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The impact of environmental variability and clothing type on the transfer of marine diatoms as trace evidence indicators in coastal locations

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ABSTRACT

Diatoms are a species-rich group of microalgae, abundant in freshwater, marine, and soil environments. Subsequently, they may be used as a form of trace evidence, reconstructing links between persons and places involved in crime. Despite previous studies demonstrating the forensic value of diatoms in freshwater and soil habitats, no previous research has explored their transfer and persistence dynamics in marine environments. This study therefore aimed to assess diatom transfer to clothing following immersion in five coastal and one freshwater location, offering a novel contribution to the rapidly expanding empirical research base within forensic ecology. Experiments were designed to consider the impact of recipient surface characteristics, environmental variability, and diatom valve characteristics (morphology and species-specific features) on the abundance, species richness, and whole assemblage composition of marine diatoms recovered from clothing. Three common materials – cotton, polyester, and acrylic – were submerged in two marine lakes, two tidal beaches, and one estuary along the Merseyside coast (NW England), and one inland pond to provide a freshwater comparison. Diatoms were recovered from all environmental and clothing transfer samples using a H_2O_2 extraction protocol before microscopic analysis including species identification. The results demonstrate significant differences in the diatom communities present within the six environmental samples, with multiple site-specific marker taxa and a variable species assemblage between coastal sites. Clothing type and environmental variability significantly influenced the abundance, species richness, morphology, and species composition of marine diatoms transferred to clothing. Notably, the whole species assemblage retrieved from each subset of transfer samples demonstrated relative similarity to the corresponding environmental control sample, supporting forensic comparisons and exclusions with questioned sites. These findings highlight that marine diatoms offer useful circumstantial trace evidence indicators during crime reconstructions involving coastal environments. Furthermore, and similarly to the findings of freshwater research, care must be taken when interpreting marine diatoms as trace evidence indicators in forensic investigations. The complexity of variables influencing the abundance and representativeness of a diatom sample transferred to clothing necessitates exclusionary interpretations to ensure robust and reliable conclusions of evidential significance.

1. Introduction

Environmental trace evidence analysis offers a valuable approach to forensic investigations, particularly when seeking to compare and exclude suspect and crime scene samples, or when profiling an unknown location of forensic interest [1]. An array of environmental indicators have been considered as trace evidence including soil quartz grains [2], fungi [3], pollen [4], microbial communities [5], and diatoms [6]. Examination of environmental materials is frequently documented within casework and, increasingly, within the empirical research base, with particular focus on evidential transfer and persistence dynamics [7,8]. Such experimental approaches are critical to ensure the development of robust frameworks required to support forensic interpretations within the criminal justice system [9].

Diatoms (*Bacillariophyceae)* are an abundant and species-rich group of eukaryotic unicellular microalgae [10]. They have a chemically resistant silica (SiO_2) cell wall which retains species-specific features, facilitating microscopic identification. Diatoms can initially be classified

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according to valve shape – either centric (radially symmetric and circular) or pennate (bilaterally symmetric and elongated) [10], before more detailed scrutiny of species characteristics. Diatoms range in size from 2 to 500μm and are found in most aquatic (e.g., freshwater, marine, estuarine) and some damp terrestrial habitats including soils [11]. Diatom populations and the species assemblage present within a site are influenced by abiotic conditions including temperature, pH, salinity, light, nutrient and silica availability [12]. Due to these characteristics, diatoms offer useful forensic intelligence to support diagnoses of death by drowning in pathology [13], and to reconstruct links between scenes and persons of forensic interest as a form of circumstantial trace evidence [6].

Previous research has focused predominantly on freshwater and soil diatoms as trace evidence indicators [14–19]. For example, Scott et al. [14] identified that diatoms transfer to clothing following as little as three minutes of submersion in freshwater environments and one minute of contact with soil surfaces. Additional studies have identified that thirty seconds of contact is sufficient to facilitate freshwater diatom transfer to footwear materials [15] and to socks immersed in a river, drainage ditch, and swamp [16]. Furthermore, recipient surface characteristics (e.g., clothing/footwear type), seasonality, spatial variability, valve morphology and species-specific features have all been shown to support or limit diatom transfer and persistence in freshwater habitats [17–19]. For example, Bogusz et al. [16] reported that diatom communities vary considerably within close-proximity freshwater sites, with such variability reflected in the assemblage transferred to immersed objects which may be encountered as forensic evidence. Finally, diatom extraction from clothing and footwear materials using a hydrogen peroxide (H_2O_2) method has been effective in the recovery of an abundant and species-rich assemblage for analysis in both freshwater and soil contexts [14].

Despite marine environments, particularly coastal habitats, frequently being encountered within crime investigations [20], very little research has sought to explore the value of environmental trace evidence, including diatoms, within such contexts. One study by Magni et al. [21] considered marine diatom transfer to clothing, however, was limited to a laboratory-controlled environment and the use of a single species - *Chaetoceros muelleri.* Their research identified that fabric type influenced the extent of transfer and that the H_2O_2 method previously described was the most effective approach for *C. muelleri* recovery from clothing [21]. To expand upon these initial findings, further, more extensive, investigations are warranted to consider diatom trace evidence dynamics (transfer and persistence) in forensically relevant marine environments.

Diatom populations inhabiting marine ecosystems retain ecological and morphological differences compared to freshwater taxa [22], emphasizing the importance of forensic research within such environments. Initially, marine diatoms have greater tolerance for salinity, with differences reported between individual species [23]. Greater salinity is also reported to reduce diatom growth and significantly impact valve morphology [24]. Further studies demonstrate that marine diatoms have less silica [25], are less species-rich [26], comprise more centric taxa [27], and have a diverse range of growth habits (solitary v. colonial cells) [28] due to spatial variability within marine ecosystems. Additionally, marine populations have fewer physical barriers to migration, with broader geographic distributions reported than in freshwater environments [29]. The forensic implications of these environmental traits are currently unknown, highlighting the importance of structured studies to address this research gap. This is especially important given previous freshwater research has identified diatom valve morphology and species-specific features significantly affect their transfer to clothing [17], and that whole species assemblage dynamics are useful to support comparisons and exclusions in freshwater and soil forensic contexts [14,16,17].

1.1. Aims and objectives

This study aims to assess the impact of clothing type and environmental variability on the transfer of marine diatoms as trace evidence indicators. Reflecting similar experimental approaches within forensic ecology research [8,17,21], three different clothing materials were immersed in five coastal environments across Merseyside (UK). A sixth freshwater location was also incorporated to provide an environmental comparison. Control samples were collected from all sites to determine how representative the transferred diatom assemblage was to the source environment. Quantitative and qualitative analysis was employed to determine whether:

- The total number of diatoms, the species richness, and the whole species assemblage was significantly different between five regional coastal locations (tidal beaches, marine lakes, estuary) in north-west England.
- Environmental patterns were reflected in the abundance, species richness, and assemblage dynamics of marine diatoms transferred to clothing following submersion.
- Marine diatom transfer to clothing was significantly impacted by material type (cotton, polyester, acrylic).
- Marine diatom transfer to clothing was impacted by valve morphology (size, shape, integrity) and species-specific features.
- Trends in marine diatom transfer were consistent with those reported in a freshwater location.
- The H_2O_2 extraction technique initially outlined in Scott et al. [14] can effectively retrieve marine diatoms from different clothing materials.

2. Methods

Ethical approval was not required in accordance with LJMU Faculty of Science guidance regarding observational work in the field. Relevant permissions were sought in advance of field sampling.

2.1. Sample materials

Three common clothing materials were used to explore transfer, reflecting the diversity of recipient surfaces encountered in casework [30] and the materials used in previous environmental trace evidence research [8,15,17]. Cotton, acrylic, and polyester were chosen due to their different surface textures and weave characteristics [Table 1]. All clothing was new (unused but washed), with 5 cm^2 swatches removed from the whole garment which were then used to assess diatom transfer within each questioned environment.

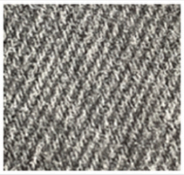
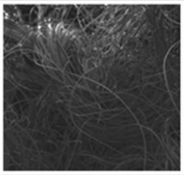
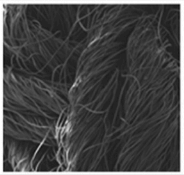
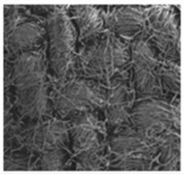
2.2. Sample collection

Similarly to freshwater studies [17], the experimental design sought to replicate the circumstances which may accompany a forensic event in or around coastal environments. Five marine and one freshwater site were sampled along the Liverpool and Wirral coastlines (Merseyside, UK), encompassing an 8.7-mile radius [Fig. 1].

Two locations – Crosby (Fig. 1a; National Grid Ref: SJ 31334 97999) and West Kirby (Fig1d; National Grid Ref: SJ 21024 86448) – are marine lakes, frequently used for recreation. One site, Speke & Garston, is an upstream estuarine site along the River Mersey (Fig1c; National Grid Ref: SJ 41228 82460). The final two marine locations are dynamic tidal beaches – New Brighton, located at the Mersey outflow to the Irish Sea (Fig1b; National Grid Ref: SJ 31252 94137), and Thursaston, at the mouth of the Dee estuary (Fig. 1e; National Grid Ref: SJ 22978 83931). The freshwater sample was collected from a pond (Fig. 1f; National Grid Ref: SJ 23793 83449) within parklands inland at Thursaston Common.

Sampling was conducted in late Spring (May 2023) at high or incoming tide. All clothing materials were fully submerged for three

Table 1
Description and images (1x and 100x magnification) of the three clothing samples used within this study. Scanning Electron Microscope (Emitech K550X) was used to capture the micrographs.

	Polyester	Acrylic	Cotton
Photograph (1x)			
SEM Image (100x)			
Fibre Type	Synthetic	Synthetic	Natural
Material Composition	100% Polyester	100% Acrylic	100% Cotton
Surface Characteristics	Pile weave rough texture	Knit weave rough texture	Twill weave smooth-medium texture

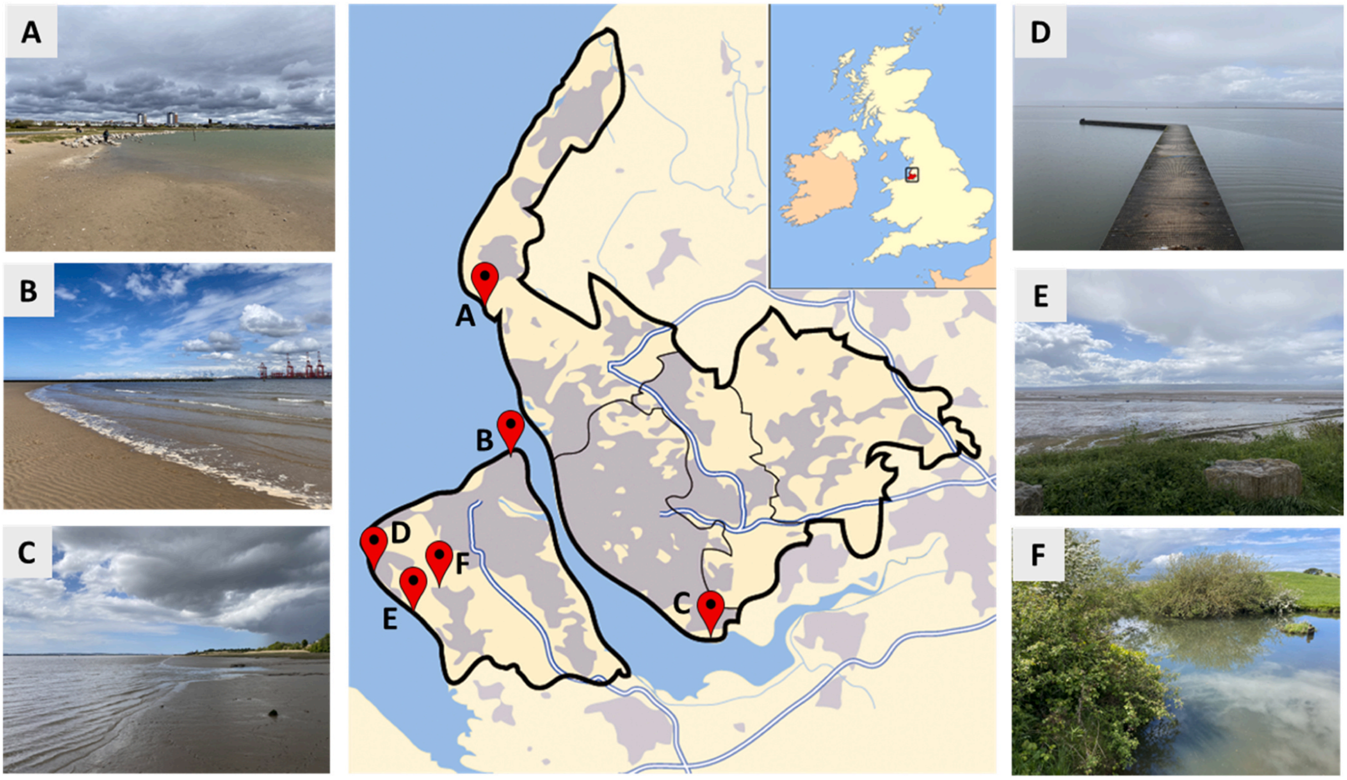


Fig. 1. Map of the five coastal and one freshwater site used to assess diatom transfer and environmental variability in this study. Sites are: Crosby marine lake (A), New Brighton tidal beach (B), Speke & Garston estuarine site (C), West Kirby marine lake (D), Thursaston tidal beach (E), and the freshwater pond in Thursaston Common parkland (F).

minutes to facilitate diatom transfer at each site. Previous research has shown that even brief periods of environmental contact such as this can result in an abundant and species-rich transfer assemblage [17]. During immersion, the water was agitated through walking, replicating movements that may take place during a forensic event. Post-transfer, each individual fabric swatch was immediately double bagged and stored (5°C) prior to laboratory analysis. Reference water samples (500 ml) were also collected following disturbance of the water as described.

2.3. Laboratory protocol

Diatoms were extracted from three 1 cm^2 subsamples for each clothing material and from three 20 ml water replicates for each site using the hydrogen (H_2O_2) method outlined by Scott et al. [14]. Eight additional 'blank' samples were prepared using distilled water to test for contamination during the lab process. Briefly, each sample was added to a pristine centrifuge tube with 20 ml of H_2O_2 (30 %) and heated in a water bath for 4 hours at 80°C. Fabric subsamples were then removed, solutions rinsed with distilled water, and a few drops of HCl (10 %) added. All samples were rinsed and centrifuged four times (1200 rpm, 4 minutes) with the final supernatant discarded to 5 ml, leaving a diatom pellet in solution.

Microscope slides were created using 500 µl of each sample solution transferred to a 19 mm glass coverslip. A second dilution of each sample was also prepared in case required for analysis. Following evaporation, each coverslip was inverted and mounted onto a microscope slide using Naphrax™ and heated at 130°C for 35 minutes.

2.4. Analysis

All slides (n = 80) were examined using light microscopy at 1000x magnification (Olympus, CX21FS1). All diatoms from a known area of the coverslip were counted and recorded, initially characterised according to their integrity (whole/fragmented). Whole diatoms were subsequently categorised according to their shape (centric/pennate) and size (<10µm/>10µm), before genus and species identification using taxonomic keys [31–36].

Results from the coverslip area studied were standardised to calculate an estimated total (whole) diatom count for each sample (per ml/per cm^2) [17,37]. Quantitative analysis involved comparisons of diatom abundance and species richness in the environmental and transferred forensic samples. Statistical analysis was performed in SPSS v. 27 using a 95 % confidence interval. Qualitative analysis compared the morphological composition of the diatoms present in each sample (by size, shape, and integrity) before consideration of species assemblage dynamics including the presence of abundant and less common taxa, the frequency of individual diatom species across the six questioned environments, and the relative similarity between all clothing and environmental samples' species assemblage. Species composition data was statistically compared using canonical correspondence analysis performed in C2 v.1.8.

3. Results

3.1. Total number of diatoms

No diatoms were present in the blank samples prepared to test for contamination. In the environmental samples, Thursaston beach (Mean ± Std Deviation: 4110 ± 514 valves/ml) and the Speke & Garston estuary (3172 ± 747/ml) were most abundant [Fig. 2]. Fewer diatoms were identified in both West Kirby (1802 ± 819/ml) and Crosby (698 ± 147/ml) marine lakes and the New Brighton tidal sample (304 ± 44/ml). The freshwater comparison comprised the lowest number of diatoms (144 ± 61/ml) of all locations sampled.

Clothing reported a greater diatom abundance following exposure to Thursaston beach (1253 ± 520 – 5494 ± 1651/cm^2) [Fig. 3]. Fewer diatoms were identified following contact with the freshwater pond (37 ± 7–59 ± 34/cm^2) and New Brighton (28 ± 14–107 ± 25/cm^2). Cotton comprised the lowest number of diatoms in all marine locations, ranging from 28 ± 14/cm^2 (New Brighton) to 1253 ± 520/cm^2 (Thursaston) [Fig. 3]. Acrylic reported the most abundant diatom assemblage at Speke & Garson (1374 ± 253/cm^2) and Thursaston (5494 ± 1651 cm^2), whereas polyester yielded more diatoms in the West Kirby (542 ± 259/cm^2), Crosby (258 ± 35/cm^2), New Brighton (107 ± 25/cm^2), and freshwater (59 ± 34/cm^2) samples [Fig. 3].

3.1.1. Statistical analysis

A one-way ANOVA identified a statistically significant difference in the total number of diatoms identified within the six environmental samples – *F (5, 18) = 31.629, p = .001*. Post hoc Tukey tests reported that the diatom assemblage identified at Speke & Garston, Thursaston beach and West Kirby marine lake was significantly greater when each

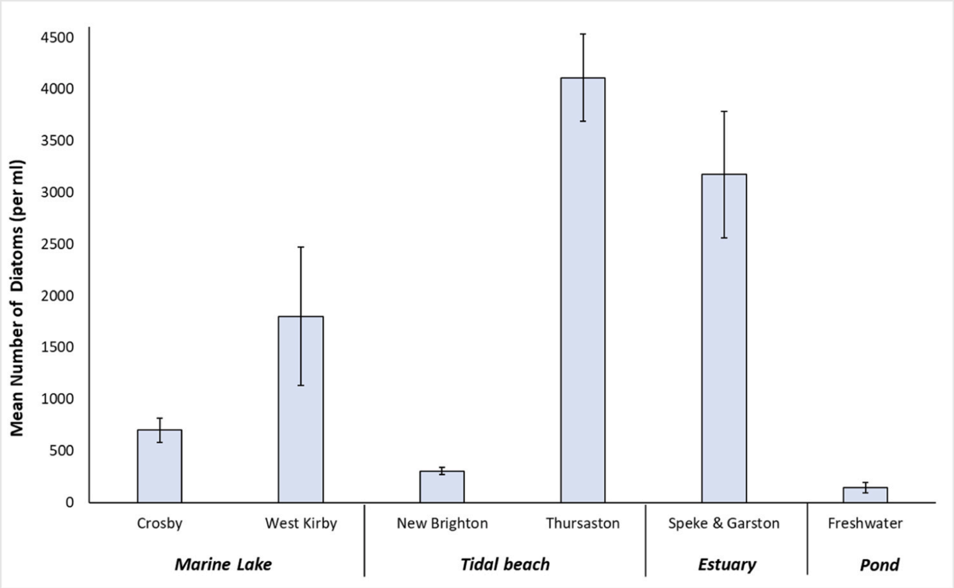


Fig. 2. The estimated total number of diatoms identified in each coastal and freshwater environmental sample (per ml) (mean ± std deviation, n = 3).

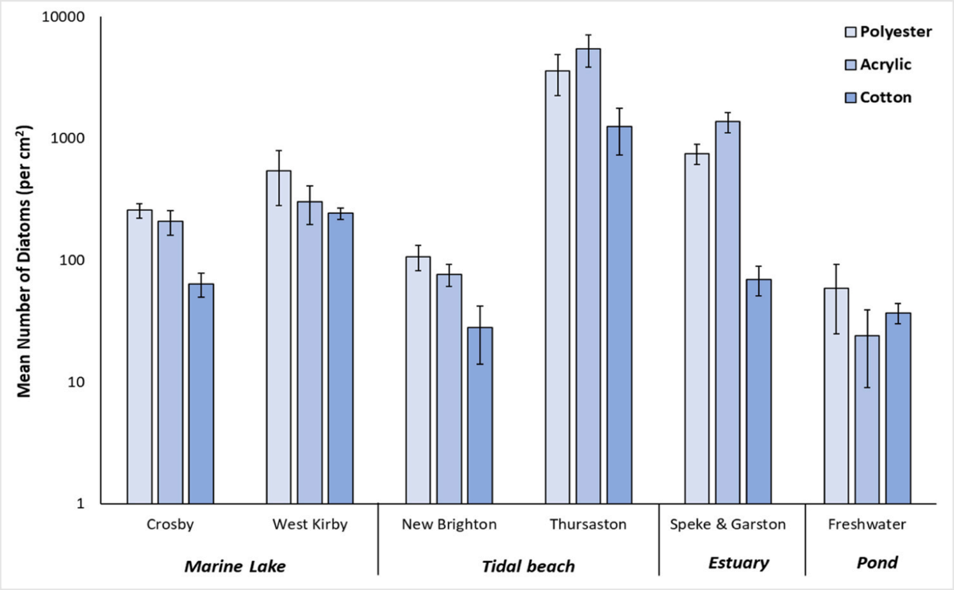


Fig. 3. The estimated total number of diatoms transferred to each clothing material (per cm^2) following submersion in each coastal and freshwater environment (mean ± std deviation, n = 3).

site was compared with New Brighton, Crosby, and the freshwater pond ($p < .05$) [Fig. 2]. No significant differences were identified between any other pairwise comparisons ($p > .05$).

A two-way ANOVA identified a statistically significant interaction between clothing type and sampling location on the total number of diatoms – $F\ (10,\ 54) = 5.171,\ p = .001$. A significant difference was identified between clothing type ($p = .001$); a post-hoc Tukey test reported significantly fewer diatoms transferred to cotton than acrylic ($p = .001$) and polyester ($p = .021$) [Fig. 3]. Differences between acrylic and polyester were not significant. A significant difference was also identified between sampling location ($p = .001$); a post-hoc Tukey test identified significantly more diatoms transferred to clothing sampled at Thursaston compared to all other sites ($p < .05$). No other significant differences were observed between sites ($p > .05$).

3.2. *Total number of species*

More diatom species were identified in the Thursaston beach (97 ± 4 *sp.*/ml) and West Kirby marine lake (70 ± 5/ml) samples compared to all other sites. The lowest species richness was reported in the freshwater pond (13 ± 3/ml). Amongst the coastal locations, Crosby (28 ± 3/ml) and New Brighton (28 ± 2/ml) contained the fewest diatom taxa [Fig. 4].

More species were also identified in the clothing samples immersed at Thursaston (52 ± 8–68 ± 6/ml) than any other site. Crosby (8 ± 2–22 ± 1/ml) and New Brighton (2–14 ± 1.6/ml) similarly comprised the lowest species richness amongst the coastal transfer samples [Fig. 5]. Fewer species were consistently recovered from cotton (2 ± 1–52 ± 8/ml) compared to polyester (5 ± 1–68 ± 6/ml) and acrylic (3 ± 1–69 ± 4/ml). Greater species richness amongst the polyester and acrylic samples reflected the initial diatom abundance trends

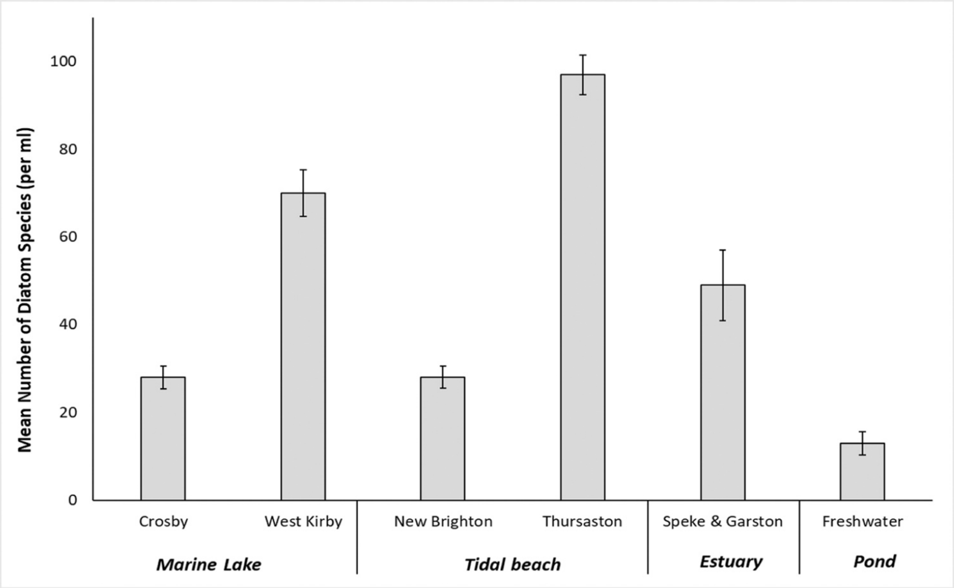


Fig. 4. The estimated number of diatom species present (per ml) within each of the coastal and freshwater environments sampled (mean ± std deviation, n = 3).

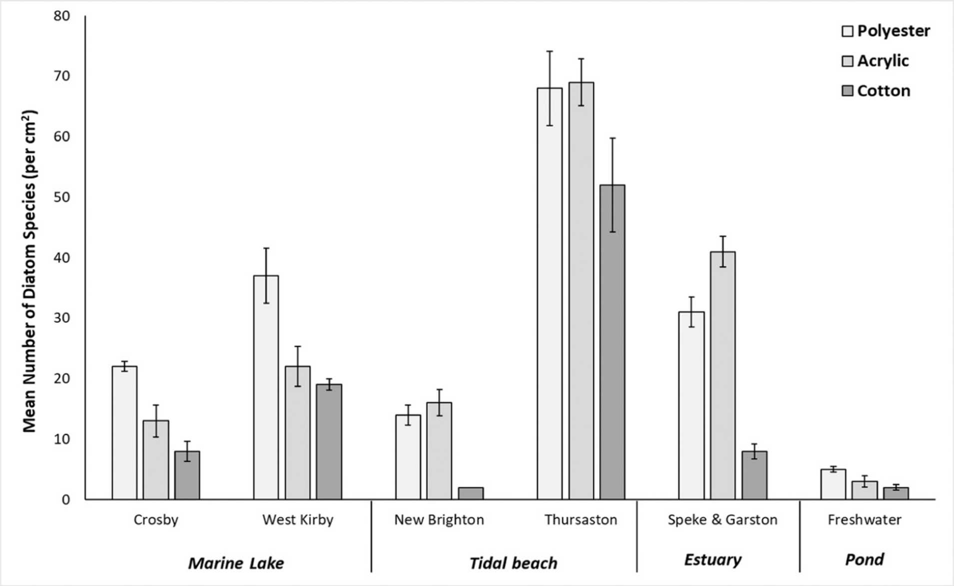


Fig. 5. The estimated number of diatom species transferred to each clothing material (per cm^2) following submersion in each coastal and freshwater environment (mean ± std deviation, n = 3).

[Fig. 3, Fig. 5].

3.3. Diatom assemblage I – morphology

Diatoms were initially identified according to their valve characteristics – shape (pennate or centric), size (greater or smaller than 10µm), and integrity (whole or fragmented) [17].

3.3.1. Diatom shape

Except New Brighton, most of each environmental sample comprised pennate taxa (59–86 %) [Fig. 6a]. The transfer samples closely reflected this distribution in West Kirby (73–81 %), Thursaston (86–91 %), and Speke & Garston (53–63 %). Greater variability was identified between the Crosby (59–93 %) and freshwater (15–88 %) samples. New Brighton was the only control environment to report more centric diatoms (57 %) than pennate [Fig. 6a]. The corresponding transfer samples demonstrated greater variability, ranging from 43 % (polyester) to 93 % (cotton) centric taxa. Greater consistency amongst the different clothing materials was identified in West Kirby, Thursaston, and Speke & Garston, compared to the other three sampling locations.

3.3.2. Diatom size

Most of the Crosby (87 %), West Kirby (89 %), Thursaston (62 %), and Speke & Garson (70 %) environmental sample assemblage comprised larger (>10µm) diatom taxa [Fig. 6b]. Smaller diatoms (<10µm) were more abundant in New Brighton (67 %) and the freshwater samples (53 %). Clothing transfer samples in Crosby (80–91 % >10µm) and West Kirby (89–91 % >10µm) demonstrated the most consistency with the corresponding site controls, and between different clothing materials. The proportion of larger taxa in the Thurstaston (43–49 %) and Speke & Garston (42–62 %) transfer samples was lower than in each of the environmental samples' assemblage [Fig. 6b]. Greater variability was reported in the New Brighton and freshwater pond samples – for example, whilst 47 % of the freshwater sample comprised larger diatoms, less than 19 % of each transfer assemblage comprised such taxa.

3.3.3. Diatom integrity

Most of the environmental control samples comprised whole diatom valves (58–91 %), with a greater proportion of fragments reported in the freshwater and Speke & Garston samples [Fig. 6c]. The clothing associated with all five coastal sites reported more fragmented diatoms, with the most identified in West Kirby marine lake (89–93 %) and the Speke & Garston estuary (75–88 %). Clothing immersed at Crosby (46–78 %) and the freshwater pond (73–90 %) had more in-tact diatoms than all other clothing samples. The proportion of whole versus fragmented diatom valves was relatively consistent amongst the three clothing types in most of the transfer sites tested. For example, 29–40 % (New Brighton), 44–52 % (Thursaston), and 7–11 % (West Kirby) of each clothing assemblage comprised of whole taxa [Fig. 6c].

3.4. Diatom assemblage II – species composition

3.4.1. Environmental samples

A total of 214 diatom species were identified across all samples analysed [Supplementary Data]. Amongst the six environmental samples, only one species – *Melosira sp.1* – was consistently identified in all locations, accounting for 3.48–46.06 % of each mean species assemblage [Fig. 7, Fig. 8]. *Cocconeis sp. 4* [Fig. 10d] was identified in all five coastal samples (absent from freshwater) with mean abundance ranging from 1.32 % (Crosby) to 18.79 % (New Brighton). Six taxa, including *Navicula tripunctata* (0.26–0.99 %), *Roperia tesselata* (0.18–2.42 %), and *Cymatosira sp. 1* (0.2–0.94 %), were common to four of the five coastal sites analysed – absent from either Crosby or New Brighton, as well as the freshwater pond. A further eight diatom species were identified in three of the environmental control samples (including *Actinoptychus senarius, Surirella sp. 1,* and *Thalassiosira sp.2*), with 37 taxa found in two of the six sample environments [Fig. 7, Supplementary Data]. Additionally, a silicoflagellate species was identified in both the Crosby marine lake (0.04 %) and Speke & Garston (0.35 %) estuarine samples.

Most diatom species (n = 150) were identified in one location only, providing environmental markers for the individual sample sites. Thursaston beach reported the highest number of site-specific taxa, with 54 diatom species including *Amphora coffaeformis, Biremis ambigua, Catenula adhaerans, Navicula humerosa,* and *Rhabdonema minutum* (Fig. 7). West Kirby was characterized by 47 marker taxa including *Coscinodiscus sp. 2, Licmophora sp. 1, N. restituta, Scoliopleura sp. 1,* and *Rhopoladia sp. 1*. Nineteen species, including *Dimeregramma sp.2, Delphineis Surirella*, and *Rhaphoneis amphiceros* were identified only in the Speke & Garston estuarine site [Fig. 7]. Crosby (n = 13) and New

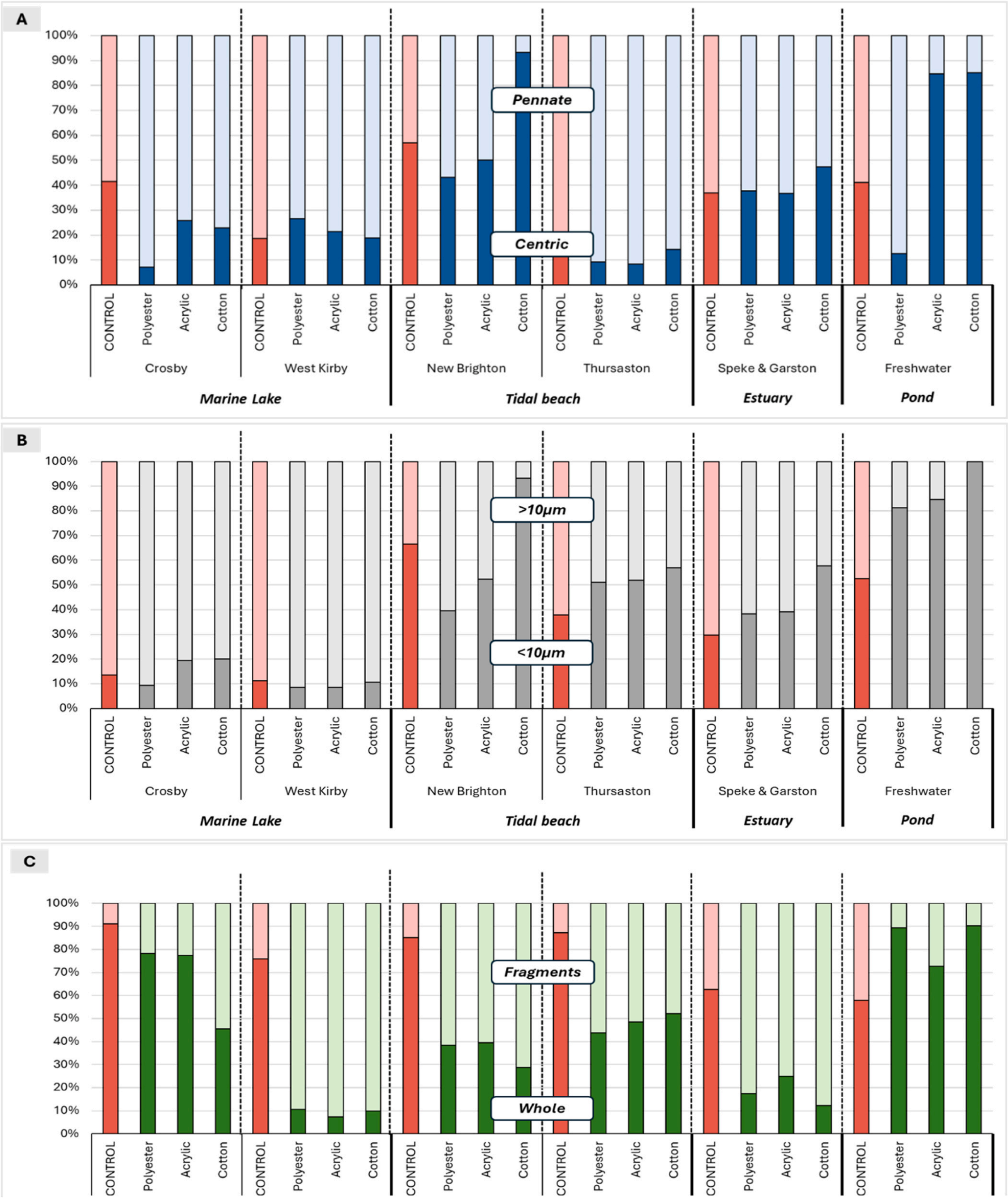


Fig. 6. The proportion of diatoms recovered from each environmental control and corresponding clothing transfer sample according to: A. shape (centric/pennate), B. size (</>10µm), and C. integrity (whole/fragmented). The top label refers to top colour of each bar chart (e.g., pennate, >10µm, fragments) and the lower label corresponds with the lower colour of each chart (e.g., centric, <10 µm, whole). Observations are a mean of three replicates.

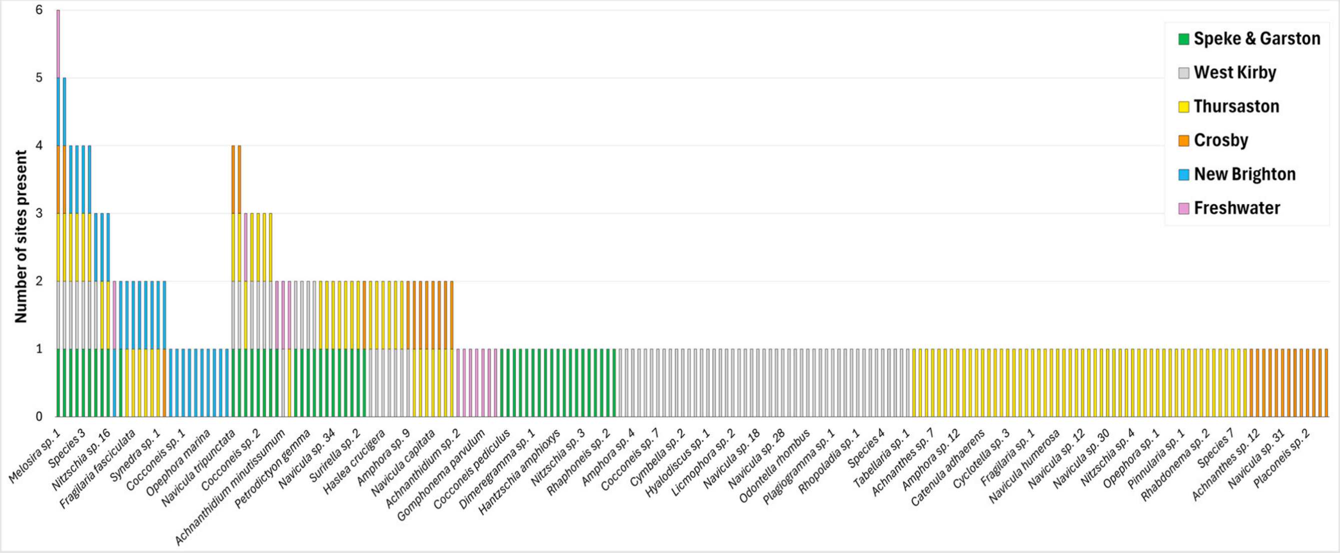


Fig. 7. The frequency that each diatom species was identified within the six environmental samples analysed. Information on the relative abundance of the taxa within each site can be found in Supplementary Data.

Brighton ($n = 10$) comprised fewer characteristic species, with the lowest number identified in the freshwater pond ($n = 7$) where species including *Cyclotella meneghiniana, Cymbella sp. 1,* and *Achnanthidium sp. 2* were exclusively identified [Supplementary Data].

3.4.2. Transfer samples

The most abundant species within each environment, were also identified within most or all corresponding clothing samples [Fig. 8]. For example, *Melosira sp. 1* was present in all environmental (3.48–46.06 %) and clothing samples (4.28–92.12 %). Within the individual sites, relatively abundant species including *Licmophora sp. 1* (12.27 % West Kirby), *Surirella sp. 1* (21.38 % Thursaston), *Thalassiosira sp. 1* (24.55 % Crosby), and *Navicula gregaria* (18.82 % Speke & Garston), were consistently identified in the corresponding clothing transfer samples [Fig. 8]. Additional less common species were also consistently identified within all environmental and clothing samples including *Achnanthes sp. 7* (Thursaston), *Nitzschia sp. 16* (Speke & Garston), *Caloneis amphisbaena* (Crosby), *Hyalodiscus sp. 1* (West Kirby), and *Navicula subforcipata* (New Brighton).

Greater variability was reported in the presence and relative abundance of less common species (<5 %). Several taxa identified in the individual environmental sample assemblages were absent from any corresponding clothing samples including *Achnanthes brevipes var. intermedia,* (0.1 % West Kirby), *Placoneis sp. 1*, (5.24 % Speke & Garston), *Coscinodiscus radiatus* (0.04 % Thursaston), *Opephora sp. 4* (0.26 % Crosby), *Actinocyclus sp. 1* (4.24 % New Brighton), and *Nitzschia dissipata* (0.61 % freshwater) [Fig. 8]. Additional species were identified only in the transfer samples; for example, *Achnanthes sp. 4* (West Kirby polyester), *Diademoides luxuriosa* (Thursaston polyester), *Plagiogramma sp. 2* (Speke & Garson polyester), and *Achnanthes sp. 10* (New Brighton polyester, acrylic) were missing from the corresponding environmental samples [Supplementary Data].

The presence and relative abundance (%) of environmental taxa in the corresponding transfer samples was more consistent in some sampling locations than others. For example, many of the species identified in Thursaston, West Kirby, and/or Speke & Gaston, including *Amphora sp. 5, Cocconeis sp. 4, Hyalodiscus sp. 1, Nitzschia sp. 16, Species 4,* and *Tryblionella sp. 1,* were present in all four samples species assemblage [Fig. 8]. In comparison, the New Brighton and freshwater samples were less consistent. Several taxa including *Biremis sp. 1, Cocconeis sp. 4* and *sp. 5, Plagiotropis sp. 2, Stauroneis sp. 1* (New Brighton), *Navicula sp. 4, Nitzschia sp. 5,* and *Synedra rumpens* (freshwater), were identified in one or two clothing samples only [Supplementary Data].

Finally, variability in species representation was also reported amongst the different clothing samples. Taxa identified in the environmental control samples were more frequently and consistently recovered from polyester and/or acrylic. For example, *Achnanthidium minutissimum* (a freshwater taxa)*, Cyclotella sp. 1, Rhaphoneis amphiceros* (Speke & Garston), *Hyalodiscus sp. 2* (West Kirby), *Nitzschia sp. 7* (Thursaston), *Thalassiosira sp. 3* (New Brighton) and *Roperia tesselata* (Speke & Garson, West Kirby, Thursaston), were all absent from the assemblage transferred to cotton [Fig. 8]. Conversely, a small number of species retrieved from the cotton clothing were absent from either polyester or acrylic including *Coscinodiscus sp. 1* (Speke & Garston), *Navicula sp. 29* (West Kirby), *Navicula tripunctata* (Thursaston), and *Achnanthidium minutissimum* (freshwater).

3.4.3. Multivariate analysis

Variability in the overall species assemblage was explored using canonical correspondence analysis [Fig. 9]. The proximity of data points demonstrates the relative similarity between the whole species assemblage of each sample. Initially, West Kirby and Crosby marine lakes demonstrated the greatest variability from all other samples, driven by the relative abundance of taxa including *Scoliopleura tumida, Licmophora sp. 1, Podosira sp. 1* (West Kirby), *Thalassiosira sp. 1,* and *Navicula sp. 6* (Crosby) [Fig. 9]. The clothing samples immersed at both West Kirby and Crosby demonstrate greater similarity in their diatom species assemblage to the respective environmental sample than any other location. Whilst the cotton, acrylic, and polyester species profiles were relatively consistent for West Kirby, the Crosby polyester sample showed more similarity to the environmental control assemblage. Variation between cotton and acrylic was driven by species including *Caloneis amphisbaena* and *Opephora sp. 4.*

The New Brighton, Thurstaston, and Speke & Garston samples species assemblage were relatively similar compared to the other sites [Fig. 9, Cluster A]. Closer scrutiny revealed subtle differences between those locations and the associated transfer samples, however. For example, the New Brighton samples can be distinguished due to the abundance of taxa including *Actinocyclus sp. 1., Opephora marina,* and *Plagiotropis sp. 2* [Fig. 9a]. The cotton transfer assemblage was more similar to the environmental control than polyester or acrylic. Variability within the Thursaston samples was driven by the relative abundance of taxa including *Catenula adhaerans, Plagiogramma sp.3,* and *Amphora sp. 12.* The water samples collected from Thursaston and Speke

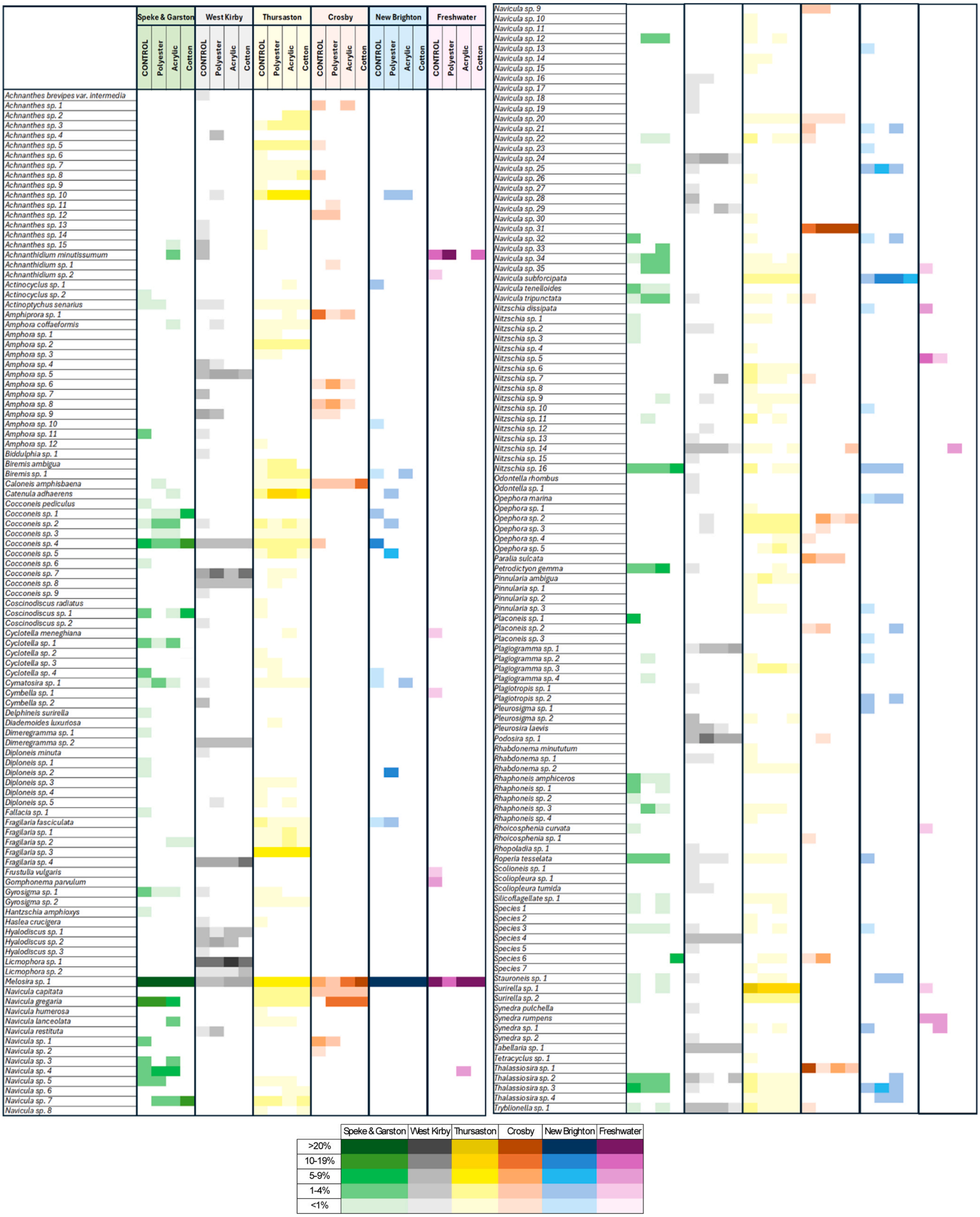


Fig. 8. The presence and mean relative abundance (%) (n = 3) of each species identified within all environmental and clothing (polyester, acrylic, cotton) samples tested. Greater abundance is demonstrated with darker colours. Greater resolution and additional species information can be accessed in Supplementary Data.

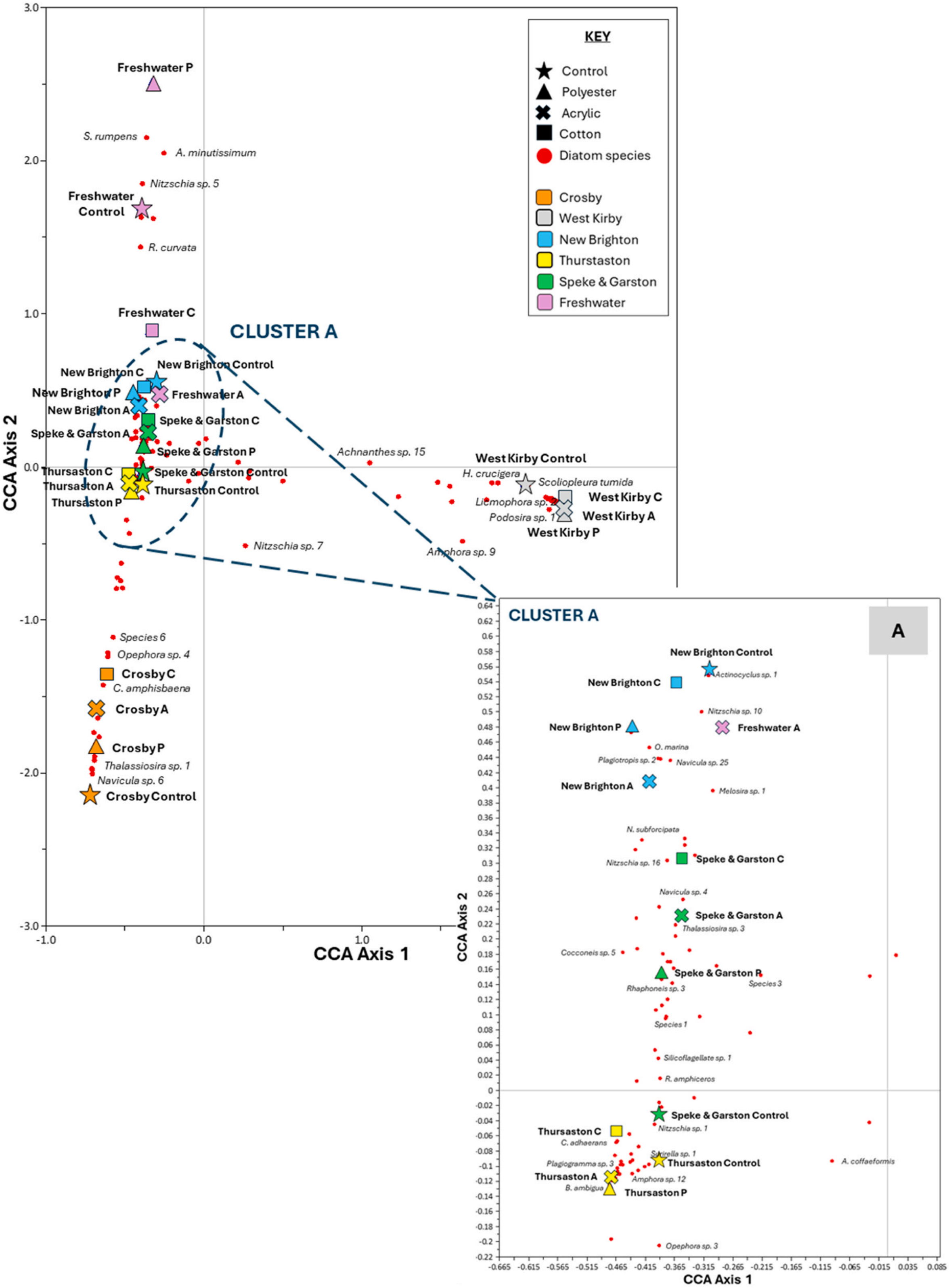


Fig. 9. Canonical correspondence analysis plot highlighting the variability between all environmental and clothing transfer samples mean species assemblage (n = 3). A) provides greater detail on the trends demonstrated in Cluster A. Greater proximity between data points demonstrates relative similarity between each sample's species composition and the relative abundance of those.

& Garston reported the greatest relative similarity of all six environmental samples analysed [Fig. 9a]. Furthermore, the Thursaston cotton assemblage was more similar to the Speke & Garston environmental sample than all three materials actually immersed at the estuarine site. Variability amongst the Speke & Garston transfer samples was influenced by taxa including *Nitzschia sp. 16, Navicula subforcipata* (cotton), *Thalassiosira sp. 3, Navicula sp. 4* (acrylic), *Species 1,* and *Rhaphoneis sp.* 3 (polyester). The polyester transfer sample demonstrated greater affinity with the corresponding environmental sample compared to acrylic and cotton [Fig. 9a].

Finally, the freshwater pond reported the greatest variability between the environmental and corresponding transfer samples [Fig. 9]. The pond control and polyester samples were the least similar to any of the coastal sites, driven by the relative abundance of *Synedra rumpens, Achnanthidium minutissimum,* and *Nitzschia sp. 5*. The assemblage transferred to cotton and acrylic was less distinctive, with those samples also indicating relative similarity with the New Brighton samples.

4. Discussion

The results highlight significant differences in diatom abundance, species richness, and assemblage dynamics within five regional coastal environments and one freshwater comparison site. Diatom populations differed between and within the different marine habitats considered (e.g., marine lakes, tidal beaches), with the freshwater pond yielding the least abundant and species-rich assemblage of all sampling locations. Diatoms transferred to all immersed clothing materials, with environmental variability and recipient surface characteristics significantly impacting the extent and species-richness of a transferred assemblage. Differences in morphology and relative species abundance (%) demonstrate the complexity of species-specific features in facilitating or limiting diatom transfer to clothing in coastal contexts. Importantly, the diatom species assemblage transferred to all clothing items demonstrated relative similarity to the corresponding transfer location, supporting forensic comparisons and exclusions in coastal environments. Furthermore, the findings indicate the effective use of the previously established H_2O_2 method for the recovery of marine diatoms from different clothing surfaces.

4.1. Environmental variability within coastal sites

More abundant and species-rich diatom samples were reported in the Thursaston beach, West Kirby marine lake, and Speke & Garston estuarine samples [Fig. 2, Fig. 4], with all three also reporting more site-specific marker species [Fig. 7]. Both Thursaston and Speke & Garston are dynamic environments, impacted by fluvial regimes and/or tidal currents. Such conditions often facilitate nutrient cycling [38], sediment suspension [39], and mixing of benthic diatoms within overlying waters [40], potentially explaining the higher diatom count and species richness reported here [Fig. 3, Fig. 5]. Such dynamic conditions are important to consider during scene investigation and evidence collection in forensic casework [41]. The timely collection of environmental control samples is imperative to ensure that they are as representative as possible of the ambient conditions encountered during a criminal event (e.g., tidal regimes), and to ensure that forensic comparisons are robust and appropriate [41]. Interestingly, significantly fewer diatoms were

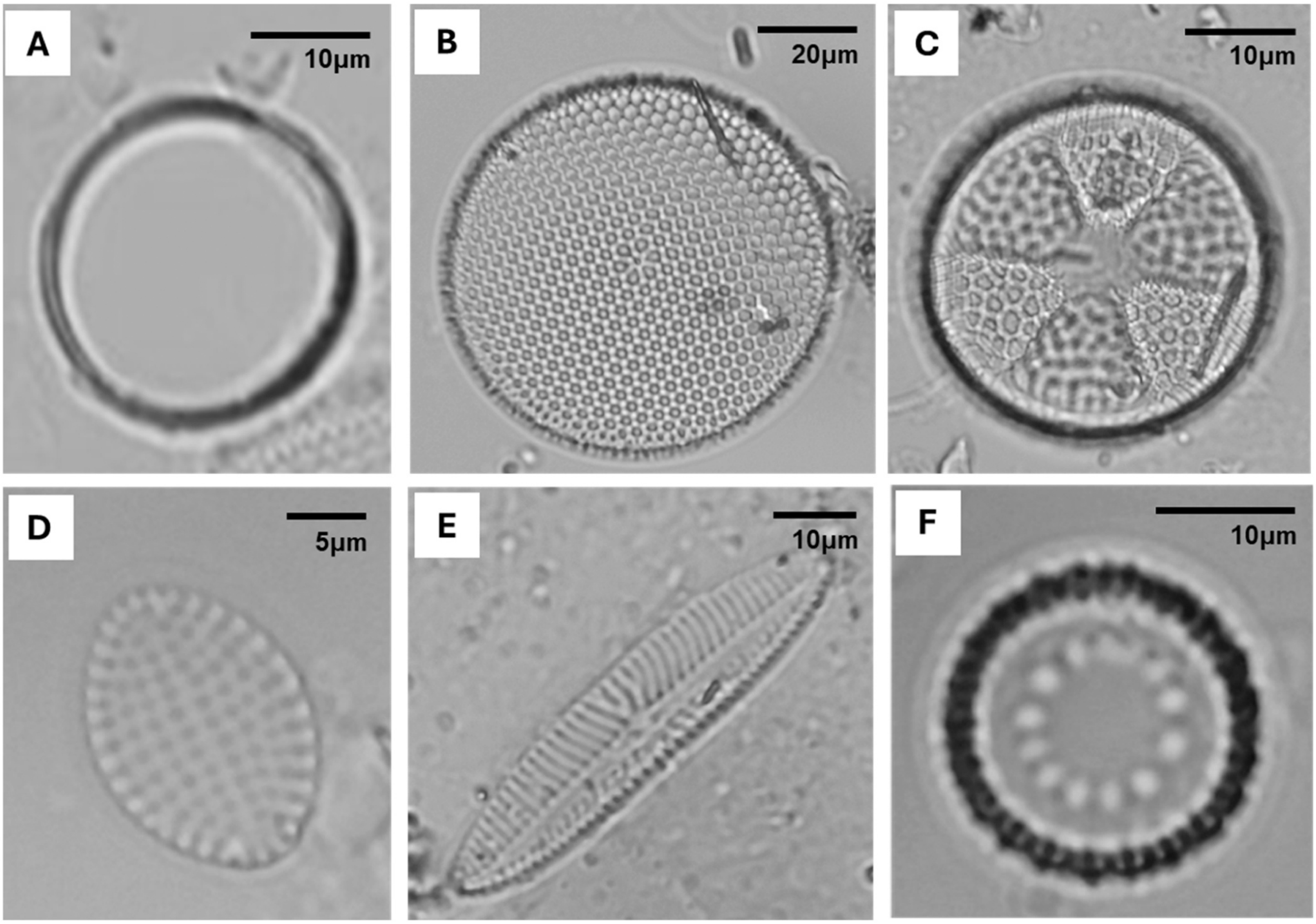


Fig. 10. Micrographs (x1000 magnification) highlighting some of the diatom species common to three or more of the coastal environments sampled: a) *Melosira sp. 1*; b) *Roperia tesselata*; c) *Actinoptychus senarius*; d) *Cocconeis sp. 4*; e) *Navicula tripunctata*; f) *Species 3*.

identified in the New Brighton samples, despite this location sharing similar tidal influence [Fig. 1]. This may be due to spatial variabilities in the environmental parameters known to influence diatom populations, including temperature, light, or silica availability [10], or alternatively, differences in land use, human-environment interactions, and/or pollution levels between the Mersey (New Brighton) and Dee (Thursaston) outflows [42,43].

The three locations impacted by tidal and fluvial activity – Thursaston, New Brighton, and Speke & Garston – demonstrated greater similarity in their species assemblage composition [Fig. 9]. Several common taxa were identified including *Biremis sp. 1, Cocconeis sp. 4, Cymatosira sp. 1, Melosira sp. 1, Roperia tesselata,* and *Thalassiosira sp. 3* [Fig. 8, Fig. 10, Supplementary Data]. These shared taxa may subsequently provide less useful circumstantial indicators when seeking to compare and exclude tidal samples in forensic investigations. Diatom species distinctive to each site, even when present in low concentrations (<1 %), offer valuable bioenvironmental markers [Fig. 11] [17,44], indicating subtle differences between otherwise similar samples [Fig. 7, Fig. 8]. These findings emphasise the value of analysing the whole species assemblage, accounting for both the ubiquitous and less common diatom taxa present, echoing recommendations made in previous freshwater transfer studies [17,19]. Such a comprehensive analytical approach is essential to support the comparison and exclusionary interpretation of diatom trace evidence in coastal contexts.

Despite significant differences in abundance and species-richness, the diatom communities present within the Crosby and West Kirby marine lakes were more variable compared to all other sites [Fig. 9]. Both habitats are characterised by distinctive diatom flora including, for example*, Dimeregramma sp. 2, Licmophora sp., 1, Navicula sp. 31*, *Opephora sp. 5,* and *Thalassiosira sp. 1* [Fig. 11], facilitating environmental, and forensic, discriminations within this study. Marine lakes are enclosed ecosystems, fed with rainwater as well as saltwater [45].

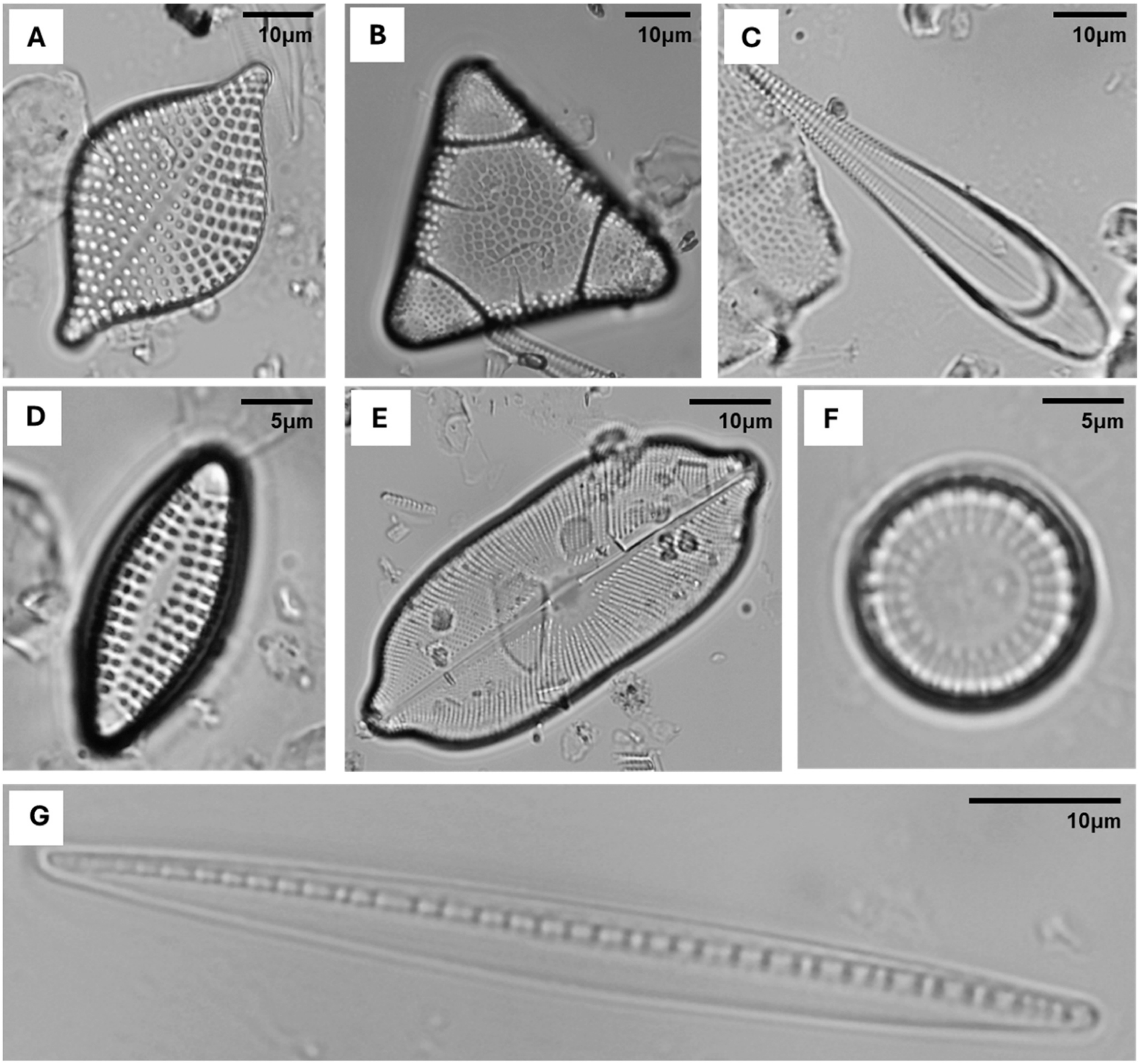


Fig. 11. Micrographs (x1000 magnification) highlighting some of those diatom species present only in one coastal environment sampled: a) *Rhaphoneis amphiceros* (Speke & Garston estuary); b) *Biddulphia sp. 1* (West Kirby marine lake); c) *Licmophora sp. 1* (West Kirby marine lake); d) *Rhabdonema* sp. 2 (Thursaston beach); e) *Navicula humerosa* (Thursaston beach); f) *Paralia sulcata* (Crosby marine lake); g) *Nitzschia dissipata* (New Brighton beach).

Consequently, they typically have reduced salinity, variable nutrient input, and delayed or reduced tidal motion [46], potentially explaining the characteristic diatom communities identified here [Fig. 9]. Interestingly, as with the tidal sites, significant differences were identified between the two marine lake habitats sampled, further indicating that coastal diatom trends are driven by localised ecological conditions [Fig. 2, Fig. 4].

The freshwater comparison site yielded the fewest diatoms, the lowest species richness, and only seven site-specific marker taxa [Fig. 2, Fig. 4, Fig. 7]. Despite this, the whole species assemblage demonstrated sufficient variability to distinguish the pond from the five coastal locations sampled [Fig. 9]. The data reported here is sufficiently lower than in previous studies, where freshwater sites have often reported thousands of diatoms [15,17,18] and/or more than a hundred different species [16,47] per ml of water sampled. Our results highlight that environmental differences can still be identified, and forensic discriminations supported, even when a site has comparatively low diatom populations and/or less diverse species compositions [Fig. 9], which may reflect the conditions encountered in forensic casework.

4.2. *Impact of coastal variability on diatom transfer*

Environmental trends were largely reflected in the clothing transfer samples; more diatoms and more species were transferred to clothing following immersion at Thursaston beach, Speke & Garston estuary, and West Kirby marine lake [Fig. 3, Fig. 5]. This suggests that environmental abundance and species richness influences the transferability of diatoms as forensic evidence in coastal contexts, consistent with previous findings in freshwater and soil environments [14,47,48]. Environmental factors including seasonality, temperature, humidity, moisture, and wind exposure, are well-known to influence trace evidence dynamics, including those of natural indicators such as soil, pollen, and diatoms [7,17,49]. This study demonstrates that, in coastal environments, water dynamism including turbulence and tidal/fluvial activity, can also influence evidential transfer [Fig. 3, Fig. 5]. Similar differences have also been observed in the abundance and species-richness of freshwater diatoms following transfer to clothing from rivers and ponds [48], potentially explaining why relatively few diatoms were identified in the pond comparison site tested in this research.

An important finding from this study is that, despite relatively low diatom populations in some coastal locations (e.g., Crosby, New Brighton [Fig. 2]), diatom transfer to all three clothing materials was consistently reported [Fig. 3]. This suggests that even minimal diatom presence within a coastal site can result in the transfer of trace materials which may yield significance to a forensic investigation [6]. The results presented are based off the preparation and analysis of relatively small (cm^2) fabric subsamples, as in previous freshwater and soil research [14,15,17,18]. A more extensive sampling effort, targeting a larger evidential surface area, may subsequently be more appropriate when diatom trace evidence is sought in forensic casework and the initial assessment of an environmental control sample indicates relatively low populations. Future research is recommended to explore and empirically test different evidence recovery strategies (e.g. tape lifting, vacuuming [48,50,51]) for diatoms in marine, freshwater, and soil contexts.

4.3. *Impact of clothing type on diatom transfer*

Significantly fewer diatoms, and a less species-rich assemblage, transferred to cotton when compared to acrylic and polyester, indicating that clothing type significantly influences marine diatom transfer to clothing [Fig. 3, Fig. 5]. These findings correspond with those of previous studies where recipient surface characteristics, including clothing type, have significantly influenced the transferability of trace evidence indicators including freshwater diatoms [17], pollen [8,55], soil [52], fibres [53], and glass [54].

The polyester and acrylic fabrics used in this study are characterized by a rough surface texture and an open pile/knitted weave, suggesting that such features support diatom entrainment upon clothing surfaces [Table 1]. This corresponds with the findings of previous freshwater diatom studies, where a more abundant transfer assemblage was identified on rough-textured and open-weaved linen, acrylic, and viscose clothing [17], and on canvas shoes [15]. Additionally, laboratory-based investigations of the marine diatom *Chaetoceros muelleri,* found greater transferability to similarly constructed acrylic and denim surfaces [21]. These findings highlight that rough-textured fabrics, where available, should be prioritised for diatom collection during a forensic investigation in both marine and freshwater contexts [17]. Where only smooth-textured and closed weave materials are available, these should still be considered, however the potential for a less abundant and species-rich sample should be accounted for within collection, analysis, and interpretation protocols.

The cotton used here was characterised by a smooth-medium texture and a closed twill weave, potentially explaining the low diatom abundance and species richness transferred [Fig. 3, Fig. 5]. In contrast, cotton clothing has yielded substantial numbers, and species, of diatoms following freshwater immersion in previous research [14,17,47]. The statistically significant interaction between sampling location and clothing type reported here ($p < .0001$), suggests that the effectiveness of diatom transfer is influenced not only by the environmental conditions at different coastal locations, but also by the specific properties of the recipient surface. This indicates that certain clothing materials may be more prone to diatom adherence depending on the characteristics of the transfer location, highlighting the need to consider both factors when analysing diatom trace evidence in forensic investigations [17,52].

These findings necessitate further research to assess diatom transfer in coastal sites to a more diverse range of recipient clothing surfaces, reflecting similar approaches in freshwater diatom [16,17] and pollen investigations [8,55]. Similarly, experimental consideration of transfer dynamics to footwear, and of coastal diatom persistence on different recipient surfaces is also warranted [15,18,19]. Importantly, future studies should aim to be as representative as possible and not limited to a single location, species, and/or evidential surface type as demonstrated in the preliminary forensic research base [14,21,47].

4.4. *Impact of valve morphology on transfer*

Diatoms were initially categorised according to their general morphology [17,19]. Most valves transferred to clothing from the coastal sites were pennate, larger than 10μm, and fragmented [Fig. 6].

4.4.1. *Diatom shape*

The distribution of centric and pennate taxa broadly corresponded with the environmental assemblage, except for the tidal New Brighton and freshwater pond samples. Polyester and acrylic more closely reflected the environmental controls than cotton [Fig. 6a]. This variability may be explained by the relatively low species richness reported in the relevant samples [Fig. 3, Fig. 5], further emphasising the significant interaction between clothing surface characteristics and environmental abundance on transfer [8].

Although some centric taxa including *Melosira sp. 1* (all sites), *Thalassiosira sp.1* (Crosby), and *Podosira sp. 1* (West Kirby) were consistently identified, other species were missing from all corresponding clothing (e.g., *Biddulphia sp. 1, Coscinodiscus sp. 2, Hyalodiscus sp. 3*) or only from the cotton transfer samples (e.g., *Actinopthychus senarius, Paralia sulcata, Roperia tesselata*) [Fig. 8]. This highlights that marine diatom transfer is also driven by species-specific features, not solely general morphology, reflecting the conclusions from previous freshwater diatom and forensic palynology research [17,52].

The lack of some centric taxa in an evidential sample may be limiting for forensic comparisons in coastal environments. For example, many of the site-specific centric marker species were absent from some or all corresponding clothing samples including *Actinocyclus sp. 1, Coscinodiscus sp. 2, Hyalodiscus sp. 3,* and *Odontella rhombus* [Fig. 7, Fig. 8]. This may potentially reduce the discriminatory potential of diatom trace evidence analysis in coastal contexts, especially given the prevalence of centric diatoms in marine environments [27]. The exclusionary interpretation of a transferred assemblage in forensic casework should therefore account for this variability when assessing how representative an evidential sample is of centric taxa.

4.4.2. Diatom size

The distribution of larger (>10μm) and smaller (<10μm) marine diatoms within clothing generally reflected the relevant environmental sample composition [Fig. 6b]. The only exceptions to this were the cotton samples immersed at New Brighton beach and the freshwater pond, where only two species were identified in each mean assemblage [Fig. 5].

Several of the smaller taxa were consistently represented in all environmental and corresponding clothing samples including *Biremis sp. 1*, *Catenula adhaerans,* (Thursaston), *Cymatosira sp. 1* (Speke & Garston, Thursaston), and *Cocconeis sp. 4* (Speke & Garston, West Kirby, Thursaston) [Fig. 8]. As with the centric taxa, however, some of the smaller site-specific marker species, were absent from some or all corresponding clothing including *Achnanthes sp. 2, sp. 12, Diploneis sp. 3,* and *Species 3*. These findings correspond with Scott *et al.* [17] in suggesting that diatom size does not entirely limit the transferability of marine diatoms to clothing surfaces, however some differences may be associated with the surface properties of different clothing materials.

4.4.3. Diatom integrity

Substantially more fragmented valves were identified in the clothing than in the equivalent environmental control samples [Fig. 6c]. This contrasts with previous freshwater studies, where most of the transferred assemblage comprised whole diatoms [17], suggesting that diatom transfer mechanisms in coastal locations, or the extraction method used, may compromise the quality of an evidential sample. Similar findings were reported in laboratory-based transfer investigations of the marine species *C. muelleri* to cotton, denim, blend, and acrylic samples [21].

The distribution of whole and fragmented diatoms was relatively consistent amongst the three clothing materials, suggesting that recipient surface characteristics do not influence the integrity of a transferred assemblage in coastal settings, similarly to the findings from freshwater research [Fig. 6c] [17]. Only whole, in-tact, diatoms were considered during the quantitative and qualitative analytical approach used throughout this study. However, in some samples where abundance was particularly low, including cotton clothing immersed at Crosby, New Brighton, Speke & Garston, and the freshwater pond (<100 diatoms/cm^2), fragments may yield sufficient evidence to initially indicate contact with water [15]. Detailed appraisal of evidential significance, including comparisons and exclusions between items and scenes of forensic interest, would, however, be limited due to the lack of identifying features within broken diatom valves.

4.5. Species assemblage dynamics

All diatoms were identified to species-level, facilitating comparisons of abundant and less common taxa within each subset of environmental and forensic samples [Fig. 8] [31–36]. The diatoms present in the five coastal sites and corresponding clothing samples comprised common marine or brackish genera, including *Actinocyclus, Biremis, Coscinodiscus, Dimeregramma, Hyalodiscus, Licmophora, Opephora, Plagiogramma, Rhaphoneis, Scoliopleura,* and *Thalassiosira* [31]. Additionally, marine species within more universal genera were also identified including *Achnanthes brevipes var. intermedia, Amphora coffaeformis, Diploneis sp., Navicula subforcipata,* and *N. humerosa* [Fig. 8, Fig. 10, Fig. 11] [32]. Common freshwater species were identified in the pond samples. Species typically inhabiting both the plankton (e.g., *Melosira sp. 1, Pleurosira laevis, Thalassiosira sp. 1*) and the benthos (e.g., *Licmophora sp. 1, Petrodictyon gemma, Pleurosigma sp. 2*) were recovered from clothing, indicating that an individual's movement within the water disturbs, mixes, and can lead to the transfer of different diatom communities to evidential surfaces [41]. A silicoflagellate species was reported within the Speke & Garston and Thursaston environmental samples, alongside both acrylic samples and one polyester assemblage, indicating that additional algal groups may yield discriminatory value in support of forensic comparisons [56,57].

Typically, the most abundant taxa within the environmental control samples were consistently identified amongst the different clothing materials. This included species found in multiple sites such as *Melosira sp. 1,* as well as site-specific markers including *Fragilaria sp. 3* (Thursaston), *Navicula sp. 31* (Crosby), and *Opephora marina* (New Brighton) [Fig. 8]. Species present as < 1 % of the whole assemblage were less consistently represented in the transfer samples, with some marker species including *Achnanthes brevipes var. intermedia* (West Kirby), *Amphora sp.11* (Speke & Garston), *Navicula humerosa* (Thursaston), and *Rhoicosphenia sp. 1* (Crosby) missing from most or all transfer samples. This suggests that species characteristics, including valve face features (e.g., raphe, striae, pores, spines) influence the evidential dynamics of marine diatoms, interacting with recipient surface features and environmental variability to limit the transfer of some taxa to clothing [Fig. 8]. This complexity has also been recognised within freshwater diatom transfer [17] and persistence [19] research, recommending that forensic comparisons and exclusions in all environmental contexts, should account for the possibility that a transferred assemblage does not reflect the entire diatom community present at an initial transfer location.

Crucially, despite differences in the transfer of individual species, the whole diatom assemblage retrieved from the clothing samples demonstrated similarity to the corresponding immersion site [Fig. 9]. The species composition retrieved from cotton, acrylic, and polyester was most characteristic of the control site in both marine lakes (West Kirby, Crosby), indicating that such locations may be successfully discriminated from other coastal environments in forensic reconstructions [45]. Variability was also identified between clothing immersed in the tidal waters of New Brighton and Thursaston, suggesting that transferred diatoms can distinguish geographical differences, as within the Wirral peninsula [Fig. 1, Fig. 9]. Clothing samples immersed in the Speke & Garston estuary, demonstrated relative similarity to the respective environmental control, as well as the New Brighton water sample [Fig. 9a]. With only 9.43 miles separating both sites along the River Mersey and its outflow, forensic comparisons and exclusions may subsequently be more challenging within the same tidal system [29] [Fig. 1]. These findings highlight the importance of species-level diatom identifications, and the use of multivariate analyses, to support forensic interpretations within empirical research and forensic casework, in coastal, as well as freshwater and soil environments [14,17].

This research highlights the value of including multiple environmental samples to support forensic comparisons and exclusions using diatom analysis in coastal contexts [Fig. 8, Fig. 9]. Most previous freshwater and soil studies have focused only on a single environment [14,15,17,18], or, on the inclusion of close-proximity comparator sites [6,16], to explore diatom trace evidence dynamics. Incorporating several control environments, representing a wider geographic area and accounting for spatial variability (e.g., up/downcoast), is valuable to support more robust and effective forensic interpretations of circumstantial intelligence in research and casework contexts [6,41]. Future research recommends maintaining a broader environmental scope, offering a more comprehensive understanding of diatom distribution patterns to assess the potential that different populations and species yield as forensic markers [44].

4.6. Effectiveness of H_2O_2 extraction protocol

This study presents the first application of the H_2O_2 extraction protocol [14] for the recovery of diatoms transferred to clothing from coastal locations. Diatoms were successfully retrieved from all clothing and environmental samples examined, with relatively consistent findings between subsample replicates also identified through the reported standard deviation values [Fig. 2, Fig. 3]. This stability differs from previous freshwater studies, where relatively high variation has been observed [14,17,18], perhaps suggesting that diatom transfer and/or the recovery method used is more consistent within coastal settings. Future research should seek to build on these findings, and those of laboratory-based investigations using singular species [21], by testing a more diverse range of extraction protocols for the recovery of marine diatoms from clothing, to ensure that, empirically, the most optimal method is applied in support of forensic casework and empirical research.

5. Conclusion

This is the first study to assess diatom trace evidence dynamics in coastal environments. The findings demonstrate that marine diatoms can yield useful trace evidence indicators following their transfer to clothing, facilitating comparisons and exclusions with environments of forensic interest. Notably, significant regional differences were identified between coastal diatom communities; spatial variability was also reflected in the diatom assemblage transferred to clothing. As with previous freshwater research, our findings demonstrate that the transferability of marine diatoms is influenced by clothing type, environmental variability, and valve characteristics including morphology and species-specific features. The complex interaction of these variables must be carefully considered during forensic investigations, to ensure robust and reliable interpretations of evidential significance.

This study provides a novel contribution to the growing empirical evidence base within forensic ecology and diatom trace evidence analysis [14–19,21,47,58]. As well as the marine focus, this study is the first to incorporate multiple sampling locations, at a regional scale, facilitating more effective comparisons and exclusions of diatom transfer samples with a site of initial immersion. It is recommended that future research studies incorporate similar focus on spatial variability, to enhance experimental findings and to reflect the complex circumstances where multiple questioned environments are encountered in forensic investigations. Finally, it is recommended that additional experimental studies are conducted to explore the full potential of marine diatoms as trace evidence indicators. These should include investigations into alternative recipient surfaces such as footwear, diatom persistence dynamics, the forensic potential of additional marine habitats, and the effectiveness of various methods for diatom recovery and analysis.

CRediT authorship contribution statement

Stevens Alice: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Scott Kirstie Rebecca:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this study.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.forsciint.2025.112461.

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