2014) acted as a preliminary assessment for diatom transfer to clothing, the results of which informed the development of a more in-depth experiment and analysis in March and November 2015. In May, the acrylic material samples were lost during sampling and subsequently excluded from further examination. All nine clothing materials were retained and available for analysis in March and November 2015.

2.3. Sample preparation

Diatoms were extracted from three 1cm^2 subsample replicates of each clothing material swatch using the hydrogen peroxide (H_2O_2) method outlined in Scott et al [11, 15]. Briefly, each subsample was added to a centrifuge tube and heated with 20ml of H_2O_2 (30%) at 70^{0}C for 4 h. Following treatment, the fabric sample was removed, rinsed with distilled water, and a few drops of HCl (10%) added to the solution to eliminate any carbonates present. All sample solutions were centrifuged, the supernatant discarded, and re-washed with distilled water a total of four times. The final supernatant was discarded to leave the diatom pellet in 5ml of solution. Three control sample replicates for each sample month were also prepared following Renberg [37] using 20ml of control site water and 20ml of H_2O_2.

After extraction, fixed microscope slides were prepared. 500µl of the final sample aliquot was transferred onto a 19mm coverslip using a calibrated micropipette. A second dilution of each suspension was also prepared to provide an additional resolution for analysis, if required. Following evaporation, each coverslip was inverted and mounted onto a microscope slide using Naphrax™ on a heated hotplate.

Appropriate measures were incorporated throughout to prevent and mitigate any contamination between samples, in line with usual forensic procedures including the preparation of several 'blank' samples (n = 9) [11, 15].

2.4. Analysis

All slides were examined under phase-contrast light microscopy at x1000 magnification. All diatoms from a known proportion of the coverslip were counted and recorded. Diatoms were initially categorised according to their general morphology (shape, size, integrity) before detailed identification to species-level (March and November 2015 data only) [38-41]. Additional observations of particular diatom taxa at higher magnifications were performed under scanning electron microscopy (SEM) (Jeol JSM-6480LV).

Results from the coverslip area studied were standardised in order to calculate the estimated diatom count for the whole sample (per ml/cm^2) [15, 48, 53]. Variability in the extent of diatom transfer to clothing was compared through assessment of the total diatom count (no. per cm^2), morphological composition, and the species richness (no. species per ml/cm^2) of a transferred assemblage when compared to the corresponding control samples. Analysis was performed

using SPSS (v. 24) [42] to statistically compare the total number of diatoms transferred to the different clothing samples. The species composition data was compared using canonical correspondence analysis (C2 [v. 1.7.7] [43]), reflecting the analytical approach adopted in casework and previous research studies [15, 22].

3. Results

3.1. Total Diatom Count (per ml/cm²)

No diatoms were identified in the nine blank samples tested for contamination throughout the experiment.

3.1.1. May 2014

The estimated mean ($\overline{x}$) total diatom count for all monthly control triplicates shows the most abundant samples were identified in May 2014 with an estimated $\overline{x}$ 85,979 diatom valves (per ml) (Table 1). Diatoms were extracted from all eight transfer materials (Figure 3). A greater number of diatom valves were recovered from the woven materials with a medium surface texture including cotton (41197 ± 32627/cm²), denim (50280 ± 11516/cm²), and viscose (61847 ± 35286/cm²). In comparison, the nylon (6924 ± 5158/cm²) and lycra (6985 ± 5692/cm²) fabrics were less efficient in the entrainment of an abundant diatom assemblage.

3.1.2. March 2015

Fewer diatoms were identified in the March controls ($\overline{x}$: 7170/ml) in comparison to the late spring (May 2014) (Table 1). Despite the control sample variation, a greater number of diatoms were extracted from most of the March clothing transfer samples (Figure 3). For example, an estimated 46594 ± 2843 diatoms/cm² were identified in the March polyester transfer samples compared to 15477 ± 8623/cm² (May) and 10264 ± 13869/cm² (November).

Diatoms were transferred to all nine clothing materials examined during March (Figure 3). A greater concentration of diatom valves were identified in acrylic (261235 ± 86864/cm²), linen (102514 ± 53273/cm²), and viscose (104632 ± 34390/cm²) – all characterised by an open weave and medium-rough surface texture (Figure 1). Similarly to the trends reported in May 2014, the smoother materials with a closed weave (nylon, PVC, lycra) reported comparatively fewer diatom valves ($\overline{x}$: 4969 – 12870/cm²).

3.1.3. November 2015

The lowest diatom counts were consistently reported in the November control ($\overline{x}$: 3461/ml) and transfer samples (Table 1, Figure 3). For example, an estimated 1955 ± 1598 diatoms/cm² were transferred to viscose in November, compared to 12870 ± 695/cm² (March) and 6985 ± 5692/cm² (May) in the spring months.

Although fewer diatoms were present, valves were still identified in all nine clothing transfer samples (Figure 3). The most abundant included cotton (18206 ± 10738 /cm^2), acrylic (18125 ± 29174/cm^2), and PVC (15558 ± 6695/cm^2). As in the spring, nylon (81 ± 71/cm^2) and lycra (1955 ± 1598/cm^2) were the least efficient transfer materials. Greater variability was observed in the extraction of diatoms from the six clothing materials constructed of an open weave and with a medium-rough surface texture in November when compared to March (Figure 1). For example, although the cotton samples were relatively abundant, fewer diatoms were recorded in linen (2362 ± 1025/cm^2) and denim (3788 ± 533/cm^2).

3.1.4. Interaction between sample month and clothing type

Statistical analysis examined the significance of the observed variation in the extent of diatom transfer to clothing. An initial two-way ANOVA (comparing the eight materials successfully retained during all three sample months) identified a statistically significant interaction between sample month and transfer surface on the estimated total diatom count: F (15, 52) = 3.29, p = .001. Significant variation was reported between the sample months (p = <.0001) and clothing materials (p = <.0001). Post-hoc Tukey tests identified a significantly greater number of diatoms were transferred in March when compared to May (p = .004) and November (p = <.0001), and in May compared to November (p = .001). Additionally, the number of diatoms transferred to linen (L) and viscose (V) was significantly greater than several of the other material types including: nylon (p = <.0001), polyester (p = .020 [L], p = .019 [V]), PVC (p = .001), and lycra (p = <.0001).

A second two-way ANOVA statistically compared the number of diatoms extracted from all nine clothing materials sampled in March and November 2015. A statistically significant interaction between sample month and transfer surface was observed: F (2, 52) = 11.504, p = <.0001. Significant variation was identified between the nine transfer surfaces (p = <.0001). A post-hoc Tukey test demonstrated that the total number of diatoms extracted from the acrylic samples was significantly higher than linen (p = .001) and all other materials (p = <.0001).

An independent t-test was used to determine whether the estimated total number of diatoms recovered from the natural and synthetic clothing types varied statistically. No significant difference was identified: t (76) = .276, p = .784.

Finally, a one-way ANOVA compared those materials constructed of an open (cotton, denim, acrylic, linen, viscose), semi-open (lycra, polyester), and closed weave (nylon, PVC). A statistically significant difference between groups was identified: F (2, 75) = 6.609, p =.002. A post hoc Tukey test demonstrated that the estimated mean diatom count transferred and extracted from the open weaved materials was significantly greater than the semi-open (p = .021) and closed weave (p = .008) fabrics. There was no statistically significant difference between the semi-open and closed weave sample groups.

3.2. Diatom assemblage I – general morphology

The influence of morphology and species-specific features on diatom transfer to clothing was assessed through an initial observation of general valve characteristics (shape, size, fragmentation), before subsequent species identification.

3.2.1. Valve shape

The diatoms present in all samples were initially categorised according to their shape: centric (round) or pennate (elongate) (Figure 4a). Although the majority of the March/November control assemblage comprised pennate taxa (86-88%), centric diatom species including *Melosira varians* were more abundant in May 2014 (52%).

The concentration of both groups varied between sample month and transfer surface. A greater and more consistent proportion, of centric diatoms were identified in May (35-61%) and March (22-60%) than November (0-57%) (Figure 4a). Fewer centric diatoms were extracted from PVC and nylon (Nov) (0-35%) than all other materials (13-61%). The distribution of centric/pennate taxa was more variable amongst the six synthetic materials than the three natural fibre types. For example, 54-58% of each diatom assemblage transferred to cotton/denim/linen in May comprised pennate taxa, in comparison to 35-61% in the synthetic clothing types.

3.2.2. Valve size

Diatoms were also categorised by size: greater than (>) or less than (<) 10µm in length (Figure 4b). Seasonal variation in the distribution of both groups was reported in the monthly control samples, with fewer diatoms <10µm in length in May (7%) than in March and November (36-51%).

The abundance of transferred diatoms </>10µm in length varied between clothing fabric and sample month, although both types were recovered from most of the materials examined. A relatively similar proportion of <10µm diatoms was identified across all transfer materials in May (6-28%) and March (9-29%) with greater variability in November (0-49%) (Figure 4b). Fewer diatoms <10µm in length were recovered from nylon (0-9% of the overall assemblage) and lycra (4-14%), when compared to all other medium-rough surface textures examined during all three sample months.

3.2.3. Fragmentation

The integrity (whole v fragmented) of each identified diatom valve was assessed in the experimental transfer samples only (Figure 4c). Most of the diatoms identified during May (75-90%) and March (69-91%) were whole, aiding additional identification to species-level. A greater proportion of the November transfer samples comprised broken diatom valves - 13% (nylon) to 90% (cotton) of the overall assemblage.

The distribution of both groups on the nine transfer surfaces varied by sample month. Although the abundance of fragmented diatoms was relatively consistent in May and March, a greater proportion of the diatoms recovered from cotton (18-22%), PVC (31% [Mar]), and lycra (25%

[May]) were broken (Figure 4c). In comparison, nylon, lycra, and PVC (74-87%) were the most successful transfer materials in the entrainment of a relatively undamaged diatom assemblage.

3.3. Diatom assemblage II – species composition

3.3.1. Control samples

All diatoms present in the March and November samples were identified by species. Although fewer diatoms were identified in the November control samples, a greater mean species richness (38 *sp.*/ml) was reported than in March 2015 (30 *sp.*/ml) (Table 1).

The most abundant species in both control samples included *Amphora pediculus, Cocconeis placentula, Melosira varians*, *Navicula radiosa* and *Rhoicosphenia curvata* (Figures 5 & 6). A number of less common species (<1% of the overall assemblage) were also identified in both control samples such as *Achnanthes lauenbergii, Caloneis bacillum, Reimeria sinuata,* and *Navicula gregaria.* Additional species including *Cyclotella planktonica, Gomphonema accuminatum, Surirella ovata* (March), *Nitzschia sigmoidea, Synedra vaucheriae,* and *Navicula capitata* (November) were observed in trace quantities in one monthly control sample only.

3.3.2. Transfer samples

The mean total number of diatom taxa recovered from the clothing surfaces tested in March ranged from 10 ± 3 (PVC) to 42 ± 5 (viscose) species/cm^2 (Figure 7). The materials constructed of a semi-open or closed weave and a smooth surface texture (nylon, PVC, lycra) recorded fewer diatom taxa (10-16 *sp.*) than all other materials (28-42 *sp.*). A lower mean species richness was consistently observed in the November transfer samples with values ranging from 1 ± 1 (nylon) to 19 ± 6 (acrylic)/cm^2. Fewer taxa were identified in cotton, viscose, nylon, and lycra (1-9 *sp.*) when compared to all other materials (14-19 *sp.*) (Figure 7).

The most abundant March control sample species, including *A. pediculus, M. varians,* and *N. radiosa*, were consistently identified as >5% of the transferred diatom assemblage in all nine clothing materials (Figure 5). Greater variation in the representation of trace species (<1%) was observed. For example, *Cyclotella planktonica, Gomphonema gracile, Navicula placentula,* and *Navicula trivalis*, were present in the control samples but were not identified in any of the clothing samples. Additional taxa (such as *Achnanthes lauenbergii, Reimeria sinuata, Encyonema minutum,* and *Sellaphora pupula*) were recorded in the assemblage of several transfer samples yet were absent (x) from others (Figure 5). All nine materials tested in March also reported the presence (*) of diatom species not previously detected within the control sample replicates. Such taxa included *Frustulia vulgaris, Nitzschia amphibia,* and *Synedra ulna.*

In November, the four most abundant control sample diatom species were identified in all clothing materials except nylon (Figure 6). Although present, *A. pediculus, C. placentula, M. varians,* and *R. curvata,* were often recorded in lower values than those reported in the control sample assemblage. For example, although *C. placentula* accounted for 20% of the control, a lower proportion was consistently identified amongst the clothing samples (1-13%). Variability

in the transfer of relatively less common species (<5%) was also observed in November (Figure 6). Although *Fragilaria construens, Fragilaria pinnata,* and *Synedra ulna* were present as less than 2% of the control sample, the same taxa were identified in one or two transfer samples only. As in March, several of the clothing samples reported the presence of diatom species not previously identified in the control replicates including *Achnanthes microcephala, F. vulgaris,* and *Gomphonema parvulum.*

3.3.3. Multivariate distribution analysis

Variability in the mean diatom species assemblage of all samples was explored further using canonical correspondence analysis (Figure 8). The proximity of data points demonstrates the relative similarity between the whole species assemblage of each sample. Initially, both control samples were relatively similar, with the presence of species including *Neidium affine, Nitzschia fonticola,* and *Gyrosigma accuminatum* driving variation from the transfer samples.

Most of the nine transfer samples tested in March 2015 are identified within the main sample cluster, with closer affinity identified between nylon, denim, and acrylic, and polyester, cotton, and linen (Figure 8). Greater variability in viscose, PVC, and lycra samples may be attributed to the relative abundance of taxa including *Achnanthes lauenbergii, Fragilaria elliptica, Gomphonema augur,* and *Sellaphora pupula* in those transfer samples.

The November clothing samples demonstrated greater variability in the transferred assemblage. The linen, denim, and viscose assemblages were comparable due to the concentration of species including *Cocconeis pediculus* and *Fragilaria bicapitata* (Figure 8). Diatom transfer to nylon, cotton, and acrylic was more variable which may be attributed to species including *Surirella sp. 1* and *Fragilaria producta* and the lower species richness in nylon. Lycra reported the most variable transfer assemblage to all other November samples, with polyester and PVC demonstrating the closest similarity to the control assemblage.

4. Discussion

The results demonstrate that recipient surface characteristics and environmental seasonality have a statistically significant impact on the extent (total count) of freshwater diatom transfer to clothing. Variability in the transfer of particular diatom species to the different clothing materials and at different times of year was also observed.

4.1. Value of H_2O_2 extraction method

This study presents the first application of the H_2O_2 extraction method for the recovery of diatoms from clothing materials other than 100% cotton [11, 15]. Diatoms were successfully retrieved from all nine clothing types examined, although a relatively large standard deviation was sometimes reported between sample triplicates in relation to both the total count (per cm^2) and total number of species identified (Figures 3 & 7). This may be attributed to variation in the extent of an initial transfer between small ($1cm^2$) sub-sample areas, or a limitation of the H_2O_2

technique when approaching different types of clothing garments. Future research may wish to test a more diverse range of trace evidence collection procedures for the recovery of diatoms from clothing [11, 21], particularly in relation to the assessment of larger transfer surface areas.

4.2. Impact of recipient clothing surface on diatom transfer

A greater number of diatoms were consistently transferred to linen, viscose, and acrylic in the three sampling efforts (Figure 3). Those samples also reported the highest species-richness and relatively little variability in the comparison of the overall species assemblage to the corresponding control samples (Figures 7 & 8). All three materials are characterised by an open weave and a medium-rough texture (Figure 1) – surface characteristics which were found to significantly enhance the total diatom count (p = .002), and the species-richness of an assemblage transferred to clothing. These findings reflect the trends reported in previous forensic studies exploring the transfer dynamics of indicators including pollen, glass, and fibres to clothing [33, 44-46], and of diatoms to footwear [21].

Significantly fewer diatoms and fewer taxa were transferred to nylon and lycra (Figures 3 & 7). The relatively limited transfer of material may be attributed to the garment construction (smooth surface, semi-open/closed weave), offering less opportunity for diatom entrainment (Figure 1). These findings recommend that rough open-weave fabrics should be sampled in the first instance during an investigation. If only smooth closed-weave materials are available, such as waterproof garments, these should also be examined, however fewer diatoms with a lower species richness may be expected.

Interestingly, the two clothing materials containing elastane (denim and lycra) had a lower mean diatom count than the other clothing garments. For example, the total number of diatoms transferred to denim was less than the cotton samples (Figure 3). Although both materials are constructed of a similar weave (open), surface texture (medium), and fibre (cotton) (Figure 1), the presence of 1% elastane in the denim fabric tested may have acted as a limitation for the successful entrainment of a transferred diatom assemblage. This is further supported by the presence of significantly fewer diatoms in the lycra samples, comprised of 14% elastane.

No statistically significant difference in the number of transferred diatoms was identified between the three natural and six synthetic clothing types (p = .784), although variation was often identified between several of the manufactured materials. For example, the mean diatom count in acrylic and viscose was often significantly greater than in the four other synthetic samples. This variability demonstrates that the approach taken during the collection and analysis of diatoms transferred to clothing, and the exclusionary interpretation of those findings, should always be considered within the context of an individual case [10]. In addition, the significant variation reported between the different clothing materials tested in this study, highlights that future experimental research within diatom trace evidence dynamics should incorporate fabrics other than 100% cotton [11, 15, 47, 48].

The overall diatom species assemblage transferred to the different clothing materials often varied from that recorded in the control sample (Figure 8). Although several of the

material samples in March (e.g. nylon, denim, acrylic and polyester, cotton, linen) and November (e.g. linen, denim, viscose) reported a relatively similar species assemblage to one another, variation from the equivalent control sample was observed. This may be attributed to the presence of less abundant species including *Nitzschia fonticola, Neidium affine,* and *Gyrosigma accuminatum* in the control samples only. Such taxa may be less likely to transfer to clothing, and subsequently identified in a forensic sample, due to their infrequent presence in the source environment (<1% of the relative abundance) (Figures 5 & 6).

Greater variability from the control sample assemblage was reported in viscose, PVC, and lycra (March) and nylon, cotton, and acrylic (November) (Figure 8). This may be due to the absence of species including *Achnanthes lauenbergii, Fragilaria elliptica,* and *Gomphonema augur* in the transfer samples, or the greater relative abundance of others such as *Amphora pediculus, Cocconeis placentula* (March), *Melosira varians,* and *Navicula radiosa* (November) in the transfer samples (Figures 5 & 6). All six materials are constructed of different textures and weave patterns (Figure 1), suggesting that factors other than surface characteristics may support or limit the transfer of particular diatom species to clothing.

4.3. Impact of seasonality on diatom transfer

The total number of diatoms transferred to the nine clothing materials varied significantly between the three sample months (p = <.0001), with a more abundant transfer assemblage in the early spring (March) than in the late spring or early winter (Figure 3). A greater number of diatom species were also identified in the March transfer samples, with greater variability in the species composition of the overall assemblage in the early winter (Figures 7 & 8). These results indicate that time of year influences the quantity and species-richness of diatoms present within the source environment, and consequently the extent of their transfer to clothing (or other recipient surfaces) as forensic evidence. Despite these temporal differences, spatial variability in diatom transfer between the nine different recipient clothing surfaces tested was relatively consistent between sample months (Figures 3, 7, and 8; Section 3.4.1). This suggests that general conclusions on the transfer dynamics of diatoms to different clothing surfaces can be inferred.

Seasonal variability has important implications throughout the different stages of a forensic investigation. Firstly, the laboratory methods used when attempting to recover diatoms from clothing and footwear surfaces should be as efficient and reliable as possible, to yield an optimal sample for comparison [15, 20, 21, 49]. Furthermore, the forensic importance of a relatively sparse diatom transfer sample should be interpreted within an exclusionary framework [10]. For example, the presence of very few, if any, diatoms in an evidential sample (as demonstrated in the November nylon replicates), may not be sufficient evidence to exclude the possibility of contact with a pertinent freshwater crime scene environment. Additional research is recommended to consider the spatial and temporal dynamics of diatom traces in alternative experimental scenarios, to generate the data needed to support the interpretation of evidence in casework [5-8].

It is well known that environmental conditions influence the transfer dynamics of other trace evidence indicators [30], and that diatom populations within a site vary temporally [23, 36]. This is the first study to consider the impact of temporal differences in the source environment within the context of diatom transfer to clothing submerged in freshwater. The experimental design of several previous diatom trace evidence studies has been based around laboratory-based transfer simulations [15, 21] with relatively little consideration, until now, of a more forensically relevant scene-based approach incorporating environmental variability. This paper provides an initial insight into the temporal influences on diatom transfer, with a more extensive and systematic study on the forensic implications of diatom seasonality recommended to complement similar approaches in the medico-legal diagnosis of drowning [50, 51].

4.4. Impact of morphological characteristics on diatom transfer

All transferred diatoms were identified and compared to the equivalent control assemblage, according to their general morphology [11, 21]. A large proportion of most transfer samples was comprised of centric taxa, despite a greater relative abundance of pennate diatoms in the March and November control samples (Figure 4a). This initially suggests that centric species including *Cyclotella radiosa* and *Melosira varians* are more inclined to transfer to clothing. Although a greater abundance of centric diatoms was identified in the environmental May sample (52%), their distribution on the clothing transfer surfaces was comparable to the trends observed in March and November. Fewer centric diatoms were recovered from nylon and PVC than all other substrates, suggesting that a smooth surface texture limits the potential for centric taxa to transfer to clothing in the context of this study (Figures 1 & 4a).

Although most of the diatoms transferred to each clothing sample were greater than 10µm in length, smaller taxa including *Achnanthidium minutissimum, Fragilaria construens,* and *Navicula cincta* were also identified in all but one assemblage (Figures 4b, 5 & 6). This provisionally suggests that size does not entirely limit the transferability of diatoms to clothing surfaces. Fewer smaller (<10µm) valves were recovered from nylon and lycra in all three sample months, indicating that the surface properties of such materials may limit the transfer of particular diatom species (Figure 1). Most of the diatoms recovered from the different clothing samples were whole and unbroken, aiding in their identification to species-level and comparison to an environmental control sample (Figure 4c). More fragmented diatoms were observed in the November transfer samples which could indicate the increased presence of broken valves in the environment during the winter months.

All diatoms present in March and November were identified to species-level, building on previous research that has considered only the most abundant taxa within an assemblage [20] or identification to genus level [15]. The diatoms present in all transfer samples comprised common freshwater species including those which are typically benthic, or bottom-dwelling (e.g. *Gyrosigma accuminatum, Neidium affine*) and those normally found suspended as plankton (e.g. *Melosira varians, Cyclotella meneghiniana*) [52]. A number of common terrestrial species were sometimes identified in the transfer samples including *Hantzschia amphioxys* and *Luticola mutica* (Figures 5 & 6), suggesting the potential for diatom transfer to clothing from habitats

other than the immediate crime scene environment. This is further supported by the presence of several diatom species in the transfer samples only including *Gomphonema parvulum, Achnanthes microcephala,* and *Frustulia vulgaris.* The diversity of diatom species identified in the clothing samples recommends the collection and analysis of several environmental control samples for appropriate comparison during a forensic investigation [22].

The presence and relative abundance of the control sample diatom species varied amongst the different clothing materials. For example, the most abundant diatom taxa in the March control was *Amphora pediculus* (48%), although this species comprised less than 25% of each transfer assemblage (Figure 5). The limited entrainment of this particular species may be attributed to morphological characteristics or surface features including the raphe or striae (Figure 9). Fewer *A. pediculus* diatoms were recovered from nylon, lycra (March), and PVC (November) suggesting that transfer is also limited by recipient surface type. A similar trend was also reported in the distribution of *Cocconeis placentula* and *Navicula radiosa* transferred to clothing (Figure 10). In comparison, *Melosira varians* was relatively more abundant in the transfer samples (particularly nylon and lycra) than in either of the two control samples (Figures 5 & 6). The successful entrainment of *M. varians* to all clothing surfaces may be explained by the microstructures of the frustule, including the presence of spines on the valve face and rim (Figure 9) [52].

A number of relatively less common diatom taxa were identified in the majority of the control and transfer samples including *Achnanthes lanceolata, Nitzschia dissipata, Rhoicosphenia curvata* (Figures 5, 6, & 10). Other species were limited in their transfer to certain clothing materials at particular times of year. For example, *Encyonema minutum, Reimeria sinuata,* and *Navicula minima* were all absent from nylon, PVC, and lycra (March) and *Nitzschia gracilis, Navicula gregaria,* and *Cyclotella meneghiniana* were identified only in linen, acrylic, PVC, and lycra (November). These results demonstrate the range of complex factors supporting or restricting diatom transfer to clothing –initial environmental abundance, recipient surface characteristics, and individual species-specific traits. The diversity of the silica cell wall is viewed as one of the most valuable traits for the analysis of diatoms in forensic science [15]. However, the results from this study provisionally suggest that different diatom morphologies and species do not transfer to clothing in a similar manner, and subsequently, the species present within the environment may not always be represented within a trace evidence sample (Figures 5-8 and 10). This has important implications for the exclusionary interpretation of evidence in forensic casework, which should be based on an assessment of both the abundant and less common components of a sample using multivariate analytical techniques for comparison [10, 11, 15, 22].

5. Conclusions

This paper presents the first structured study of three variables which impact upon the transfer of diatoms from freshwater crime scene environments to clothing. The results highlight that recipient surface characteristics (clothing type) and environmental variability (seasonal fluctuations) have a statistically significant effect on the total number of diatoms, and the species richness of an assemblage, transferred as evidence in forensic scenarios. Consideration

of all diatom species present in the experimental samples (rather than simply the most abundant), provides a novel empirical approach towards understanding the influence of valve morphology on transfer to clothing. The findings from this study provisionally suggest that general characteristics including size and shape, as well as species-specific features, may support or limit evidential transfer to clothing. Consequently, the forensic identification of diatoms to species-level should always be recommended where possible in an investigation. Finally, the H_2O_2 technique developed by Scott et al [15] was effectively used for the recovery of diatoms transferred to a range of different clothing garments.

It has been shown that, although clothing offers a valuable transfer medium when seeking to retrieve freshwater diatoms in forensic investigations, the interpretation of evidential material should be approached within an exclusionary framework [10, 48]. This research has generated valuable data to contribute towards the necessary empirical evidence bases in forensic ecology, demonstrating that the initial transfer of freshwater diatoms to clothing varies both spatially and temporally. Further experimental studies are recommended to consider the pre- and post-transfer dynamics of diatoms, in line with international priorities for forensic research [2, 3, 8].

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Highlights

- Freshwater diatom transfer to nine clothing items at three times of year is tested.
- More diatoms (with a greater species richness) transferred to woven fabrics.
- More diatoms (with a greater species richness) transferred in the spring.
- Diatom characteristics appear to influence their transfer to clothing.
- Spatial and temporal variability in transfer has important forensic implications.

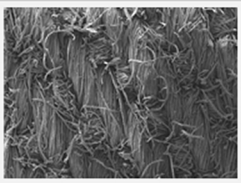
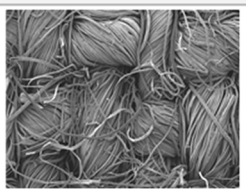
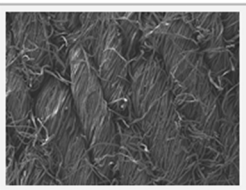
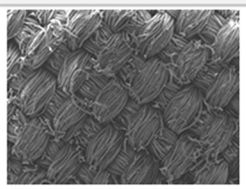
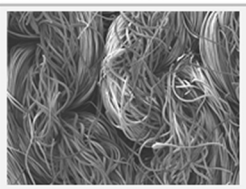
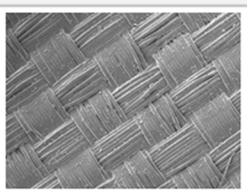
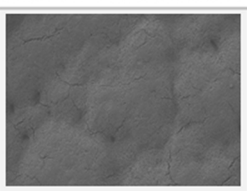
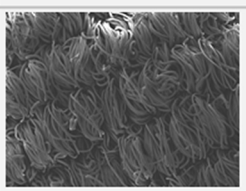
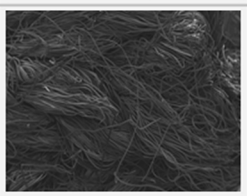
	Photograph	SEM image *(x50mag)*	Item description	Material composition	Fibre type	Surface characteristics
COTTON			Cotton cargo trousers	100% cotton	Natural	Coarse, open-weave, medium texture
LINEN			Linen trousers	63% linen, 37% polyamide	Natural	Open weave, rough texture
DENIM			Denim jeans	99% cotton, 1% elastane	Natural	Coarse open weave, medium texture
POLYESTER			Utility trousers	100% polyester	Synthetic	Semi-open weave, medium texture
VISCOSE			Knitted jumper	83% viscose, 17% polyamide	Synthetic	Open weave, medium texture
NYLON			Outdoor waterproof jacket	100% nylon	Synthetic	Closed weave, smooth texture
PVC			Outdoor jacket	100% polyurethane	Synthetic	Closed weave, smooth texture
LYCRA			Legging trousers	84% polyamide, 14% elastane, 2% cotton	Synthetic	Semi-open weave, smooth texture
ACRYLIC			Knitted cardigan	100% acrylic	Synthetic	Open weave, rough texture

Figure 1

	Temperature (°C)	pH	Conductivity (µ/s)	Dissolved O_2 (mg/L)	Depth (cm)
May 2014	11.5	7.1	452	10.14	8
March 2015	8.9	7.7	687	11.54	20
November 2015	12.7	7.5	629	8.63	17

Figure 2

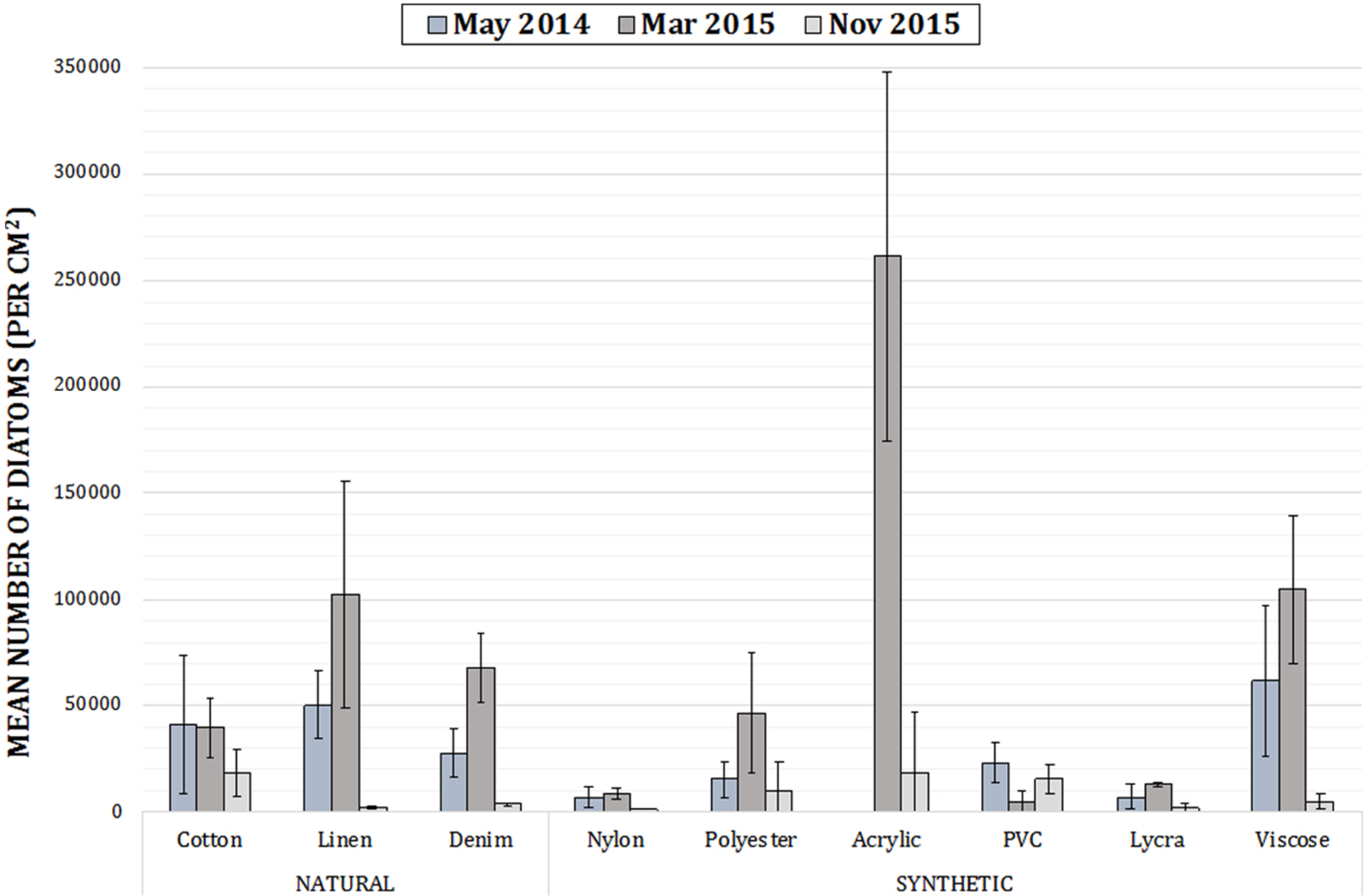
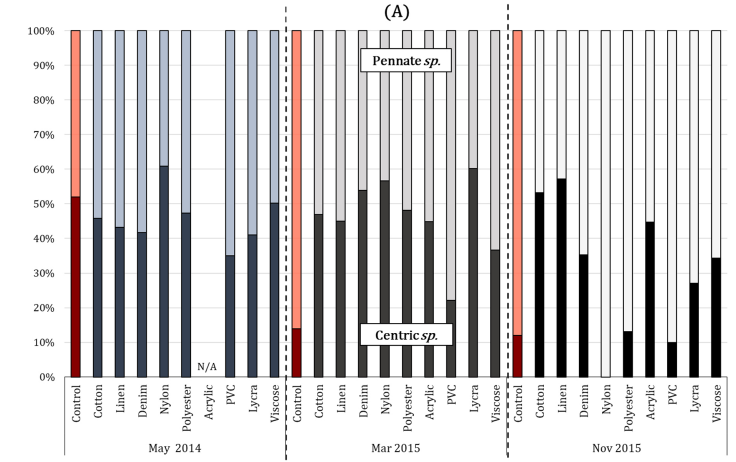
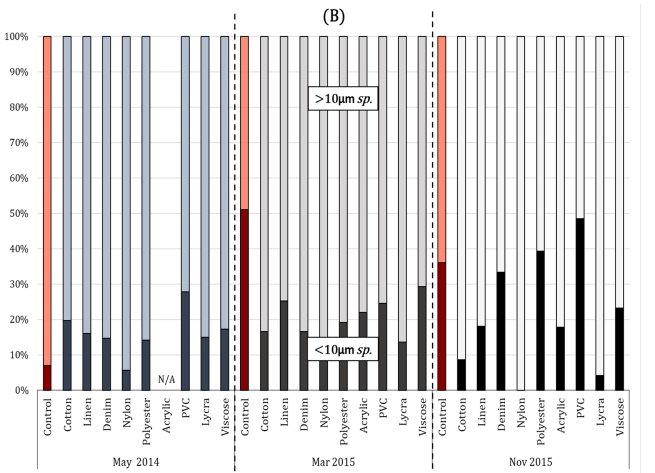

May 2014
Mar 2015
Nov 2015
MEAN NUMBER OF DIATOMS (PER CM²)
350000
300000
250000
200000
150000
100000
50000
0
Cotton
Linen
Denim
Nylon
Polyester
Acrylic
PVC
Lycra
Viscose
NATURAL
SYNTHETIC

Figure 3


(A)
Pennate sp.
Centric sp.
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
N/A
Control
Cotton
Linen
Denim
Nylon
Polyester
Acrylic
PVC
Lycra
Viscose
May 2014
Mar 2015
Nov 2015


(B)
>10µm sp.
<10µm sp.
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
N/A
Control
Cotton
Linen
Denim
Nylon
Polyester
Acrylic
PVC
Lycra
Viscose
May 2014
Mar 2015
Nov 2015

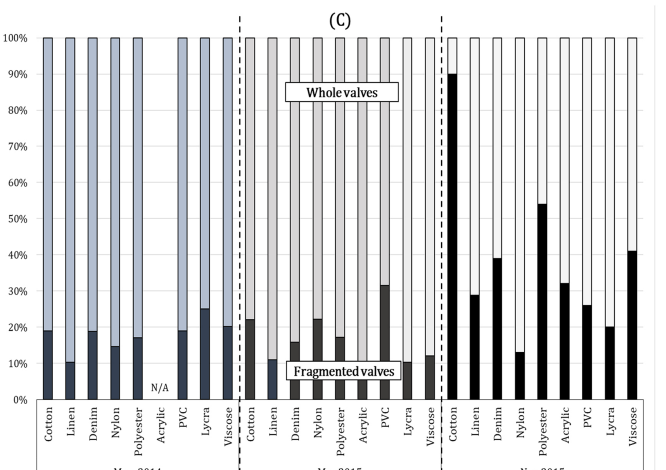

(C)
Whole valves
Fragmented valves
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
N/A
Cotton
Linen
Denim
Nylon
Polyester
Acrylic
PVC
Lycra
Viscose
May 2014
Mar 2015
Nov 2015

Figure 4

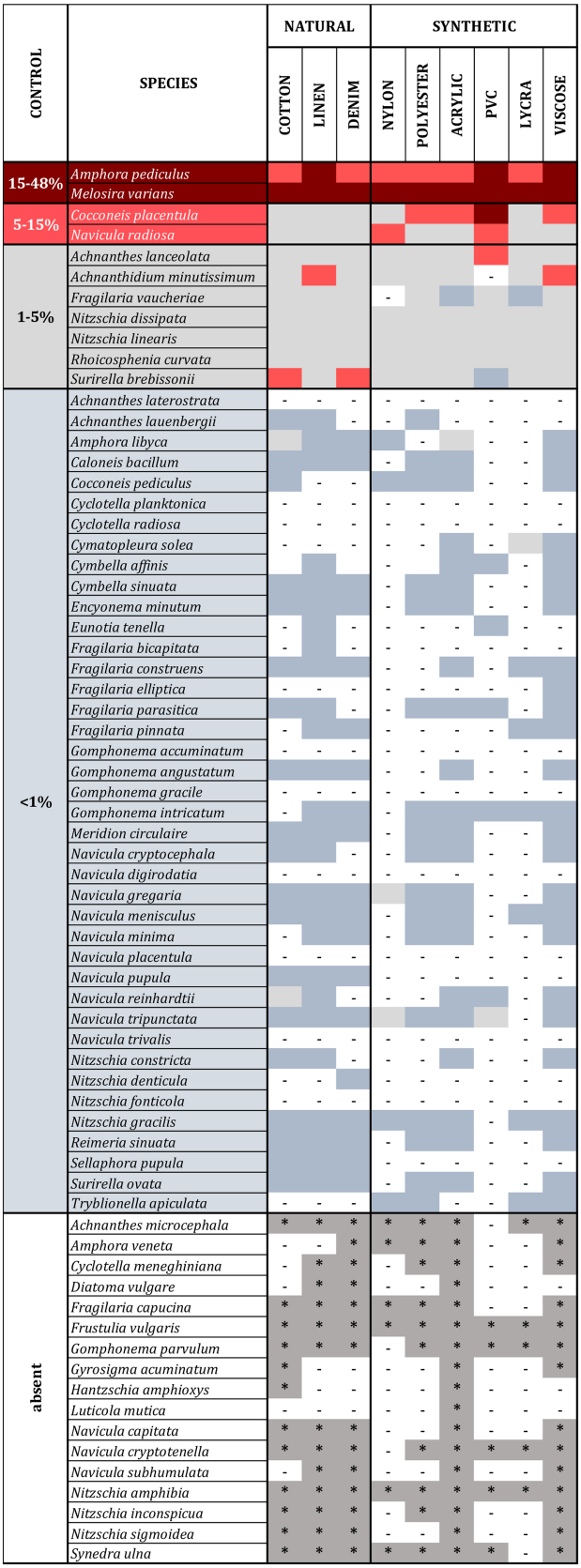

CONTROL
SPECIES
NATURAL
SYNTHETIC
COTTON
LINEN
DENIM
NYLON
POLYESTER
ACRYLIC
PVC
LYCRA
VISCOSE
15-48%
Amphora pediculus
Melosira varians
5-15%
Cocconeis placentula
Navicula radiosa
1-5%
Achnanthes lanceolata
Achnanthidium minutissimum
Fragilaria vaucheriae
Nitzschia dissipata
Nitzschia linearis
Rhoicosphenia curvata
Surirella brebissonii
<1%
Achnanthes laterostrata
Achnanthes lauenbergii
Amphora libyca
Caloneis bacillum
Cocconeis pediculus
Cyclotella planktonica
Cyclotella radiosa
Cymatopleura solea
Cymbella affinis
Cymbella sinuata
Encyonema minutum
Eunotia tenella
Fragilaria bicapitata
Fragilaria construens
Fragilaria elliptica
Fragilaria parasitica
Fragilaria pinnata
Gomphonema accuminatum
Gomphonema angustatum
Gomphonema gracile
Gomphonema intricatum
Meridion circulaire
Navicula cryptocephala
Navicula digirodatia
Navicula gregaria
Navicula menisculus
Navicula minima
Navicula placentula
Navicula pupula
Navicula reinhardtii
Navicula tripunctata
Navicula trivalis
Nitzschia constricta
Nitzschia denticula
Nitzschia fonticola
Nitzschia gracilis
Reimeria sinuata
Sellaphora pupula
Surirella ovata
Tryblionella apiculata
absent
Achnanthes microcephala
Amphora veneta
Cyclotella meneghiniana
Diatoma vulgare
Fragilaria capucina
Frustulia vulgaris
Gomphonema parvulum
Gyrosigma acuminatum
Hantzschia amphioxys
Luticola mutica
Navicula capitata
Navicula cryptotenella
Navicula subhumulata
Nitzschia amphibia
Nitzschia inconspicua
Nitzschia sigmoidea
Synedra ulna

Figure 5

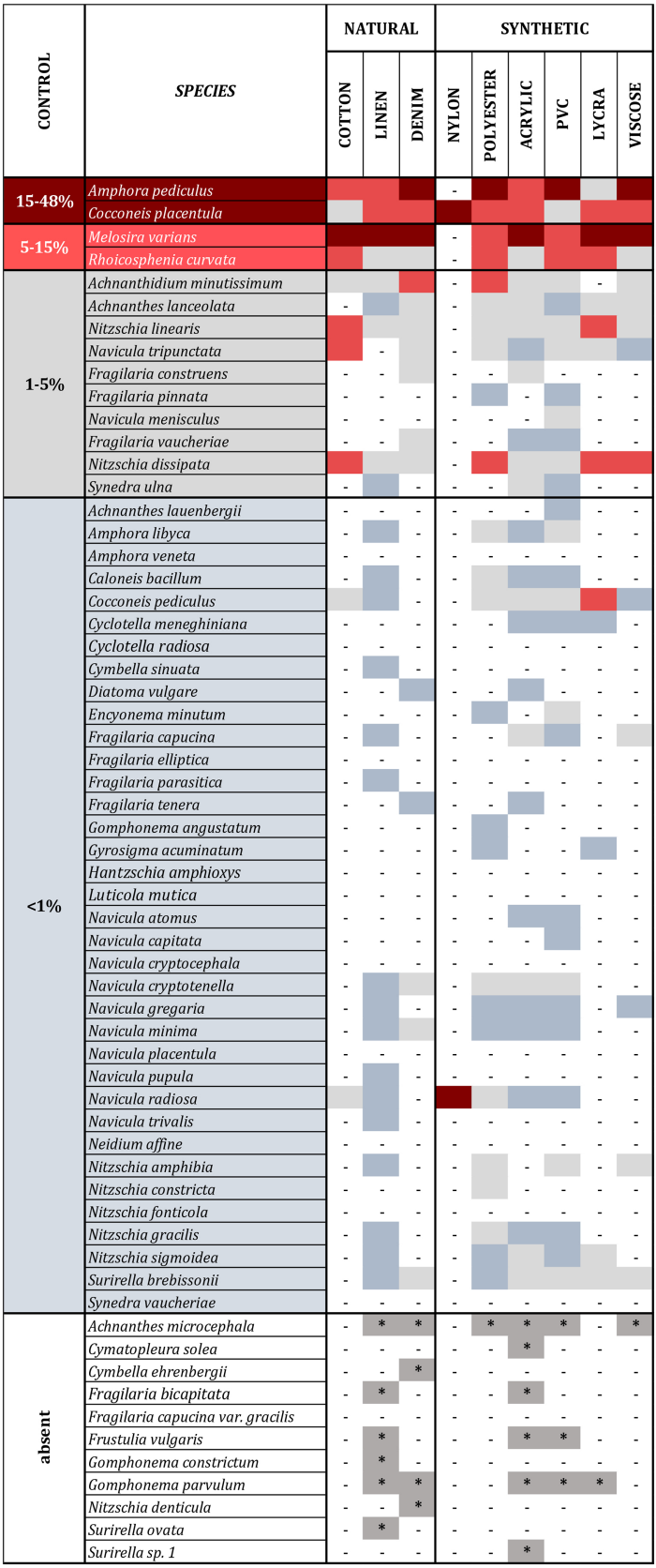

CONTROL
SPECIES
NATURAL
SYNTHETIC
COTTON
LINEN
DENIM
NYLON
POLYESTER
ACRYLIC
PVC
LYCRA
VISCOSE
15-48%
Amphora pediculus
Cocconeis placentula
5-15%
Melosira varians
Rhoicosphenia curvata
1-5%
Achnanthidium minutissimum
Achnanthes lanceolata
Nitzschia linearis
Navicula tripunctata
Fragilaria construens
Fragilaria pinnata
Navicula meniscculus
Fragilaria vaucheriae
Nitzschia dissipata
Synedra ulna
<1%
Achnanthes lauenbergii
Amphora libyca
Amphora veneta
Caloneis bacillum
Cocconeis pediculus
Cyclotella meneghiniana
Cyclotella radiosa
Cymbella sinuata
Diatoma vulgare
Encyonema minutum
Fragilaria capucina
Fragilaria elliptica
Fragilaria parasitica
Fragilaria tenera
Gomphonema angustatum
Gyrosigma acuminatum
Hantzschia amphioxys
Luticola mutica
Navicula atomus
Navicula capitata
Navicula cryptocephala
Navicula cryptotenella
Navicula gregaria
Navicula minima
Navicula placentula
Navicula pupula
Navicula radiosa
Navicula trivalis
Neidium affine
Nitzschia amphibia
Nitzschia constricta
Nitzschia fonticola
Nitzschia gracilis
Nitzschia sigmoidea
Surirella brebissonii
Synedra vaucheriae
absent
Achnanthes microcephala
Cymatopleura solea
Cymbella ehrenbergii
Fragilaria bicapitata
Fragilaria capucina var. gracilis
Frustulia vulgaris
Gomphonema constrictum
Gomphonema parvulum
Nitzschia denticula
Surirella ovata
Surirella sp. 1

Figure 6

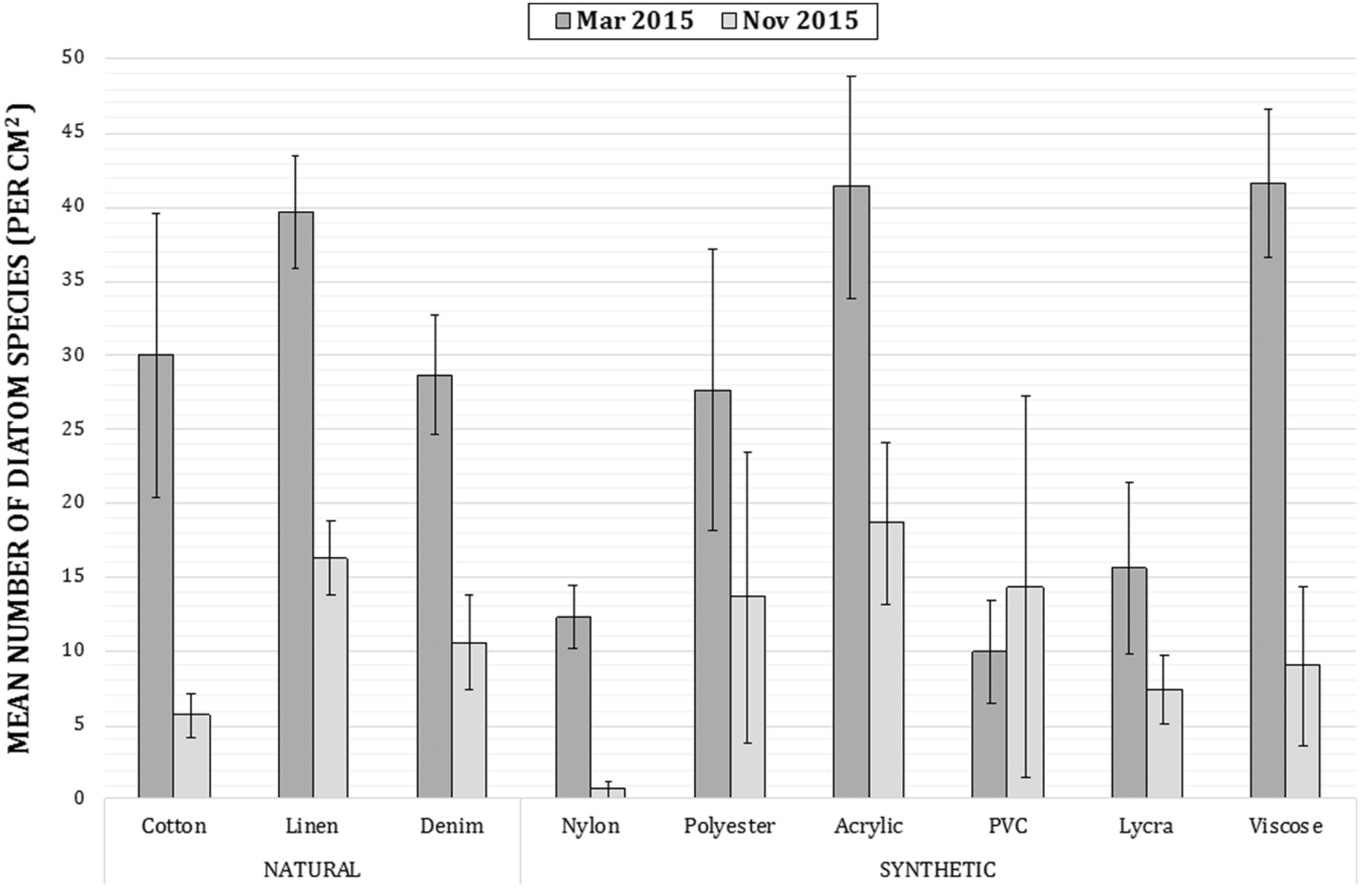

Mar 2015
Nov 2015
MEAN NUMBER OF DIATOM SPECIES (PER CM²)
50
45
40
35
30
25
20
15
10
5
0
Cotton
Linen
Denim
Nylon
Polyester
Acrylic
PVC
Lycra
Viscose
NATURAL
SYNTHETIC

Figure 7

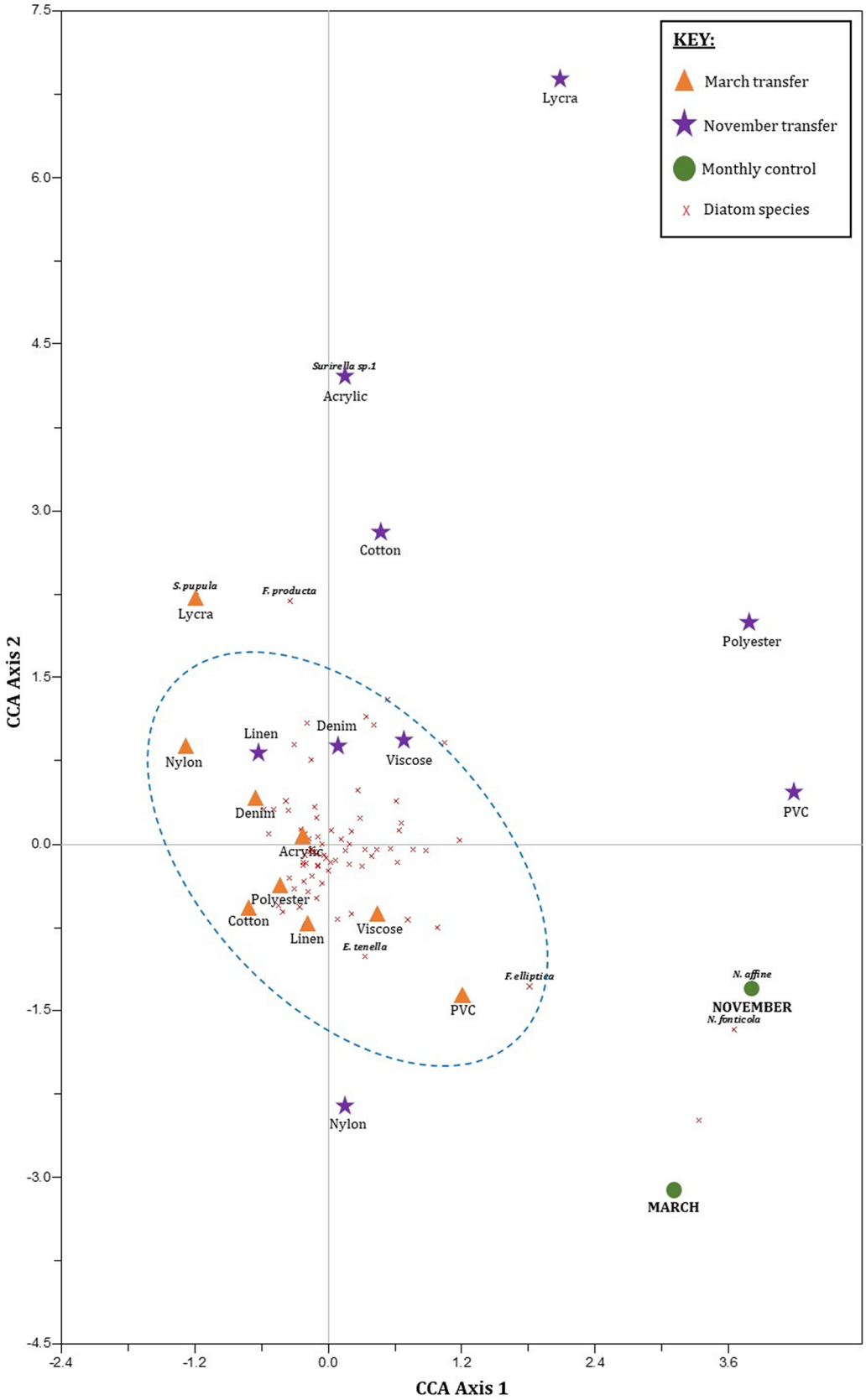

KEY:
March transfer
November transfer
Monthly control
Diatom species
Lycra
Surirella sp.1
Acrylic
Cotton
S. pupula
Lycra
F. producta
Polyester
Linen
Denim
Nylon
Viscose
PVC
Denim
Acrylic
Polyester
Cotton
Linen
Viscose
E. tenella
F. elliptica
PVC
N. affine
NOVEMBER
N. fonticola
Nylon
MARCH
CCA Axis 2
CCA Axis 1
7.5
6.0
4.5
3.0
1.5
0.0
-1.5
-3.0
-4.5
-2.4
-1.2
0.0
1.2
2.4
3.6

Figure 8

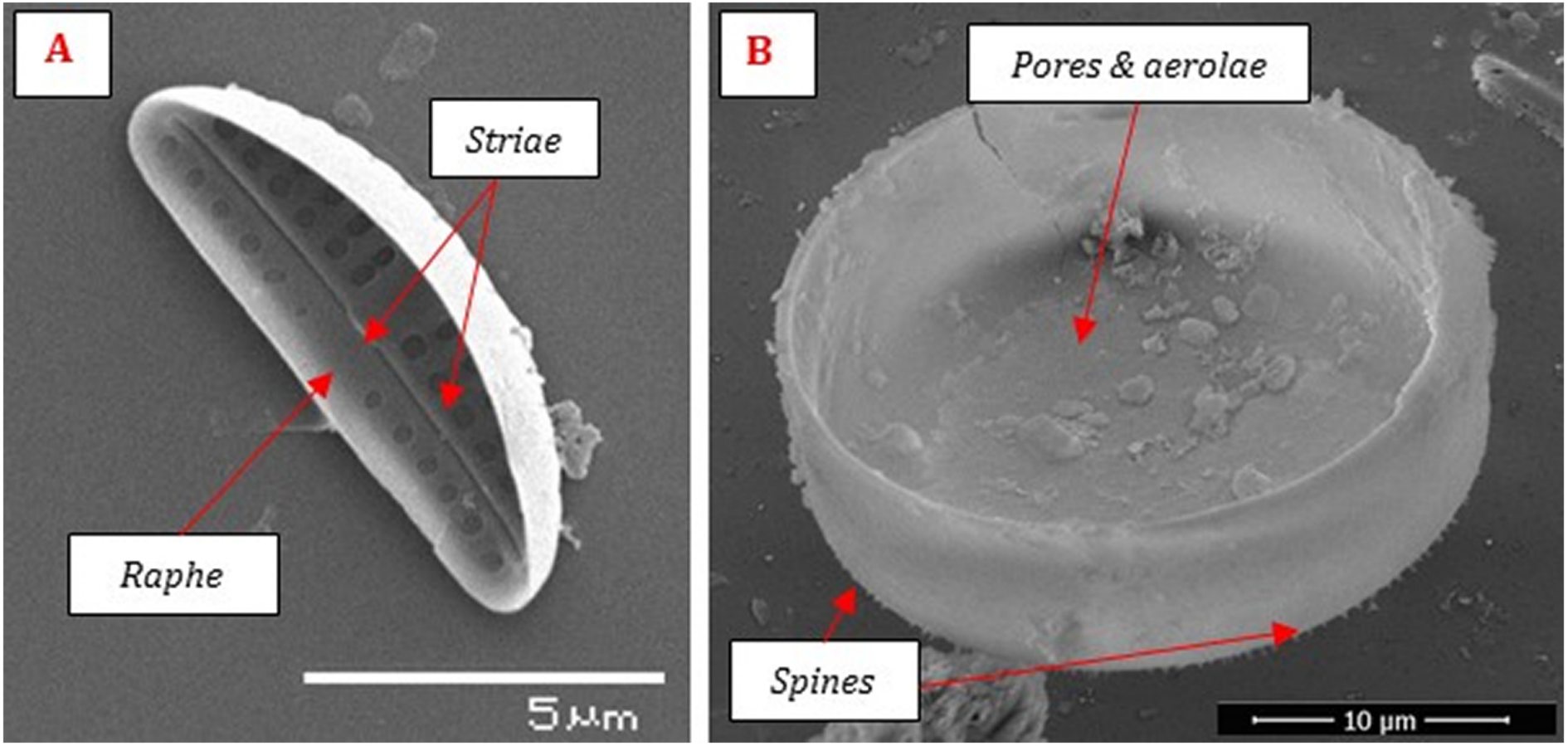


Figure 9

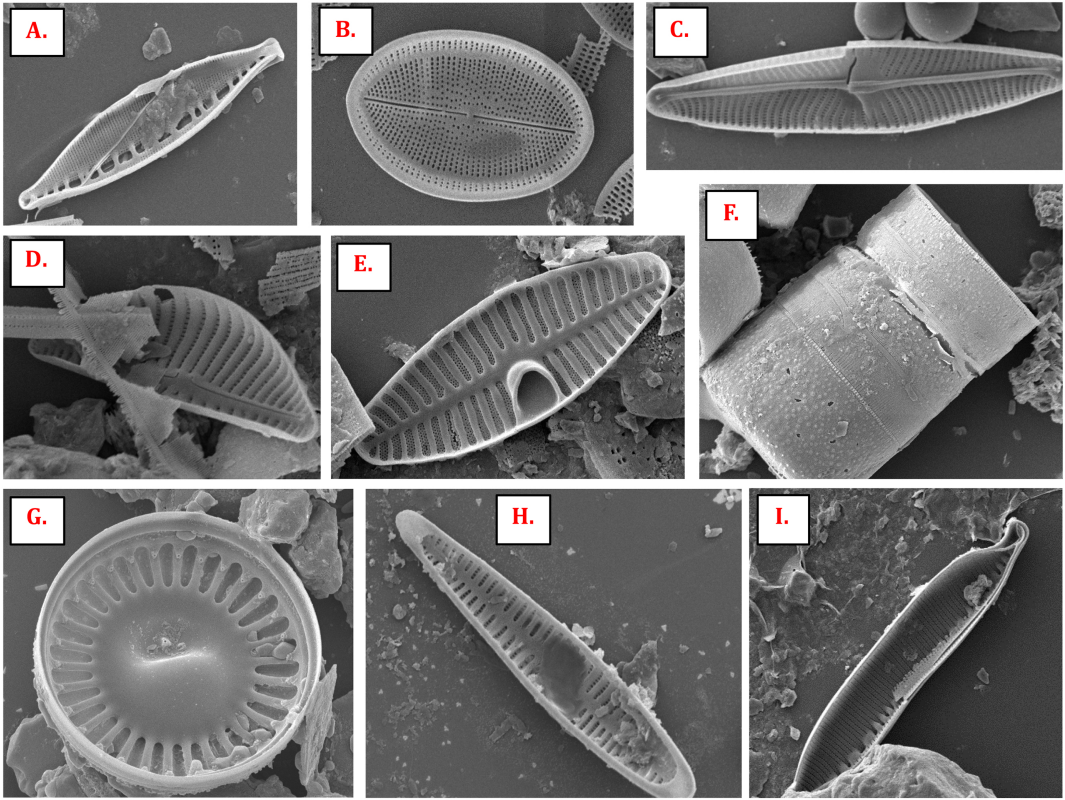


Figure 10