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# Freshwater diatom persistence on clothing I: A quantitative assessment of trace evidence dynamics over time

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ABSTRACT

Freshwater diatoms offer valuable circumstantial forensic indicators, with a growing empirical research base aiming to identify and understand some of the spatial and temporal factors affecting their validity as trace evidence. Previous studies demonstrated that recipient surface characteristics, environmental variability, and individual species traits influence the initial transfer of freshwater diatoms to clothing. However, no previous research has sought to consider the impact of these and other variables on the persistence of transferred diatoms over investigative timescales. Therefore, this study aimed to identify and explore diatom retention dynamics on clothing following wear over time (hours to weeks). A series of experiments were designed to examine the impact of clothing material, seasonality, and time since wear (persistence interval) on the total number and species-richness of diatoms recovered and their relative retention (%) over time. Nine clothing swatches were immersed in a freshwater environment and then worn for one month in the spring. Subsamples were retrieved at regular intervals (e.g. 30 mins, 1 h, 8 h, 24 h) up to one month, diatoms were extracted using a $H_2O_2$ method, and examined microscopically. Three clothing materials were subject to the same experiment in the winter to generate a seasonal comparison. The results broadly identified three stages of diatom persistence on clothing – rapid initial loss, variable intermediate decay, and sustained long-term presence. Clothing material significantly impacted the number of diatoms recovered and retention dynamics over time, with complex interactions identified with seasonality. Although fewer diatoms were recovered in the winter, overall retention trends were consistent at the different times of year. The findings demonstrate that diatoms can be recovered from clothing, even weeks or months after an initial transfer, yielding a useful environmental trace indicator for forensic reconstructions over investigative timescales. The impact of clothing material and seasonality on persistence identified cotton, acrylic, and viscose clothing as the most reliable temporal repository of diatom trace evidence, with a more abundant forensic assemblage available for forensic comparisons in the spring.



## 1. Introduction

Empirical research within the forensic sciences is critical to support expert analysis and interpretations [1]. The importance of targeted research within trace evidence dynamics has consistently been raised as a priority for forensic development [2]. Structured studies pertaining to evidence transfer, persistence, and preservation allow inferences made in forensic casework to be evidence-based and the significance of any findings transparently established [3]. This paper contributes to the growing research literature within forensic diatom analysis [4–6], through assessment of the persistence dynamics of diatoms as circumstantial trace indicators hours, days, and weeks post- initial transfer.

For trace evidence to be appropriately evaluated within the context of a forensic case, it is imperative to examine the dynamics of that evidence after a crime has occurred and before retrieval by investigators. From the significant amount of existing forensic research, it is widely accepted that the availability and quality of

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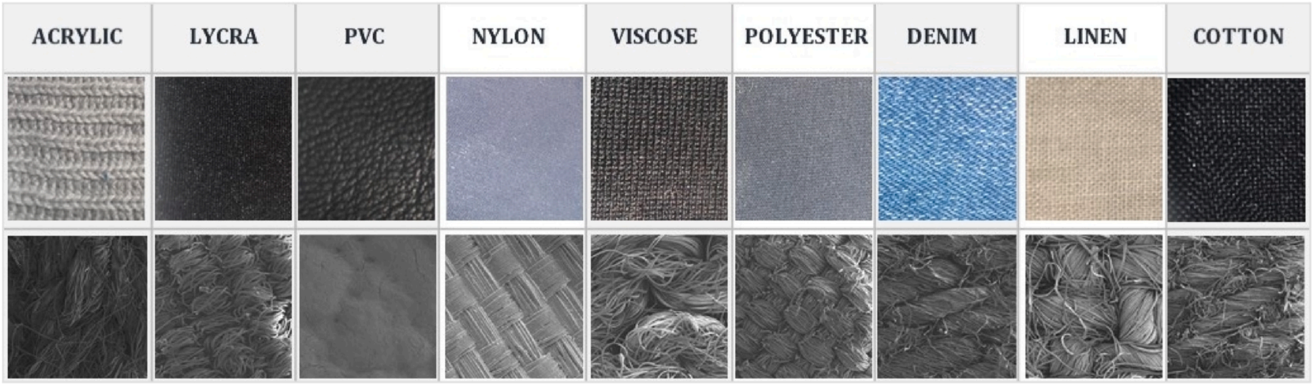


**Fig. 1.** Images (1x mag.) and SEM micrographs (50x mag.) of the nine clothing materials tested throughout the study. Additional detail on fabric composition is provided in [6].

evidence generally declines over time [7]. Different variables including offender activity (e.g. wear over time), recipient surface characteristics (e.g. clothing type), environmental conditions (e.g. seasonality), and the properties of the trace itself have been shown to influence the persistence dynamics of indicators including paint, glass, fibres, pollen, human hair and DNA [8–13].

Environmental indicators including soil, pollen, diatoms, and microbial communities are commonly encountered as circumstantial evidence in forensic investigations, addressing key questions including where, when, and how a crime was committed [14]. Due to the abundance and diversity of environmental indicators in our surroundings, and in anticipation of additional (inevitable) transfers that may occur pre- and post- forensic event, it is important to empirically assess environmental trace persistence [15]. Such research has primarily considered soil mineralogy [16] and forensic palynology [8,11]. For example, the persistence dynamics of six pollen types (10–100 µm) on six clothing materials identified a significant loss of material over time, with only 2% of the initial assemblage retained after one month [11]. Loss was influenced by clothing type rather than pollen grain morphology. Despite a growing empirical research focus exploring diatoms as a form of trace evidence, there is relatively little research addressing the dynamics of that evidence over time [5,6].

Diatoms (*Bacillariophyceae*) are an abundant and species-rich group of unicellular algae. They are found in most aquatic and various terrestrial environments and are characterised by a highly resistant silica cell wall ($SiO_2$). The diatom population density and species assemblage at a site fluctuates with changes in biotic and abiotic conditions including temperature, silica, and light availability [17]. The abundance and the spatial and temporal diversity of diatoms within the environment, supports their forensic application as indicators of death by drowning [18] and to establish contact between scenes and persons of forensic interest as a form of trace evidence [19,20].

Although diatom trace evidence analysis is documented in casework [19–21], the corresponding research base is relatively recent in its development. Previous studies have tested different techniques for diatom recovery from clothing and footwear, with a $H_2O_2$ extraction protocol shown to generate an abundant and species-rich forensic sample [4]. Diatoms are transferred to clothing and footwear following brief periods of immersion [4,5] although the extent of this transfer is significantly affected by environmental variability, recipient surface characteristics, and diatom morphology [6,22]. Persistence has briefly been tested on footwear, with diatoms retrievable after one week of continued wear [5]. Additional research is needed to further develop this evidence base, and to explore some of the variables known to affect trace evidence persistence within the context of freshwater diatoms. This will contribute to the exclusionary frameworks required to support the reliable interpretation of diatom trace evidence and the communication of evidential findings in forensic casework.

This research aimed to explore the persistence of freshwater diatoms on clothing following wear over timescales pertinent to forensic investigations, building upon the initial evidence bases developed within diatom transfer [6]. A series of experiments were designed to determine the extent (no. per $cm^2$), overall retention (% loss), and species-richness of a forensic diatom assemblage recovered from clothing following brief and extended periods of wear - 30 min, 1 h, 2 h, 4 h, 8 h, 16 h, 24 h, 48 h, 168 h (one week), 336 h (two weeks), 720 h (one month). Nine common clothing garments were sampled to consider the impact of substrate characteristics on diatom retention in the spring, before a seasonal comparison in the winter sought to determine whether persistence trends are consistent when fewer diatoms are present in the environment [23], which subsequently yields a less abundant forensic transfer assemblage [6].

## 2. Methods

### 2.1. Clothing samples

Nine common clothing fabrics were used to explore diatom persistence (Fig. 1). Natural and synthetic fabrics with different surface textures and woven structures were chosen to account for the diversity of clothing items that may be recovered as evidence exhibits. The same substrates were used as in [6] to build upon initial transfer research. All clothing samples were new (unused but washed) and removed from the whole garment prior to contact with the site.

### 2.2. Sample collection

Three 4 x 10 cm swatches of each clothing type were attached to the bottom of a pair of waterproof trousers, which were worn, fully immersed and walked through a 5 m transect of the River Beane (Hertfordshire, UK) (National Grid Ref: TL313148). Diatom transfer was initiated for a period of 3 min [4]. To provide an environmental control sample, the same transect of the river was walked through and a 500 ml water sample (with any suspended) material collected downstream.

Each clothing swatch was removed from the trousers and immediately re-attached to the back of a coat. The coat, unwashed throughout, was worn daily (both in and outdoors) for a minimum of 4 h up to one month (30 days). 1 $cm^2$ subsamples were removed from pre-marked specific locations on the swatch at regular intervals: 0.5, 1, 2, 4, 16, 24, 48, 168, 336, and 720 h post-transfer (Fig. 2). Following recovery, each sample was double bagged and refrigerated in dark storage (5 °C).

To account for seasonal variability, the experiment was conducted in two phases. All nine clothing materials were initially

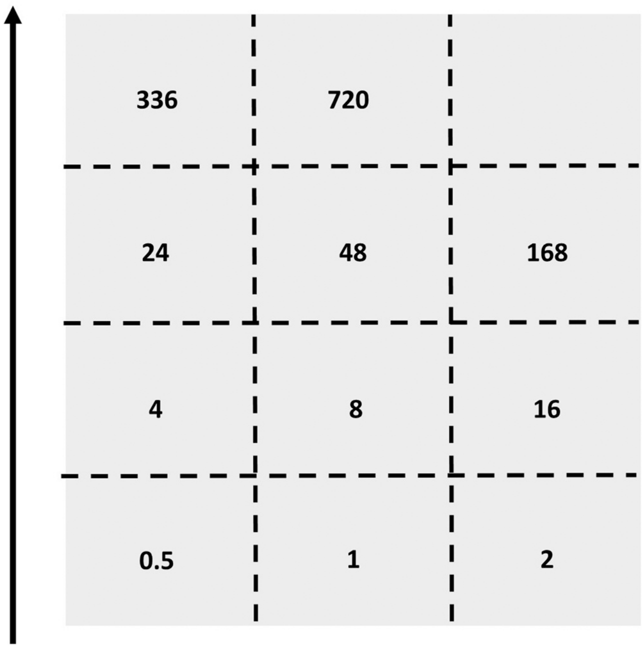


**Fig. 2.** The sections of each material swatch subsampled following the eleven persistence intervals. All four corners of the swatch were secured to an overcoat which was worn daily for a minimum of 4 h, prior to subsample removal at the specified times. All values presented are in hours (h) and the arrow shows the top of the material swatch.

tested in the spring (March-April 2015). Three materials (cotton, acrylic, nylon) were chosen for a seasonal comparison in the winter (November-December 2015), as they represented the three different diatom retention patterns as observed in the first part of the study.

### 2.3. Laboratory preparation

Diatoms were extracted from all 1 $cm^2$ clothing subsamples using the $H_2O_2$ method outlined in [4]. Each sample was added to a centrifuge tube, 20 ml of $H_2O_2$ (30%) was added, before heating in a water bath for 4 h (70 °C). The clothing material was then removed before distilled water and a few drops of HCl (10%) were added to the solution. The sample solutions were centrifuged (1200 rpm, 4 mins), the supernatant discarded, and re-washed with distilled water four times. Most of the final supernatant (45 ml) was removed to provide a final aliquot of concentrated diatoms in solution. Three control replicates for each sample month were prepared using the same protocol; 20 ml of well-mixed control site water was treated with 20 ml of $H_2O_2$ for each subsample [4,24].

Fixed microscope slides were prepared using 500 µl of the final sample aliquot. Each solution was transferred to a 19 mm coverslip using a calibrated micropipette. After evaporation, slides were produced using the high refractive index mountant Naphrax™ (RI: 1.73) on a heated hotplate [24].

Various measures were incorporated throughout the laboratory process to prevent cross-contamination between samples. This included the use of pristine (or clean and sterile) equipment and the preparation of blank control samples throughout the laboratory process (n = 45) [4].

### 2.4. Analysis

All samples (n = 477) were examined using phase-contrast light microscopy (x1000 magnification). All diatoms from a known proportion of the coverslip were counted, recorded, and identified to species-level. Results from the coverslip area studied were standardised to provide an estimated calculation of the total diatom count for the whole sample (per ml/$cm^2$) [4,6]. Persistence was compared through assessment of the total diatom count (no. per $cm^2$), overall retention (expressed as a % of the initial count recorded at time 0.5 h), and changes in species-richness over time. Statistical analysis was performed using SPSS v. 25.

**Table 1**
The estimated mean ± standard deviation number of diatoms and the number of species identified in the spring and winter control samples (per ml) (n = 3).

| | March | November |
|---|---|---|
| **N. of diatoms** | 7170 ± 4382 | 3461 ± 1036 |
| **N. of species** | 30 ± 3 | 38 ± 10 |

## 3. Results

No diatoms were identified in the blank samples prepared to test for contamination. The spring control samples were more abundant (mean ± std. deviation = 7170 ± 4382 diatoms per ml) than the winter comparison (3561 ± 1036/ml) (Table 1).

### 3.1. Spring

#### 3.1.1. Mean diatom count (per $cm^2$)

Diatoms were recovered from all spring persistence samples (Table 2). More diatoms were identified in acrylic ($\bar{x}$ [mean]: 188,865–608,968/$cm^2$), viscose ($\bar{x}$: 10,264–180,571/$cm^2$), and cotton ($\bar{x}$: 8003–203644/$cm^2$). Fewer diatoms were recovered from nylon, although they were still present following each period of wear (Table 2). An initial two-way ANOVA identified no statistically significant interaction between clothing type and time since immersion on the estimated mean diatom count: $F$ (80, 198) = 0.987, $p$ = .518. Post-hoc tests showed that the number of diatoms recovered from acrylic was significantly higher than all other clothing types ($p < .0001$), and that significantly fewer diatoms were present beyond 0.5 h of wear across all clothing materials ($p < .05$).

Diatoms were identified after one month of continuous wear in all clothing samples ($\bar{x}$: 1100–204,412/$cm^2$) (Table 2). A one-way ANOVA reported no statistically significant difference between clothing type and the number of diatoms identified in the 720 h samples: $F$ (8, 18) = 1.894, $p$ = .124.

#### 3.1.2. Diatom retention (%)

To enable a meaningful comparison of diatom persistence over time, overall retention trends (expressed as a % of the initial count recorded at time 0.5 h) are presented ( Figs. 3 and 5). Percentage retention was calculated for each replicate separately with mean and standard deviation values (n = 3) then determined and presented here.

Diatom retention varied amongst the nine materials (Fig. 3). Although diatoms were always present in the later samples (e.g. 4–37% at 720 h), persistence trends were not consistent between sampling interval and clothing type. An immediate loss of material (0.5–1 h) was identified in most clothing samples (1 h: 24–92% retention), except viscose (206%) and lycra (208%) where more diatoms were recovered following 1 h of wear (Fig. 3). Diatom retention beyond 1 h was variable between the different clothing types, although three main patterns can be observed:

1. Cotton, linen, and polyester exhibit a relatively typical exponential decay curve, with consistent diatom loss over time (Fig. 3a, b, c). For example, the retention of diatoms on cotton decreased from 56.9% (1 h), to 47.8% (4 h), 25.4% (24 h) and 5.2% (720 h). Although the initial loss of material from polyester was greater (43% – 1 h), a similar proportion of the initial count remained after 720 h of wear (4%) (Fig. 3c). The initial loss of transferred diatoms from linen was less pronounced with mean

**Table 2**
The estimated mean number of diatoms identified in each spring clothing sample (per $cm^2$) following each persistence interval of wear (n = 3).

| | 0.5 h | 1 h | 2 h | 4 h | 8 h | 16 h | 24 h | 48 h | 168 h | 336 h | 720 h |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Cotton** | 203,664 | 48,040 | 66,144 | 74,473 | 60,177 | 29,243 | 52,438 | 15,966 | 10,610 | 9531 | 8003 |
| **Linen** | 91,620 | 84,125 | 62,111 | 70,481 | 54,694 | 31,931 | 21,688 | 24,356 | 22,564 | 11,363 | 12,341 |
| **Denim** | 67,243 | 30,567 | 59,301 | 25,232 | 34,538 | 18,491 | 37,674 | 49,200 | 28,714 | 28,245 | 7372 |
| **Nylon** | 4826 | 3869 | 6293 | 13,990 | 1731 | 3238 | 3258 | 1181 | 1568 | 1914 | 1100 |
| **Polyester** | 69,382 | 36,819 | 36,880 | 45,596 | 19,692 | 17,350 | 19,407 | 5111 | 6842 | 4745 | 3116 |
| **Acrylic** | 608,968 | 540,520 | 334,853 | 575,912 | 257,125 | 188,865 | 417,213 | 224,976 | 191,585 | 202,763 | 204,412 |
| **PVC** | 56,023 | 33,051 | 42,602 | 129,742 | 25,476 | 25,659 | 27,818 | 45,290 | 59,138 | 21,586 | 20,609 |
| **Lycra** | 47,938 | 99,867 | 80,012 | 37,267 | 27,891 | 22,380 | 17,025 | 64,698 | 34,497 | 6191 | 9225 |
| **Viscose** | 18,0571 | 130,862 | 101,211 | 96,792 | 64,168 | 66,938 | 71,459 | 140,555 | 119,376 | 25,639 | 10,264 |

retention ranging from 88% (1 h), 64% (4 h), 21% (24 h), and 12% (720 h) (Fig. 3b). There was relatively little variation between sample replicates across the three materials, with less variability after extended periods of wear.

2. Diatom retention on denim and acrylic also decreased although loss was not consistent over time (Fig. 3d, e). For example, diatom retention on acrylic initially decreased to 85.5% (1 h) and 53.4% (2 h), before an increase in the amount of material at 4 h (92.8%). A similar peak is observed at 24 h (69.9%) (Fig. 3e). Similarly, within denim, a greater abundance of diatoms was identified after 2 h (67.6%) and 8 h (45%) than in the preceding samples (1 h: 42.4%; 4 h: 32%). Diatom retention in the latter stages (24–336 h: 37.5–76.5%) was higher than in the earlier persistence intervals (1–16 h: 27.2–67.6%) (Fig. 3d). More diatoms were retained in the 720 h acrylic samples (34.4%) than most other clothing samples, although there was a large standard deviation between replicates. Greater variation was also reported in denim and acrylic (1–64%) when compared to cotton, linen, and polyester, although the lowest values were still identified at 336 h and 720 h (1–34%).
3. Diatom retention on PVC, nylon, lycra, and viscose demonstrated the most complex persistence trends. Increased and unexpected relative abundances were identified in several intermediate persistence intervals (Fig. 3f, g, h, i). Within viscose, the retention at 1 h, 2 h, and 8 h (173–293.3%) was 2–3x higher than the initial 0.5 h assemblage (Fig. 3f). Similar peaks were identified at 4 h and 168 h (120.4–266.9%) in PVC, 2 h and 4 h in nylon (108–196%), and from 1 to 8 h in lycra (159.3–472.4%). Greater variability between triplicates accompanied increases in diatom retention. For example, nylon retention at 4 h (196%) also reported a deviation of 168% (Fig. 3 h). Triplicate variability ranged from 179% to 523% (lycra 1–8 h), 206–363% (viscose 1, 2, 8 h) and 134–256% (PVC 4, 168 h) in the most abundant retention samples. Although diatom loss was not consistent between consecutive persistence intervals, fewer diatoms were always identified at 336 h (15.1–42.3%) and 720 h (6.7–80.7%). Variability between sample replicates at the later stages was also lower than in the preceding intervals of wear.

Differences in diatom retention (%) were statistically tested through a two-way ANOVA accounting for clothing type and time of wear. To aid analysis, the persistence intervals were grouped into five categories (0.5–1 h, 2–8 h, 16–48 h, 168–336 h, and 720 h). There was no significant interaction between clothing type and persistence interval on diatom retention: $F(32, 252) = 0.568$, $p = .972$. There was a significant difference between clothing type ($p = .006$) and persistence interval ($p < .0001$). Post-hoc tests identified diatom retention on lycra was significantly higher than cotton, linen, denim, polyester, and acrylic ($p = .001$ −0.028). Additionally, significantly more diatoms were retained in the 0.5–1 h and 2–8 h assemblage when compared to the latter three intervals ($p = .004$ – $p = .016$).

### 3.1.3. Species richness

The mean number of diatom species decreased over time in all spring samples (Fig. 4). As with overall retention, species loss was not always consistent between persistence interval, although the least species-rich assemblage was always identified at 336 h or 720 h ($\bar{x}$: 5–50 sp.). Most clothing samples retained >17 diatom species after one month except nylon ($\bar{x}$: 5).

Acrylic ($\bar{x}$: 50–70 sp.) and viscose ($\bar{x}$: 19–61 sp.) reported the most species-rich assemblage over time, with consistently fewer species in nylon ($\bar{x}$: 5–24 sp.) (Fig. 4). PVC and lycra demonstrated the most consistent species richness overtime . For example, from 0.5 to 168 h, the mean number of diatom taxa retained on PVC ranged from 40 to 52 *sp*. Over time, more species were lost from cotton, polyester, and viscose, with 42–45 sp. lost between 0.5 h and 720 h.

## 3.2. Winter

Cotton, acrylic, and nylon were chosen for the seasonal comparison as all three demonstrated different retention trends in the spring (Fig. 3a, e, h).

### 3.2.1. Mean diatom count (per $cm^2$)

Fewer diatoms were identified in all winter persistence samples, corresponding with similar decreases in control sample abundance (Table 1, Table 3). As in the spring, acrylic yielded more diatoms ($\bar{x}$: 10,774–335/$cm^2$) and the nylon samples were less abundant ($\bar{x}$: 0–177/$cm^2$). Although persistence counts were lower, diatoms were identified in most nylon and acrylic samples including at 720 h. The persistence of diatoms transferred to cotton was more variable, with very few diatoms recovered in winter ($\bar{x}$: 1–14/$cm^2$).

A three-way ANOVA compared the number of diatoms identified in the spring and winter persistence samples (cotton, nylon, acrylic only). No statistically significant interaction was identified between the three variables ($F(20, 132) = 1.142$, $p = .316$) and between clothing material and time of wear ($p = .271$). The interaction between clothing material and sample season was significant ($p < .0001$). Significantly more diatoms were recovered from the spring persistence samples ($p < .0001$) and from acrylic clothing ($p < .0001$) as determined by post-hoc tests.

### 3.2.2. Diatom retention (%)

Although there were fewer diatoms in the winter cotton samples, a similar trend in diatom retention was observed (Table 3, Fig. 5a). The initial loss of material was slower, with an initial increase in diatom presence at 1 h (124% v 57% in spring). Beyond 4 h, diatom retention was comparable – e.g. 4 h (48–50%), 24 h (25–29%), and 336 h (15–18%). As in spring, there was little variability between replicates in the later stages of wear (Fig. 5a).

Complex trends in diatom persistence on nylon were also reflected in the winter (Fig. 5b). Relative retention abundances were identified at 1 h, 8 h, and 720 h (105–166%), similarly to peaks in spring. In both sample runs, the loss of material was not consistent between consecutive wear intervals. Variability was greater in winter (15–225%), perhaps reflecting the less abundant assemblage (Table 3). No diatoms were identified in the 24 h samples.

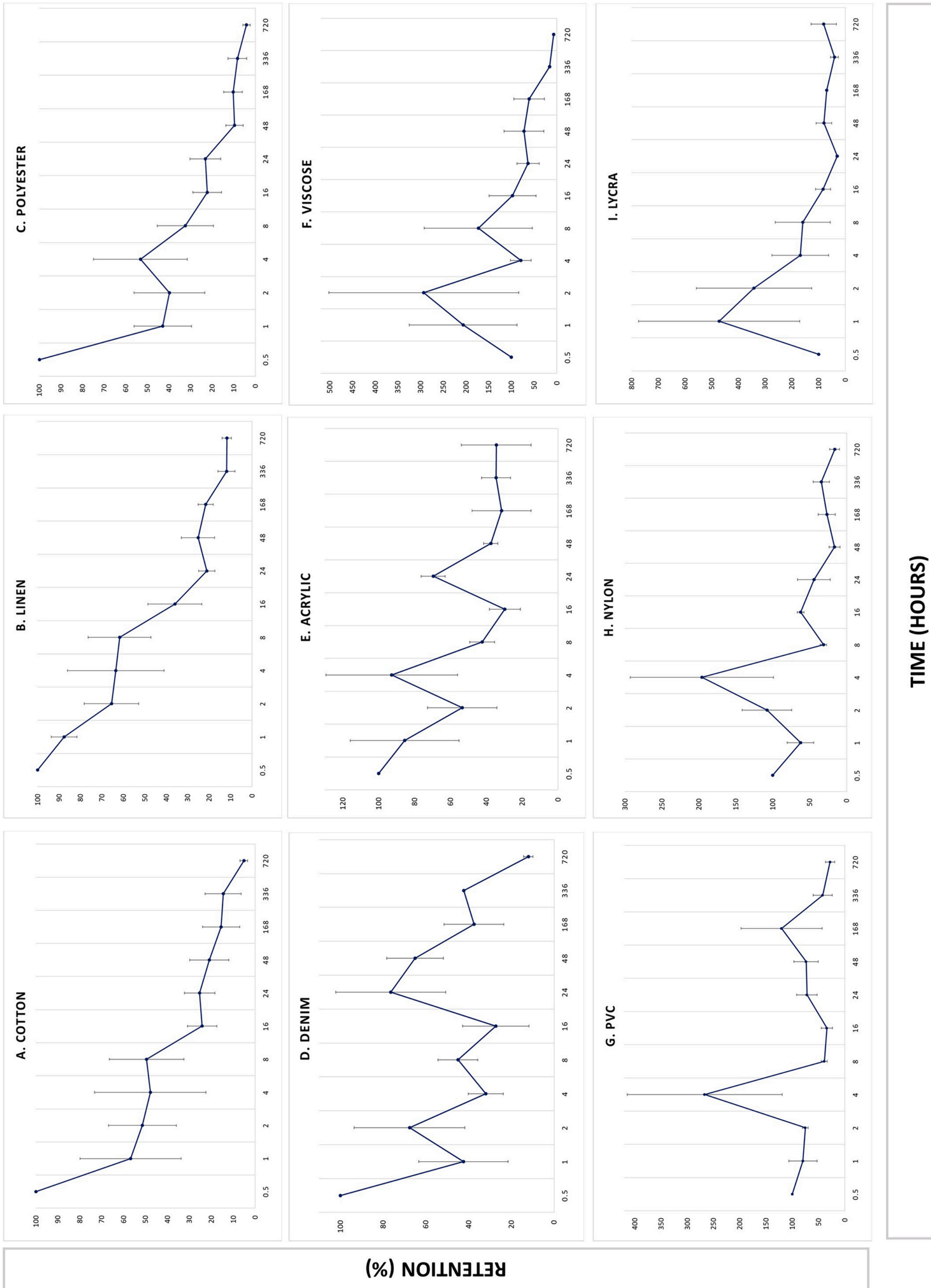


**Fig. 3.** a-i**:** The overall retention (%) of diatoms recovered from the nine clothing materials tested, *f*ollowing up to one month (720 h) of wear in the spring. Mean values are presented (n = 3) with error bars showing the standard deviation between replicates.

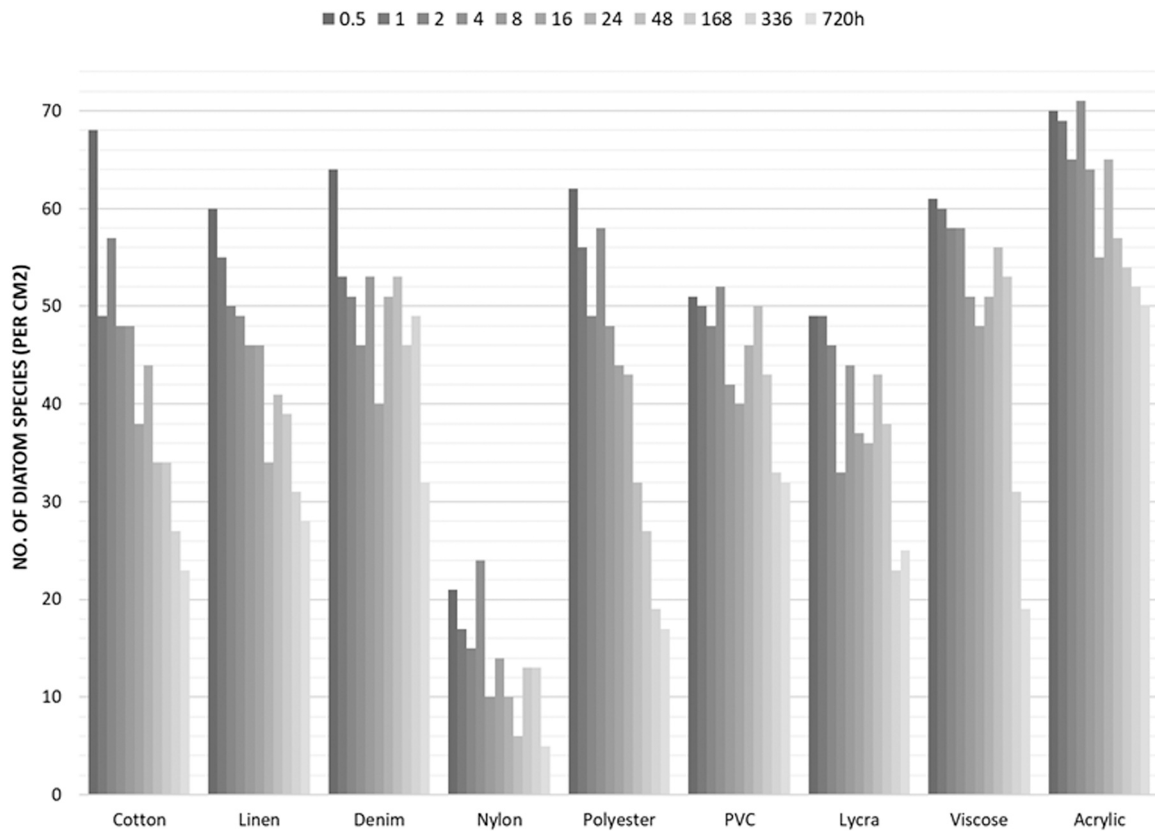


**Fig. 4.** The mean number of diatom species identified in each spring clothing persistence sample (n = 3).

Diatom retention on acrylic was the most variable between season (Fig. 5c). Although loss was relatively consistent in spring, the winter values demonstrated relative increases throughout. For example, diatom retention was greater from 1 to 168 h (141–217%) than in the initial 0.5 h assemblage and in the same interval samples in spring (32–93%). A similar abundance of diatoms was retained after 720 h of wear in both seasons – 34–35%. As with cotton and nylon, variability was also greater in winter (40–411%).

A three-way ANOVA compared diatom retention between the three clothing materials, sample seasons, and the five persistence groups (0.5–1, 2–8, 16–48, 168–336, and 720 h). There was no significant interaction between the three variables: $F$ (8, 36) = 0.559, $p$ = .804, or between each two-way comparison ($p$ > .05). There was a significant difference between clothing materials ($p$ = .003), with diatom retention significantly greater on nylon than cotton ($p$ = .001) and acrylic than nylon substrates ($p$ = .009). As in the spring, there was a significant difference between persistence interval ($p$ < .0001) with a greater proportion of diatoms retained in the 0.5–1 h and 2–8 h samples compared to all other intervals ($p$ < .05). Diatom retention was not significantly different between spring and winter ($p$ = .653).

### 3.2.3. *Species richness*

Although there was a greater species-richness in the winter controls (Table 1), fewer species were identified in the persistence samples (Fig. 6). Acrylic was the most species-rich with $\bar{x}$:16–55 *sp.* over the duration of the study. More species were lost from cotton over time ($\bar{x}$: 23–4 sp.), although a greater number of taxa were identified in the 720 h nylon samples ($\bar{x}$: 11 sp.) than in the 0.5 h assemblage ($\bar{x}$: 6 sp.). Finally, as in spring, the temporal loss of species was consistent in cotton and acrylic. In comparison, diatom species-richness on nylon was more variable over time, with more species identified in the 2 h and 720 h samples than in preceding persistence intervals.

## 4. Discussion

The results demonstrate that diatoms are retained and can be recovered as forensic trace evidence from common clothing items up to one month of wear post-immersion. The number of diatoms and overall retention (%) was affected by clothing material and persistence interval, with significantly fewer diatoms recovered beyond 8 h of wear. Although fewer diatoms were identified in the winter, retention patterns were not significantly different between sample month.

### 4.1. *General persistence trends*

Diatom retention on clothing broadly conforms with the two-stage model of decay identified by Pounds & Smalldon (1975) [26] and frequently demonstrated in subsequent particulate persistence studies [9–12,27–29] – a rapid initial loss of material followed by the

**Table 3**
The estimated mean number of diatoms identified in each November clothing sample (per cm$^2$) following each persistence interval of wear (n = 3).

| | 0.5 h | 1 h | 2 h | 4 h | 8 h | 16 h | 24 h | 48 h | 168 h | 336 h | 720 h |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Cotton** | 14 | 10 | 5 | 8 | 7 | 3 | 2 | 1 | 2 | 1 | 1 |
| **Nylon** | 118 | 177 | 107 | 103 | 144 | 7 | 0 | 44 | 55 | 22 | 114 |
| **Acrylic** | 10,774 | 3655 | 3585 | 2001 | 1286 | 1500 | 1905 | 1654 | 2030 | 531 | 335 |

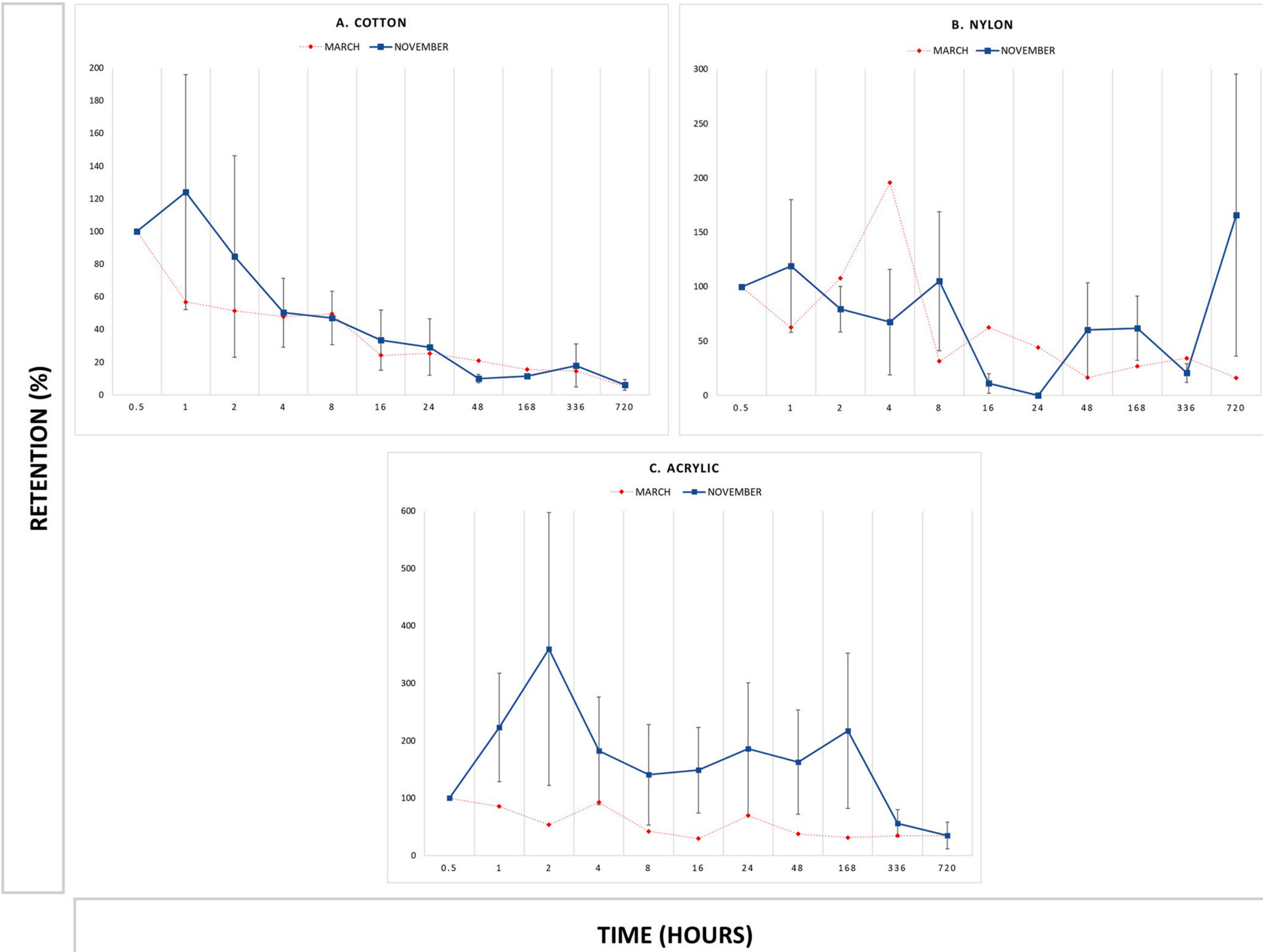


**Fig. 5.** a-c: The overall retention (%) of diatoms recovered from the three clothing materials tested following up to one month (720 h) of wear in spring and winter. Mean values are presented (n = 3) with error bars showing the standard deviation between winter replicates.

sustained presence of traces over time (Figs. 3 and 4). The findings from this study also introduce a third trend within the context of diatom persistence on several (but not all) clothing types – the relatively inconsistent loss of diatoms over time. Although fewer diatoms were always identified following weeks of wear, the number of diatoms yielded between 1 h and 48 h was often greater than in preceding samples or the initial transfer assemblage (Tables 2 and 3). This added complexity has important implications for forensic interpretations and highlights the need for additional empirical consideration of species dynamics over time [25].

#### 4.1.1. *Rapid initial loss (0–8 h)*

Across all seasonal persistence samples, fewer diatoms were identified beyond 0.5 h of wear, with a statistically significant reduction in overall retention after 8 h (Figs. 3 and 4). This reflects similar findings in glass [9], fibre [10,26,29], hair [12] and pollen [11] persistence research exploring wear over time, although most of these previous trends have not been statistically compared. For example, [10] found that more than 75% of wool and acrylic fibres transferred to a range of clothing garments were lost following up to 8–10 h of wear.

In this study, the initial loss of diatoms between thirty minutes and one hour varied substantially between the nine clothing materials, with some relative abundance increases noted in viscose and lycra (spring) and all three fabrics tested in the winter. Initial consideration of diatom persistence on footwear demonstrated a similar trend, with a greater number of diatoms recovered from leather and suede following one hour of wear than in the initial transfer assemblage [5]. Overall diatom retention for those fabrics in this and the previous study, are accompanied by high levels of variability between sample triplicates. This may indicate that diatom persistence dynamics, as with initial transfer [6], are diverse within small substrate areas (1 $cm^2$). To account for this variability and to ensure that the full potential of diatom recovery is realised in forensic casework, we recommend sampling a number of discrete subsample areas within a clothing garment using the current methodology [4], or to adopt alternative collection measures in pursuit of a larger composite sample from the whole garment [22,30].

#### 4.1.2. *Variable intermediate decay (4–48 h)*

Unlike most previous trace evidence persistence studies (including pollen [11], fibres [26], and glass [27]), the loss of diatoms from clothing was frequently inconsistent. Several intermediate time intervals (4–48 h) contained more diatoms, and subsequently a % increase in overall retention, than earlier periods of wear including the initial transfer assemblage (Figs. 3 and 4). Similar findings were reported when testing diatom persistence on footwear [5]. The three materials tested in [5] exhibited peaks in the relative abundance of diatoms after one, twelve, and thirty-six hours of wear - consistent with the persistence intervals generating variability within this study.

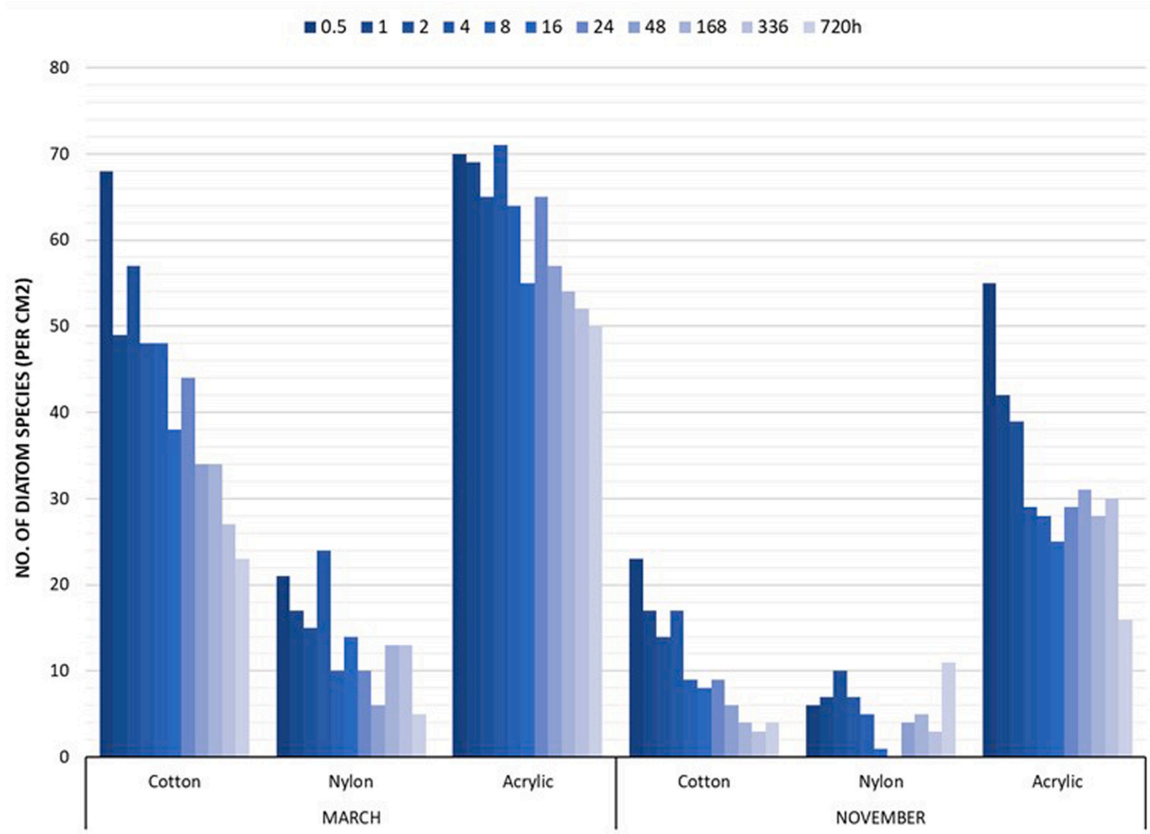


**Fig. 6.** The mean number of diatom species identified in the cotton, nylon, and acrylic persistence samples tested in the spring and winter (n = 3).

All relative intermediate peaks in diatom retention also reported increased standard deviation values (Figs. 3 and 4). This further highlights that diatom transfer and persistence dynamics are highly variable within a small subsample surface area (1 $cm^2$). Furthermore, due to the scene-based experimental approach adopted within this study and the need to chemically prepare all samples prior to analysis, separate clothing areas were subsampled following each interval of wear. Some previous environmental trace studies have seeded clothing with a known quantity of particulates before consecutive sampling of the same area [8,11]. Whilst the approach taken within this paper more closely reflects the conditions that may be encountered within a forensic case, future research may also wish to consider non-destructive collection and analysis techniques (e.g. SEM taping [22]) on the same fabric area over consecutive time points.

Intermediate variability in decay may be attributed to various factors: the reincorporation of diatoms within a clothing garment during activity, or the introduction of additional diatoms from post-crime scene environments during each experimental run. Previous research has shown that forensic indicators including pollen, UV powder, and fibres can be redistributed within a clothing garment during wear, and when machine washed or packaged as an evidential exhibit [31–34]. For example, one study demonstrated that despite shedding from the initial transfer area, pollen grains were not lost from the entire system but rather redistributed within a clothing item [31]. Although no previous study has considered diatom reincorporation, the nature of the clothing swatch tested (Fig. 2), and the relative abundance of diatoms within the intermediate sampling intervals (4–48 h), strongly suggests the need for future research to consider if and how this may impact forensic reconstructions and the recovery of environmental trace evidence from clothing.

Diatom abundance increases could also be attributed to secondary diatom transfers from subsequent environments visited during wear. The complexity of pre- and post-crime scene transfers has previously been demonstrated in relation to soil evidence [16,34,35]. One study highlighted the succession of soil quartz grains on the soles of footwear during wear, generating mixed-provenance forensic samples requiring careful analysis and exclusionary interpretation [16]. Due to the prevalence of diatoms within natural and anthropogenic environments [36] and given that the clothing in this study was worn both indoors and outdoors, the potential for secondary post-crime scene diatom transfers should not be discounted. Additional qualitative analysis of individual species persistence dynamics would offer a useful insight into the retention of the transferred assemblage and/or whether new diatom species are identified over time [25]. Further research is also recommended to assess multiple transfers of environmental traces including diatoms and pollen within forensic samples. Such empirical focus can identify potential limitations which may impact source environment inference or understanding the chronology of pertinent forensic events.

#### *4.1.3. Long-term preservation (168–720 h)*

The continued presence of diatoms following extended periods of wear (weeks-months) is encouraging for forensic reconstructions. Up to 36% (spring) and 97% (winter) of the initial diatom count was retained in the 720 h samples tested, with differences in overall retention between clothing materials (Figs. 3 and 4). Interestingly, there was no significant difference in the total diatom count recovered from the nine clothing samples after one month, although significant differences have previously been identified in the initial transfer to the same clothing surfaces [6]. This indicates that, although the transfer and early temporal dynamics of diatom persistence may vary greatly between recipient substrates, the likelihood of recovering an evidential assemblage over extended periods is relatively consistent.

Although this research accounted for a longer duration, the findings are comparable with Levin et al. [5], who reported that 10–90% of transferred diatoms were retained on footwear after one week of wear. Diatoms were more successfully retained on clothing than pollen based on findings from previous studies [8,11]. For example, less than 5% of the initial pollen transfer assemblage was consistently recovered from the six clothing items after one month of wear in [11]. This, and other, pollen persistence research has primarily considered the dynamics of individual species following laboratory-controlled transfer [8]. The experimental design of this study facilitated diatom transfer and persistence under forensic conditions at a proposed crime scene location. The abundance and diversity of diatoms within the environment may have contributed to the recovery of a more extensive diatom persistence assemblage over time, with an in-depth analysis of species composition presented in [25]. Furthermore, to ensure the findings from empirical environmental trace evidence studies are pertinent to the conditions encountered in forensic casework, future research should consider adopting an environmental (rather than laboratory controlled) approach to transfer and persistence.

As evidential exhibits including clothing, are often identified and retrieved days, weeks, or months into an investigation, it is useful to understand whether various lines of forensic enquiry are still worthwhile to pursue after such time has elapsed [37]. The findings from this study indicate that diatoms may contribute useful circumstantial trace evidence several weeks post-transfer.

### 4.2. Species richness over time

The number of diatom species recovered from the persistence samples followed a similar trend as the overall retention trends, with the fewest taxa consistently identified beyond one week of wear (Figs. 5 and 6). Despite this loss, most of the clothing materials tested in spring contained more than 15 diatom species after one month of wear. This is particularly useful for forensic reconstructions following delays in the recovery of evidence, as a species-rich evidential diatom assemblage is useful when comparing and excluding samples from a proposed transfer location [20]. Fewer species were identified in all winter persistence samples, reflecting similar trends in the transfer of a less species-rich assemblage to clothing in the winter months (Fig. 6) [6]. Subsequently, the capacity to reliably compare and exclude evidential and environmental diatom samples may be limited in the winter.

Temporal variability highlights the need for close consideration of diatom trace evidence collection, analysis, and interpretation protocols at different times of year. Extraction methods should aim to be as sensitive and non-destructive as possible, to ensure a representative species assemblage is retrieved and available for comparison to environmental samples [4]. Furthermore, interpretation of the forensic value of species information following wear over time should always maintain an exclusionary approach [38]. This could be aided further by assessment of temporal species dynamics, as in [25]. For example, if the > 15 species recovered from the 720 h persistence samples are the same as those within the control sample, this may prove useful associative evidence. If the species are different however, this may indicate the introduction of new species over time, limiting the potential for forensic comparisons to be made.

### 4.3. Impact of clothing type on persistence

Clothing type had a significant impact on the number of diatoms recovered and overall retention trends in both seasons (Tables 2 and 3, Figs. 3 and 4). As in a previous diatom transfer study, a significant interaction was identified between clothing type and seasonality on the diatom count over time, highlighting the complexity of variables affecting the spatial and temporal dynamics of diatoms as trace evidence [6]. The results correspond with those from previous studies within forensic palynology, which have also demonstrated that clothing fabric affects the temporal dynamics of pollen on clothing following simulated light and heavy wear [8] and up to one month of wear in/outdoors [11].

More diatoms, and a more species-rich assemblage, were identified in acrylic, viscose, and cotton, replicating similar findings exploring diatom transfer to clothing [6]. All three fabrics have an open weave and medium-rough surface texture, suggesting that these recipient surface characteristics can effectively retain, as well as collect, diatoms over time. Fewer diatoms and species were recovered from nylon, lycra, and PVC, with the greatest intermediate variability in overall retention (Fig. 3). This was also reflected in relation to diatom transfer [6], suggesting that a less abundant initial assemblage may lead to a less consistent decay curve over time. Subsequently, certain clothing items – those composed of acrylic, viscose, and cotton - may provide a more stable repository of diatoms as a source of trace evidence over early and intermediate investigative timescales and should be appropriately prioritised for diatom collection in casework.

Interestingly, although significant differences have been identified in the number of diatoms transferred [6] and retained over time on the nine clothing materials, no significant difference was reported in the count recovered after one month (Table 2). This indicates that variability in the initial transfer and early persistence of diatoms associated with clothing type decreases following weeks of consecutive wear. When substantial amounts of time have passed since an anticipated diatom transfer, it may be recommended to sample multiple clothing items when attempting to yield sufficient evidence to assist with environmental comparison and exclusion.

Diatoms were shed more rapidly from cotton, denim, and polyester, whilst fewer diatoms were initially lost from fabrics with an open weave and rough surface (acrylic, linen) and smooth textured materials (nylon, PVC) [6] (Fig. 3). Similar diversity relating to initial loss has been demonstrated in previous persistence studies focusing on pollen, glass, hair, and foam particles [9,11,12,28]. For example, the initial loss of pollen (0–1 h) from cotton and acrylic was much more extensive in comparison to nylon and polyester [11].

Diatoms were consistently lost from cotton, linen, and polyester with more intermediate variability identified in most of the synthetic fabric types tested (Fig. 3). This may suggest that different substrates may be more susceptible to the reincorporation of previously shed diatoms or the introduction of secondary transfers from post-crime scene environments visited over time. Although this has already been demonstrated within the context of soil [16], pollen [31], and fibre [39] persistence studies; additional research is recommended to consider the potential for multiple secondary diatom transfers to a range of clothing substrates worn over time.

### 4.4. Impact of seasonality on persistence

Although significantly fewer diatoms were retrieved in the winter persistence samples, there was no significant difference in % retention trends at different times of year (Table 3, Fig. 4). This suggests that persistence trends are relatively consistent, despite variation in the abundance of an initial transfer assemblage [6] and fluctuations in prevailing environmental conditions at different times of year.

Fewer diatoms were consistently identified in the winter persistence samples, corresponding with seasonal transfer differences [6] (Table 3, Fig. 4). This may potentially limit the exclusionary capability of diatom analysis when source environment communities are relatively less abundant [25]. The interaction between seasonality and clothing type may impact on the reliability of diatoms as temporal indicators. For example, the winter cotton persistence samples all retained fewer than 15 diatoms, although the persistence curve was relatively similar in each sample month (Fig. 4a). In contrast, the winter acrylic samples were similarly less abundant, however diatom retention trends were more variable between sample month. These findings highlight that the value of diatoms recovered as trace evidence in forensic cases should be carefully considered within the context of the case presented, including the timing of and interval since proposed freshwater contact, as well as the empirical research base [3].

Species richness after weeks of wear was lower in the winter, potentially limiting the ability to reliably compare and exclude forensic samples (Fig. 6). Temporal fluctuations in the presence and abundance of different diatom taxa has important implications for the interpretation of forensic samples, with lower species-richness often limiting forensic comparisons and exclusions with the source environment [6,38].

## 5. Conclusion

Although diatom persistence broadly conforms with the two-stage model of decay observed in other trace evidence studies [26], the loss of material over time was not consistent. Subsequently, a third retention trend (variable intermediate decay) was identified when reconstructing diatom loss from clothing and footwear [5]. The added complexity of this variability may be attributed to diatom abundance within the environment; secondary transfers may potentially incorporate diatoms acquired from post-crime scene locations visited during wear within a recovered assemblage. Further research is recommended to empirically assess the implications of such transfers during forensic reconstructions.

As with other forms of environmental trace evidence, clothing type significantly impacted diatom count and retention over time. This indicates that certain clothing items – those constructed of cotton, acrylic, and viscose – should be prioritised for diatom recovery when extensive periods of time have elapsed since proposed contact with a freshwater crime scene. Fewer diatoms were recovered in the winter persistence study, although overall retention trends were similar at different times of year. This consistency suggests that diatoms can contribute useful circumstantial intelligence when attempting to understand the temporal dynamics of evidence at different times of year. The complex interaction between clothing type and environmental variability reflects similar trends within pollen persistence research [8,11] and highlights the need for an evidential diatom assemblage to be carefully considered within the context of the individual crime investigation and the supporting empirical evidence base.

Finally, the data generated in this study highlights, for the first time, that diatoms can be retained and retrieved from different clothing items following brief (hours) and extended (weeks) periods of wear post-immersion. The persistence of multiple species over time is especially promising and generates additional opportunity to further scrutinise the temporal dynamics of diatoms as a form of trace evidence. This paper has initiated the development of the empirical evidence bases required to support the exclusionary interpretation of diatom trace evidence over pertinent investigative timescales with multiple avenues for additional future research highlighted.

## CRediT authorship contribution statement

**K.R. Scott:** Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **V.J. Jones:** Conceptualization, Methodology, Writing - review & editing, Supervision. **N.G. Cameron:** Conceptualization, Methodology, Writing - review & editing, Supervision. **J.M. Young:** Methodology, Writing - review & editing. **R.M. Morgan:** Conceptualization, Methodology, Writing - review & editing, Supervision.

## Declaration of Competing Interest

The authors declare no competing information or conflicts of interest.

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