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Quantification of a pretreatment procedure for organic-rich calcareous nannofossil samples

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Abstract A new sodium hypochlorite (NaClO) pretreatment technique for calcareous nannofossil samples rich in marine and terrestrial organic components was tested on samples containing moderate amounts of organic matter (~1–3%). In the untreated control slides, organic matter at moderate levels obscured individual specimens or caused cohesion of nannofossils with background material, such as clays and opaque minerals. While samples with >7% background organic matter require two iterations of the pretreatment procedure (Shamrock *et al*., 2015), samples with average background values up to 3% were sufficiently treated with one exposure to the primary oxidizing reagent (NaClO). Pretreated samples showed notable increases in both relative abundance and species richness. In addition, we examined the potential loss of nannofossil specimens while decanting the supernatant after centrifuging. Despite some negligible loss in nannofossil specimens, the enriched abundance and diversity of the data justifies this enhanced processing procedure.

1. Introduction

Interest in unconventional resource exploration in the petroleum industry, as well as both academic and industrial interest in carbon excursions, has resulted in a subsequent increase in the number of nannofossil samples analyzed from organic-rich lithologies. These samples are significantly more difficult to analyze than calcareous chalks and oozes due to the high proportion of marine and terrestrial organics, clays and opaque minerals. Specimens are frequently obscured by organic matter and may adhere to larger clumps of background material, which can lead to skewed abundance counts and reduced species richness. To minimize these issues, a sodium hypochlorite pretreatment method for organic-rich samples was developed with excellent results (Shamrock *et al*., 2015). Pretreated samples show significant reduction in residual organic matter as well as increases in both the number of identifiable specimens and in species richness.

Samples from this original study had high (~7–14%) values of μ_{org} (average residual organic matter per field of view [FOV]; N = 50 at 630x magnification), and require at least two exposures to the oxidizing reagent (NaClO) to produce significant results. However, samples with only 1–2% μ_{org} can still significantly inhibit sample preparation and subsequent data collection. In the present paper, we examine whether samples with a lower μ_{org} can be significantly improved while reducing pretreatment to only one exposure to the NaClO solution and documented the relative improvement in these lower μ_{org} samples.

One potential pitfall of the procedure is the loss of nannofossil specimens when decanting after centrifuging the treated samples. Decanted specimens would likely be very small in size, and several of these very small species are used as marker taxa. In addition, changes in the relative abundance of some small species can act as significant bioevents (e.g., acme events) or be important in paleoenvironmental analysis. As this step is repeated several times throughout the pretreatment, the residual sediment in the supernatant liquid was examined with respect to both dried weight and relative floral composition of the residues, to quantify any negative impact this step may have on nannofossil assemblage compositions.

2. Localities and samples

A total of twelve samples were examined for calcareous nannofossil content. Seven samples were collected from the late Cenomanian Clubhouse Formation and an unnamed Turonian formation from the Holland Ball Park core, located in the City of Suffolk, VA (Figure 1; 36.68211°N, 76.78058°W). One sample was collected from the Eocene Exmore Formation of the Ashby Subdivision core, located in Carrollton, VA (Figure 1; 36.93317°N, 76.52973°W). Three samples were collected from the late Campanian Donoho Creek Formation in southern North Carolina (Figure 2) from two outcrops on the Cape Fear River: Donoho Creek Landing (34.48065°N, 78.40995°W), and Brown's Landing (34.47505°N, 78.39767°W). One sample was collected from the early Maastrichtian Peedee Formation at Mitchell's Landing (34.3567°N, 78.20947°W). Sample depths and sample codes are detailed in Table 1.

Cored samples were extracted from the central portion of freshly broken core in order to avoid contamination by drilling fluid. Outcrop samples were collected by digging back into the exposed sediments on the outcrops to obtain fresh, unweathered material. Samples from the Cape Fear River, which is tidally controlled near the river mouth, are

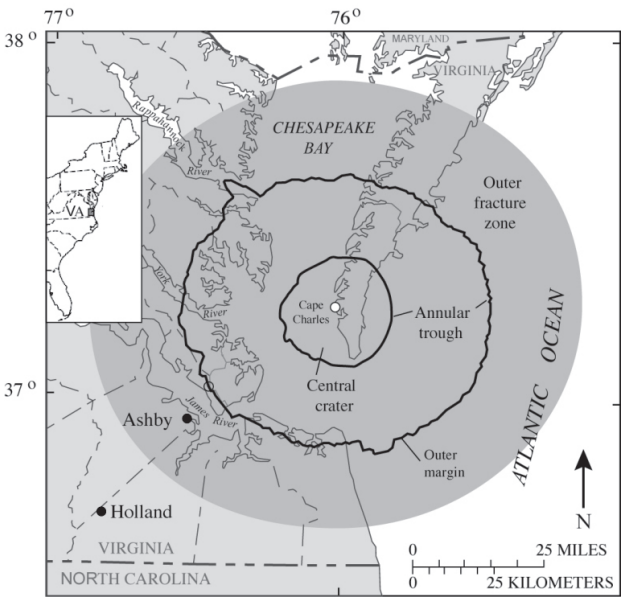

Figure 1: Map showing the location of the Holland Ball Park core (HC) and the Ashby Subdivision core (AC). Note that the Ashby core is located in the outer fracture zone of the Chesapeake Bay impact crater. Modified from Edwards & Powars (2003). Central crater outline updated according to Powars *et al*. (2009)

recorded as "Taped Feet". These samples are measured upward from a permanent spike drilled into the base of each section, with the location of each spike recorded by GPS and tied to base sea level.

3. Methodology and procedures

A complete and detailed description of the pretreatment procedure and background methodology utilized for this study is documented in Shamrock *et al*. (2015). Details on procedure times, volumes, etc. are provided in Table 2. Samples for this study were processed by first placing a small amount of sample (~0.1g) in a 15ml Kimax centrifuge tube with 3ml NaClO reagent, a ~5–6% sodium hypochlorite solution as found in basic household bleach. Samples were shaken then submerged in a 100°C hot water bath for the prescribed time, then centrifuged using a Thermo Scientific CL2 centrifuge with a 16.5cm rotor diameter. The supernatant liquid was decanted, and samples were then washed with a 9.5pH bicarbonate solution to neutralize any residual acidity within the sample from the break-down of organic matter. Finally, samples were rinsed several times with distilled water (See Table 2). It is critical to rinse the bicarbonate ions out of the treated sample, otherwise these residual ions will crystalize on the coverslip, obscuring much of the final preparation.

3.1 Residual organic material and treatment duration

All samples were initially prepared both as untreated smear slides using the double slurry method of Watkins & Bergen (2003) and using the pretreatment method above. Samples were examined in PPL (plane polarized light) on a Zeiss AxioImager A.2 microscope to assess μ_{org}. Relative abundance estimates of μ_{org} were made using Compton (1962) for abundances of 2–50% per FOV and Reid (1985) for abundances ≤1% per FOV. A select set of samples with the highest μ_{org} were reprocessed with only one exposure to the NaClO solution to assess oxidation potential in samples with lower residual organic material than those studied in Shamrock *et al*. (2015). In addition, we reexamined some samples with high nannofossil abundance to assess potential effects of pretreatment on nannofossil assemblages.

The pretreatment method was originally developed using samples which all contained significant amounts of marine and terrestrial μ_{org} (≈7–14%). A single exposure to the sodium hypochlorite solution was insufficient for such organic-rich samples, and optimal results (μ_{org} < 1%) were achieved with two exposures to the NaClO solution

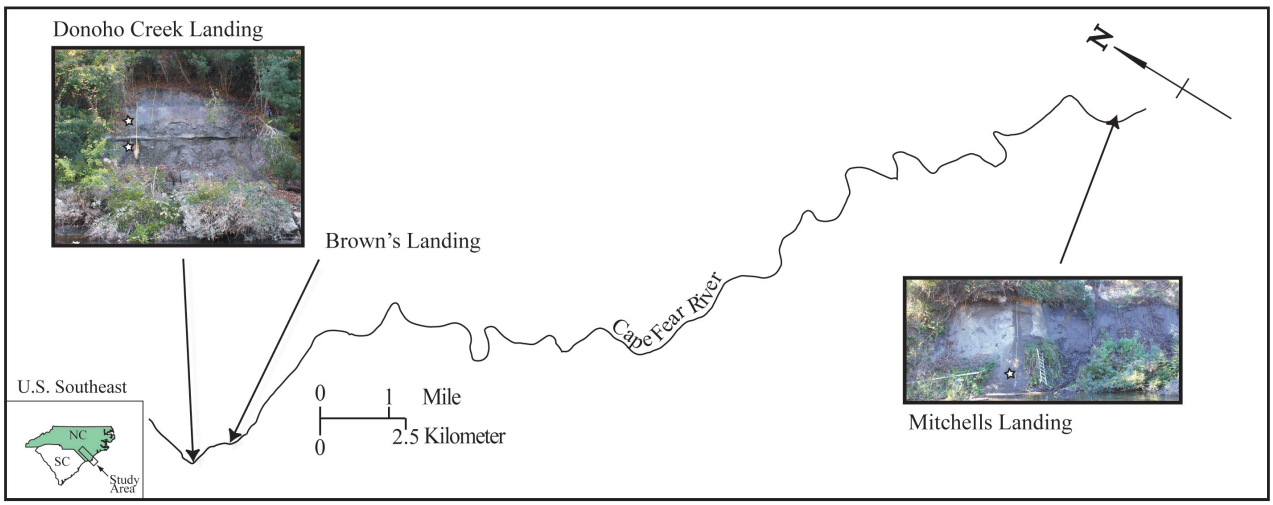

Figure 2: Map showing the location of Donoho Creek Landing, Brown's Landing and Mitchell's Landing on the Cape Fear River. Stars indicate the level at which a sample was taken. Modified from Self-Trail *et al*. (2012)

Sample	Locality	Depth (ft)	Depth (m)	Sample Code	Formation	Latitude (N)	Longitude (W)
	Holland Core (VA)						
1	Holland Core	273.8	83.45	HC 273.8	unnnamed Turonian	36.68211	76.78058
2	Holland Core	317.0–317.2	96.62–96.68	HC 317.2	Clubhouse		
3	Holland Core	337.4	102.84	HC 337.4	Clubhouse		
4	Holland Core	347.2	105.83	HC 347.2	Clubhouse		
5	Holland Core	369.0	112.47	HC 369.0	Clubhouse		
6	Holland Core	376.2–376.3	114.67–114.70	HC 376.3	Clubhouse		
7	Holland Core	397.0–397.2	121.01–121.07	HC 397.2	Clubhouse		
	Ashby Core (VA)						
8	Ashby Core	332.7	101.41	AC 332.7	Exmore	36.93317	76.52973
	Cape Fear River (NC)						
9	Donoho Creek Landing	18.0	5.49	CFR-DCL 18.0	Donoho Creek	34.48065	78.40995
10	Donoho Creek Landing	35.0	10.67	CFR-DCL 35.0	Donoho Creek		
11	Brown Landing	23.0	7.01	CFR-BL 23.0	Donoho Creek	34.47505	78.39767
12	Mitchell's Landing	6.0	1.83	CFR-ML 6.0	Peedee	34.35670	78.20947

Table 1: Sample list detailing sample locality, sample depth (in ft and m), sample code (used throughout text and figures), geologic formation and latitude/longitude. VA = Virginia, NC = North Carolina, USA

Step	Reagent	Volume	Vortex	H_2O Bath	Centrifuge Speed	Centrifuge Time	Iterations
		(ml)	(sec)	(min)	(rpm)	(min)	
1	Sodium hypocholrite (NaClO)	3	3–5	15	2000	7	1–2*
2	Bicarbonate solution ($NaHCO_3$)	5	3–5	15	2500	10	2
3	Distilled H_2O	5	5–10	NA	2000	10	3
4	Distilled H_2O	5	>4 hrs†	NA	2000	10	1

Table 2: Summary of reagents, volumes, mixing times, heating durations, centrifuge parameters and number of iterations. The sodium hypochlorite solution is 5–6% NaClO by volume, as found in basic household bleach. The bicarbonate solution ($NaHCO_3$) is mixed to 9.5pH. *The number of oxidation steps is dependent on average residual organic matter of the sample. Two iterations are required when μ_{org} is ≥7% per FOV. Only one exposure to the reagent is required when μ_{org} is ≤3% per FOV. †For the final water wash, samples are swirled on low for a minimum of four hours. For a detailed methodology and procedure, see Shamrock *et al*. (2015)

(Shamrock *et al*., 2015). In comparison, samples in this study contained notably less μ_{org}, typically between 1–3%, thus we reexamined the potential for reducing treatment.

3.2 Nannofossil abundance and species richness

Nannofossil samples were examined in XPL (cross-polarized light) and PPL at 1000x magnification. Species richness and relative abundance counts were made by identifying all specimens across a full traverse on a 40mm coverslip, or approximately 200 FOVs. Two additional traverses were made to scan for rare taxa, which were given a value of one. Specimens covered by organic material or those contained in sediment clumps were identified when possible. Obscured specimens were listed as unidentifiable. Relative abundance is significantly affected by the amount of material used in sample preparation, so care was taken to ensure similar sediment thicknesses on all sample preparations. After pretreatment, a similar amount of sediment was collected from the centrifuge tube on the tip of a flat-headed toothpick, and evenly distributed over the coverslip using the double-slurry method. Even sediment distribution was fairly easy to achieve, as the repeated mixing of the sample and washing with distilled water during pretreatment is very effective in deflocculating clays as well as individual nannofossil specimens.

3.3 Sample loss

The loss of sample while decanting was examined with respect to the weight of the dried residue in the supernatant liquid, the number of nannofossil specimens, the species richness and the average size (length) of the specimens observed in smear slides.

The supernatant from Steps 1 and 2 cannot be collected due to the presence of either sodium hypochlorite or bicarbonate in the solution (Shamrock *et al*., 2015). The supernatant liquid from the three water washes of Step 3 (Table 2) were collected in a 50ml beaker. The liquid was allowed to sit for 48hrs, which exceeds the required settling rates detailed by Geisen *et al*. (1999), to permit all residual suspended material to settle to the bottom. The liquid was gently pipetted off, and the residues dried in an oven at 100°C for two hours. The dried residues were weighed and then prepared as smear slides to assess the composition of the sediment (Table 4).

4. Results

4.1 Residual organic material and treatment duration

Several untreated smear slides showed relatively low residual organic matter ($\mu_{org} \approx 1\%$); however, six samples yielded significant amounts of μ_{org} (>1.5%) to warrant further testing (Table 3). Though relatively low in μ_{org}, sample AC 332.7 was also included due to the high nannofossil abundance and clay content.

Those samples with the highest μ_{org} were processed with only one exposure to the NaClO solution to examine

the potential for reducing pretreatment time. All samples showed significant reduction in residual organic matter after only one pretreatment (Table 3). These results are best seen in photomicrographs in plane polarized light. Plate 1 shows sample CFR-BL 23.0 from all three preparations in both XPL and PPL. Residual organic matter and clumping of nannofossil specimens was seen in the untreated smear slides (Plate 1–1). Preparations treated with one and two exposures to the NaClO solution (Plate 1–2 and 1–3, respectively) showed a significant reduction in residual organic matter. In addition, sediment clumps were disaggregated and a significant amount of clay-sized particles had been removed. These factors helped to improve sample preparation and aid in data analysis.

Two exposures to the NaClO solution are necessary for samples with ≥7% μ_{org} (See Shamrock *et al*., 2015: Figure 1, Table 1, 2); however, the most organic-rich sample examined here (CFR-BL 23.0; μ_{org} = 3.2%) achieved optimum results (μ_{org} < 1%) with only one exposure to the oxidizing reagent (Table 3, Plate 1–2), indicating that samples with μ_{org} ≈ 3% or less could be sufficiently treated with only one oxidization step. There was no data for samples with μ_{org} ≈ 4–6% in either study and, as a result, we were unable to define the value where one would need to increase from one to two iterations.

4.2 Nannofossil abundance and species richness

Samples were examined for relative nannofossil abundance and species richness as described above. As in the original study, there was a decrease in the number of 'unidentifiable' specimens in treated samples (Table 3). This term refers to specimens obscured by organic matter or aggregated into clumps with other background components, such as clays, organics and opaque minerals. The pretreatment process lightened organic matter, removed a portion of clay-sized particles (μ_{length} ≈ 2μm) and dissociated sediment aggregates, thus freeing individual specimens for identification and more robust relative abundance counts.

All fossiliferous samples from the late Cenomanian-Turonian age Holland core displayed an increase in relative abundance and species richness with pretreatment (Table 3). As expected, the sample with the highest residual organic matter (HC 376.3, μ_{org} = 2.3%) showed a notable increase in species richness, though not as dramatic as the AC and CFR localities.

Sample AC 332.7 (late Eocene) is taken from the Exmore Formation, the sedimentary resurge material that filled the Chesapeake Bay impact crater following a bolide impact ~35.0Mya (Edwards *et al*., 2009) (Figure 1). The sample contained a relatively rich, well-preserved Priabonian assemblage, intermixed with reworked specimens of Late Cretaceous, early Eocene (Ypresian) and late Eocene (Bartonian) age. Both reworked and *in situ* assemblages showed good preservation with the pretreatment, as well as increased relative abundance and species richness (Table 3). The observed increases in relative abundance with pretreatment, in this and the original study, had been primarily attributed to changes in background organic matter; however, μ_{org} was very low in the untreated AC 332.7 smear slide (μ_{org} = 0.3% per FOV). Examination of the supernatant liquid from this sample showed a significant fraction of clay-sized particles (~2–3μm) (Plate 2–3). This, accordingly, played a significant role in the enrichment

Sample Code	Smear Slide					Pretreatment 1x					Pretreatment 2x				
	μ_{org} per FOV	Total Specimens	Undentifiable	Identifiable	Species Richness	μ_{org} per FOV	Total Specimens	Unidentifiable	Identifiable	Species Richness	μ_{org} per FOV	Total Specimens	Unidentifiable	Identifiable	Species Richness
HC 273.8	1.0	4	2	2	2	NA					0.1	5	0	5	5
HC 317.2	1.1	35	2	33	21	NA					0.1	46	1	45	22
HC 337.4	2.6	0	0	0	0	0.1	0	0	0	0	0.1	0	0	0	0
HC 347.2	2.5	0	0	0	0	0.1	0	0	0	0	0.1	0	0	0	0
HC 369.0	2.3	7	3	4	3	0.1	19	2	17	10	0.1	17	3	14	11
HC 376.3	2.4	63	8	55	18	0.0	76	1	75	20	0.1	81	0	81	21
HC 397.2	1.3	62	3	59	21	NA					0.1	71	0	71	23
AC 332.7	0.3	895	23	872	70	0.1	1129	2	1127	89	0.1	1177	3	1174	93
CFR-DCL 18.0	0.7	1349	25	1324	78	NA					0.0	1959	4	1955	96
CFR-DCL 35.0	1.0	410	46	364	56	NA					0.1	3073	0	3073	99
CFR-BL 23.0	3.2	1297	106	1191	75	0.1	1996	0	1996	97	0.1	2063	3	2060	90
CFR-ML 6.0	1.7	687	57	630	70	0.1	2824	1	2823	93	0.1	2628	1	2627	96
						Sd ±0.02					Sd ±0.03				

Table 3: Summary of average residual organic matter, relative nannofossil abundance and species richness data with varying sample preparations. Organic matter estimates (μ_{org}/FOV) were made at 630x magnification in plane polarized light (PPL) where N = 50 FOV. Estimates were made using Compton (1962) for abundances of 2–50% per FOV and Reid (1985) for abundances of 1% or less per FOV. "Unidentifiable" refers only to specimens obscured by organic material, clay minerals or clumping of background sediments. Samples treated once show increases in both relative abundance and species richness, linked to lightening of organic matter, dissociation of sediment aggregates and removal of clays during sample processing. Sd = standard deviation

Sample Code	Sample Weight (g)	Residue Weight (g)	% Loss	$L_{min-max}$ (µm)	μ_{length} (µm)	Untreated Smear Slide		Pretreated Slide		Supernatant Residue	
				Nanno Specimens		RA	SR	RA	SR	RA	SR
HC 376.3	0.136	0.004	2.9	3.4	3.4	63	18	81	21	1	1
HC 397.2	0.125	0.000	0.0	NA	NA	62	21	73	23	0	0
AC 332.7	0.108	0.000	0.0	1.6–5.6	2.6	872	70	1176	93	28	6
CFR-DCL 18.0	0.117	0.003	2.6	2.0–6.1	3.2	1324	78	1955	95	25	11
CFR-DCL 35.0	0.126	0.001	0.8	1.9–3.6	2.8	364	56	3073	99	13	5
CFR-BL 23.0	0.128	0.006	4.7	1.5–4.9	2.8	1191	75	2060	90	18	5
CFR-ML 6.0	0.129	0.002	1.6	1.7–4.8	2.9	630	70	2627	96	26	9

Table 4: Data comparison from decanted supernatant residues. Residue weights are a minor portion of the original sample weight, and are comprised primarily of clay-sized (1–3µm) non-nannofossil background components. Maximum, minimum and average length is provided for residue nannofossil specimens. Three rightmost columns show relative abundance (RA) and species richness (SR) of selected untreated smear slides, pretreated samples, and pretreated sample residues. Nannofossil specimens observed in supernatant residues show low nannofossil abundance and species richness relative to the parent samples

of the >3µm sediment fraction and relative abundance of smaller calcareous nannofossils (Table 4). This is further discussed in the *Sample Loss* section, below.

Samples from the Cape Fear River outcrops showed very abundant, diverse and relatively well-preserved assemblages of Late Cretaceous (Campanian) age. The notably high species richness was a result of a well-developed oligotrophic assemblage combined with a well-preserved shelf assemblage. All pretreated samples showed a decrease in the number of unidentifiable specimens and an increase in relative abundance and species richness (Table 3). Photomicrographs of untreated smear slides and pretreated slides of sample CFR-ML 6.0 again showed a decrease in residual organic matter, clay-sized particles and clumped sediments (Plate 3). Comparable results were obtained with either one or two exposures to the oxidizing reagent (Table 3).

4.3 Sample loss

While our results with the pretreatment method are predominantly positive, the increase in sample processing also provides the opportunity to negatively impact the fossil assemblage. A potential for specimen loss occurs while decanting the supernatant liquid after centrifuging. While this step enriched the samples by removing significant portions of clay-sized particles (≈2–3µm), there are also some coccoliths in the 2–3µm size range that are subject to loss.

Seven samples with the greatest abundance of nannofossil specimens were tested for this type of potential specimen loss. The dried weights of sediment in the decanted liquid are provided in Table 4. Residues are produced from six decanting steps but could only be collected from three of these steps, as described above. Therefore, actual residue weights may be twice as high, or higher, than the measured values. Nevertheless, the weight of decanted residues is still a very small proportion of original sample weight (Table 4).

Smear slides of the residues showed the primary components to be very small (2–3µm) carbonate and siliciclastic background particles (Plate 2–3, Plate 4). Still, some nannofossil specimens were identified in all of the residues except the relatively low-abundance sample HC 397.2 (Table 4). Nannofossil specimens in these residues represented a very small fraction of the total abundance and were generally restricted to very small specimens of particular taxa. In addition, all species observed in the residue slides were also identified in the pretreated slides, indicating no impact on sample diversity. Table 4 compares relative abundance and species richness for smear slides, pretreated samples and supernatant residues produced from the pretreatment process. Table 5 details the distribution of specimens identified in the supernatant residues and compares these values to the pretreated samples from which the residues were derived. Species identified in residues were generally very abundant in the pretreated sample, and represented only a fraction of the total species richness of the sample (Tables 4, 5).

The lengths of all nannofossil specimens observed in the residue slides were measured to understand the size fractionation of specimens during the pretreatment. The mean length (μ_{length}) of all nannofossils specimens identified in the residue slides is 2.9µm (min. = 1.5µm, max. = 6.1µm, N = 111). As expected, most specimens (74%) were very small (length ≤3.0µm, ±0.2µm), and the majority (92%) were ≤4.0µm (±0.2µm) (Table 4). The largest specimen identified was 6.1µm; however, this specimen is one of the very few larger-sized outliers identified from all of the residue slides.

The abundance of individual taxa in the residues is linked to the relative abundance of those taxa in the pretreated sample, with the most abundant small taxa being observed in the supernatant residue as well. For example, we observed sample four specimens of *Repagulum parvidentatum* in the residue slide of sample CFR-BL 23.0, while >250 were counted in the pretreated slide. Similarly, two specimens of *Eiffellithus gorkae* were identified in the residue of sample CFR-ML 6.0, while 163 were identified in the pretreated slide. Abundance data for the residues is compared to the abundance of these taxa in the pretreated slides in Table 5. Sample HC 376.3 is omitted from Table 5 as only one specimen of *Watznaueria barnesiae* was observed in the residue slide.

Sample Code	% Relative to Parent Assemblage	Slide Preparation	Relative Abundance	Species Richness	*Angulofenestrellithus snyderi*	*Biscutum constans*	*Chiastozygus synquadriperforatus*	*Corollithion signum*	*Discorhabdus ignotus*	*Eiffellithus gorkae*	*Helicolithus trabeculatus*	*Placozygus fibuliformis*	*Prediscosphaera cretacea*	*Prediscosphaera spinosa*	*Prediscosphaera stoveri*	*Repagulum parvidentatum*	*Retecapsa crenulata*	*Tranolithus minimus*	*Zeugrhabdotus diplogrammus*	*Zeugrhabdotus erectus*	*Zeugrhabdotus trivectus*	*Coccolithus minimus*	*Cruciplacolithus* sp.	*Reticulofenestra haqii*	*Reticulofenestra minuta*	*Reticulofenestra minutula*	*Pontosphaera sigmoidalis*
AC 332.7	2.33	P	1176	93																		15	3	105	126	57	15
		R	28	6																		1	1	5	19	1	1
CFR-DCL 18.0	1.89	P	1195	95	5	56	68	6	27					68	14	214	37		27	38								
		R	23	11	1	1	3	1	2					1	1	8	1		1	3								
CFR-DCL 35.0	0.42	P	3073	99		183				196					77	322				56							
		R	13	5		3				2					1	6				1							
CFR-BL 23.0	0.72	P	2060	90		53					4		160			286				84	29						
		R	15	6		2					1		1			4				6	1						
CRF-ML 6.0	0.98	P	2627	96		78			71	163		58				153		55	3	66	30						
		R	26	9		7			1	2		1				6		2	1	4	2						

Table 5: Comparison of nannofossil assemblage data from pretreated samples and supernatant residues from centrifuging. Sample HC 376.3 is not included as only one specimen of *W. barnesiae* was observed. P = pretreated parent sample; R = sample residue. The relative abundance of nannofossil specimens in one full traverse of the residue slide is very low when compared to the pretreated parent sample (Column 2). Specimens tend to be small taxa, with species such as *R. parvidentatum* and *B. constans* most frequently observed. Please refer to Nannotax (ina.tmsoc.org/Nannotax3) for species concepts of taxa listed in the table

The Ashby core sample shows a very abundant and diverse mixed Cretaceous and Eocene assemblage. This is the first sample containing Cenozoic forms that has been examined using the pretreatment method, and contains a high abundance of small reticulofenistrids (2–5µm). No large taxa were documented in the Eocene residues and, as expected, the observed size distribution of small reticulofenestrids is similar to that of Cretaceous taxa such as *Biscutum constans*, *Repagulum parvidentatum* and *Zeugrhabdotus erectus* (Table 5).

5. Summary and conclusions

The nannofossil pretreatment method of Shamrock *et al*., (2015) was reexamined to determine its utility on samples with considerably lower residual organic material than the original study. Results indicated samples with as low as ~1–3% μ_{org} can be notably improved with sample pretreatment. The pretreatment process lightens organic matter, dissociates sediment aggregates and reduces the 1–2µm fraction of background sediment. As a result, pretreated smear slides showed consistent increases in relative abundance and species richness. In contrast to samples with >7% μ_{org} that required two iterations of the NaClO treatment, samples with ~3% or less were sufficiently treated by reducing the procedure to only one exposure to the oxidizing reagent.

Nannofossil specimen loss was observed during the decanting process, but was negligible when compared to the primary assemblage. Decanted specimens were small to very small, with $\mu_{length} \approx 3\mu m$. The relative abundance of small taxa in the residue was related to the relative abundance of the taxa in the sample. This was generally a few percent, or less, of the total relative abundance observed in the treated samples, and does not appear to affect relative abundance schemes, general biostratigraphy or paleoenvironmental analysis. In fact, it may be argued that with the recovery of additional taxa and increases in both abundance and species richness, the pretreated samples may give more accurate data with respect to both biostratigraphy and paleoenvironmental interpretation. Results of both studies show nannofossil samples with varying amounts of residual organic matter can be significantly improved with pretreatment, and this procedure may both enhance paleoenvironmental interpretations and increase potential biostratigraphic resolution in organic-rich lithologies.

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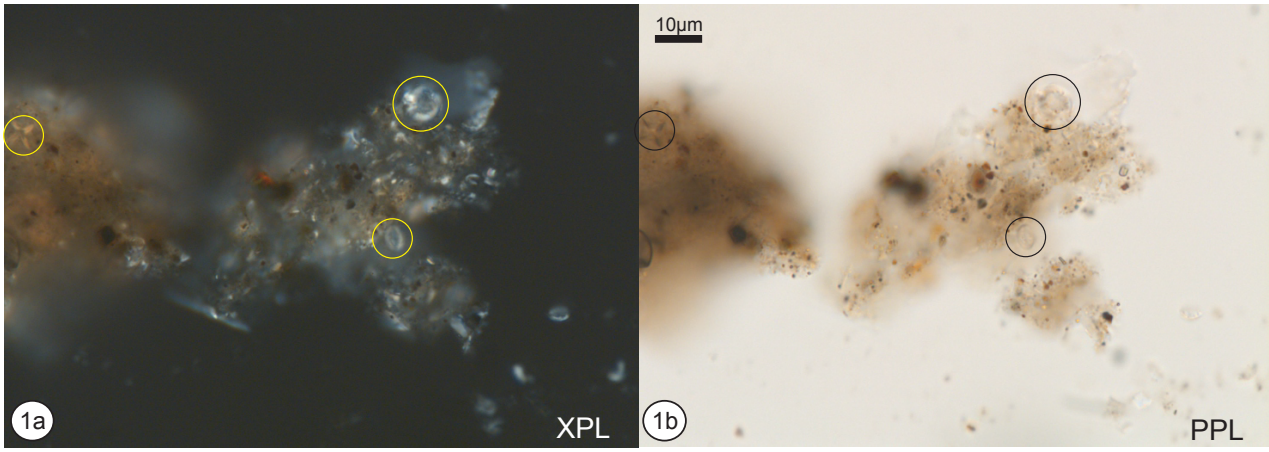
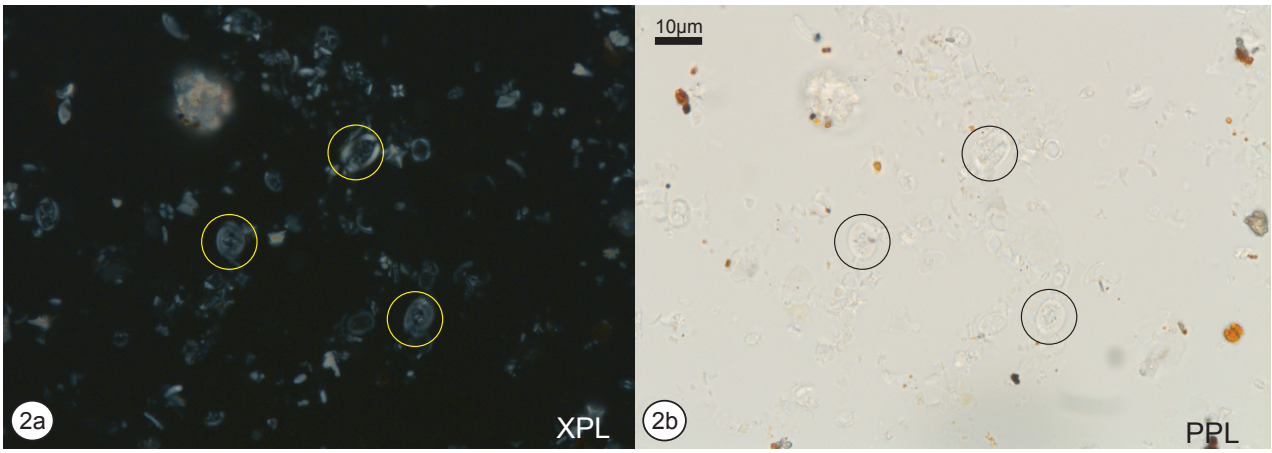
Plate 1

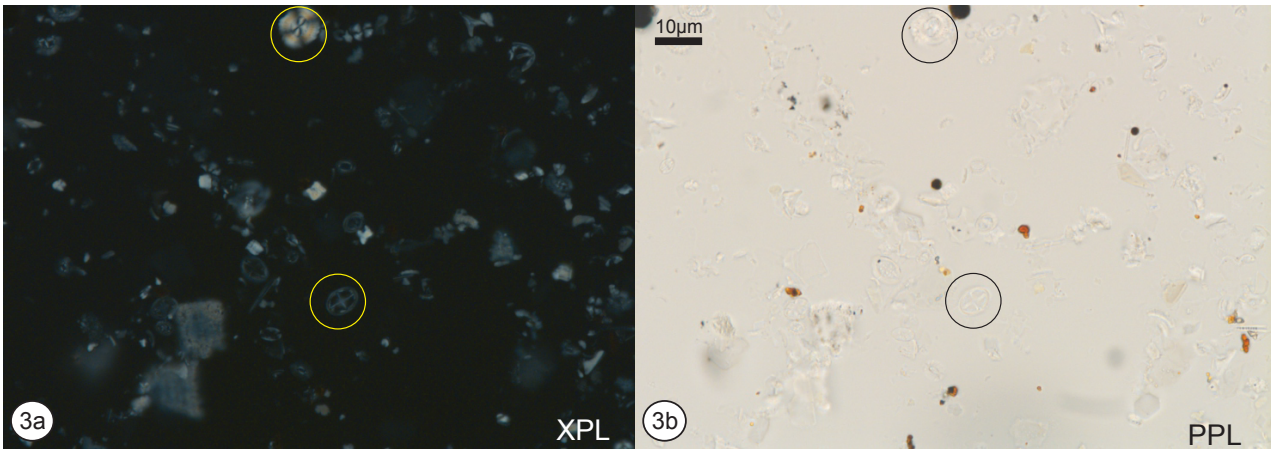
Sample CFR-BL 23.0
Photomicrographs taken at 630x for wider FOV; Selected specimens highlighted for comparison



Untreated slurry-smear slide; organic matter particularly evident in PPL



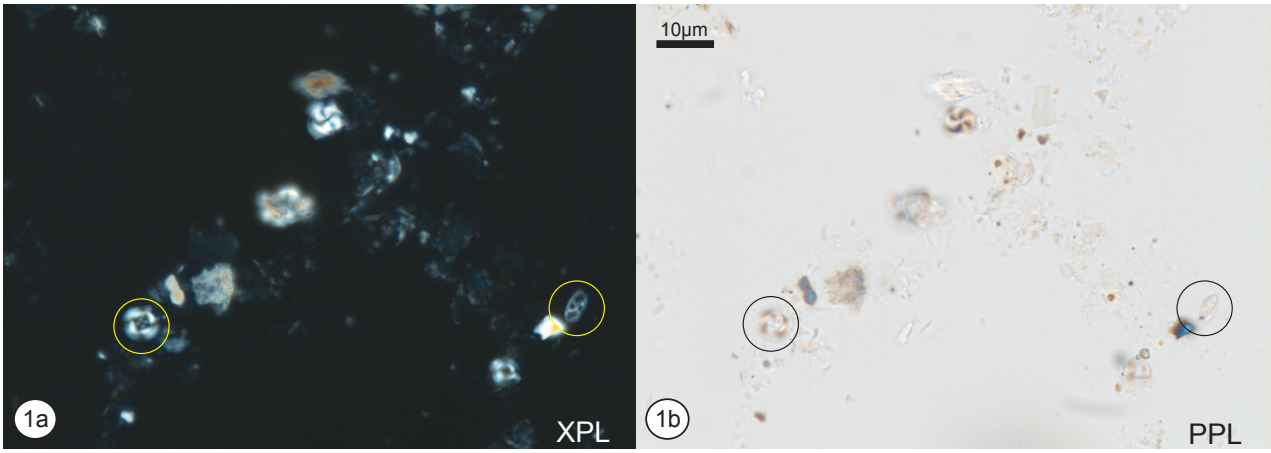
Pretreated with one exposure to NaClO solution



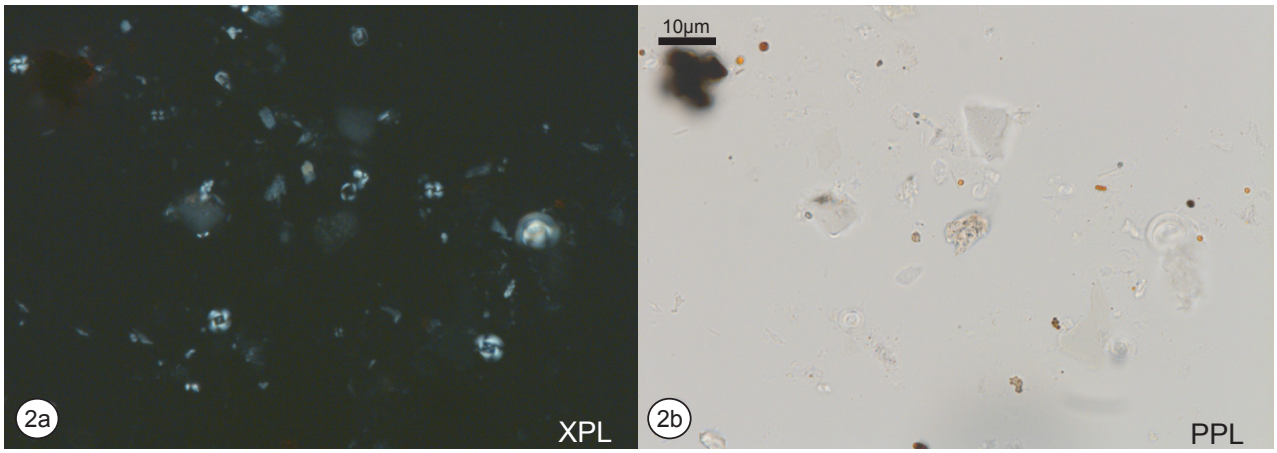
Pretreated with two exposures to NaClO solution

Plate 2

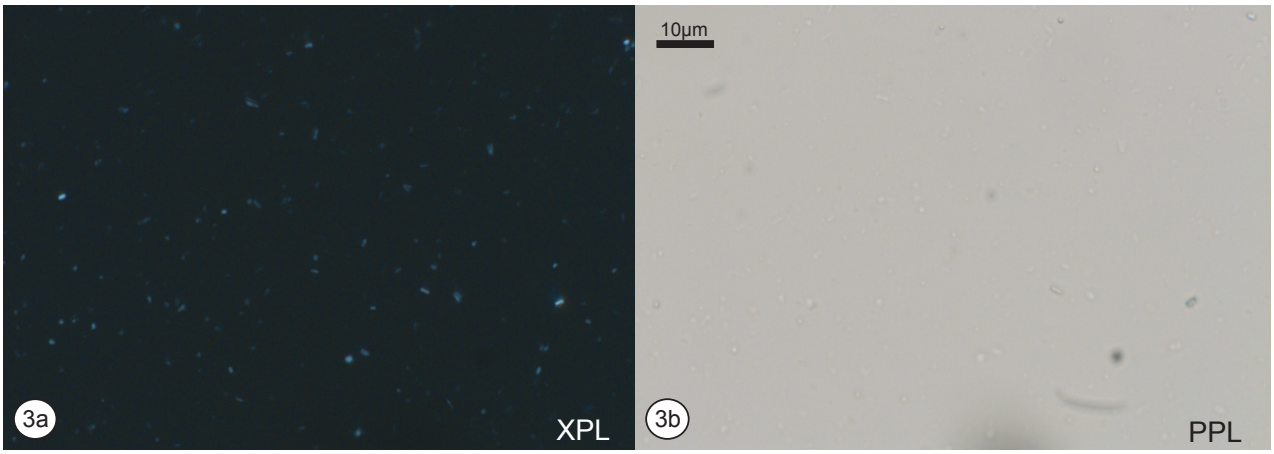
Sample AC 332.7 - Photomicrographs taken at 630x for wider FOV; Selected specimens highlighted for comparison. Sample shows low μ_{org}, but is improved by processing through dissociation of sediment clumps and removal of clay-sized background material (3a-b)



Untreated slurry-smear slide



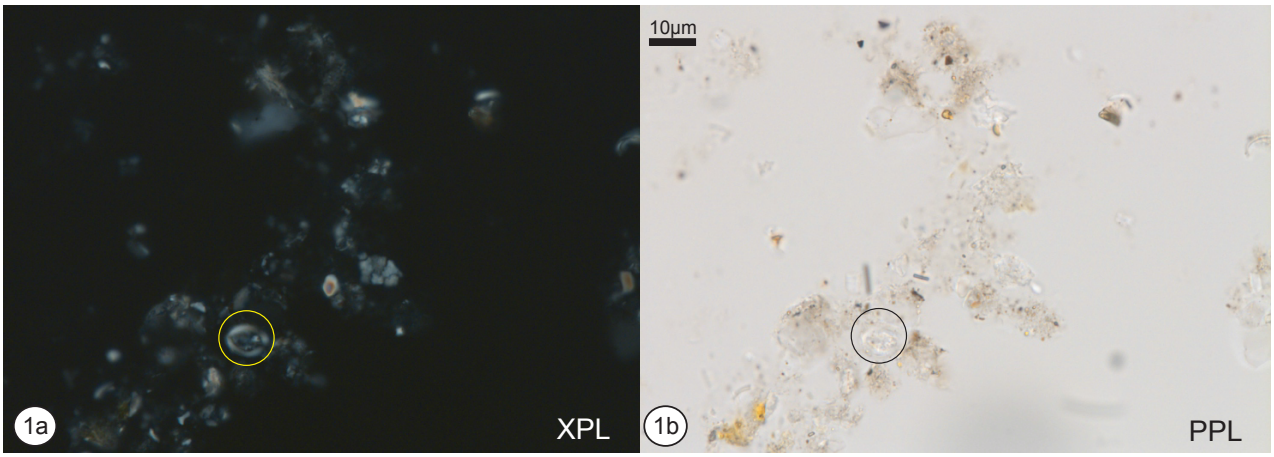
Pretreated with one exposure to NaClO solution



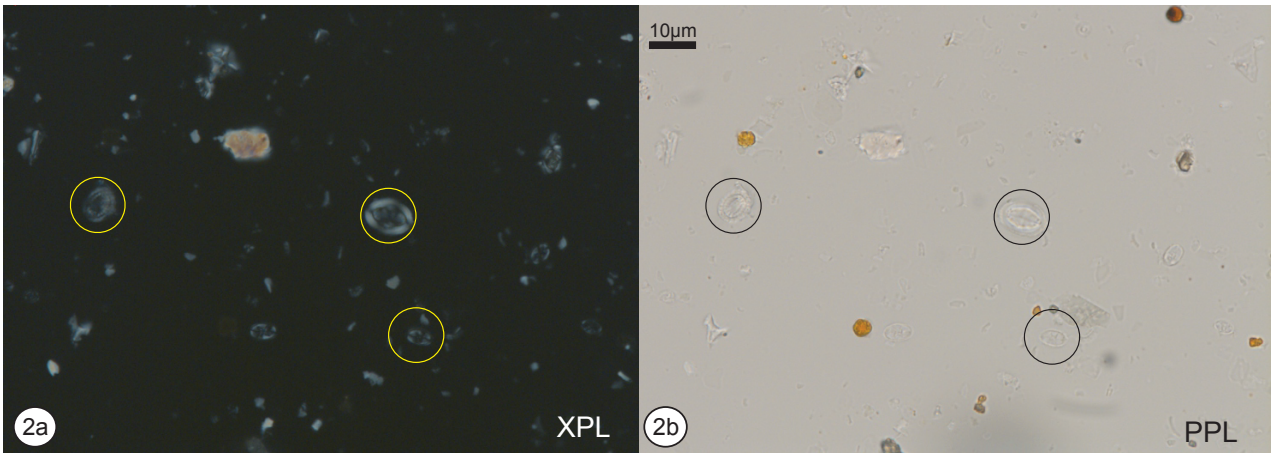
Residue slide produced from decanted supernatant

Plate 3

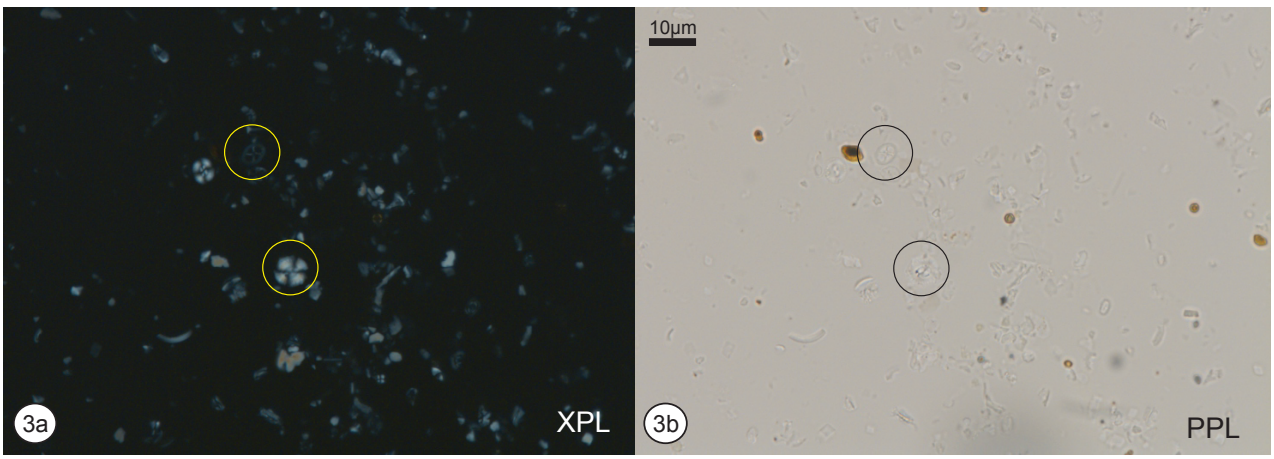
Sample CFR-ML 6.0
Photomicrographs taken at 630x for wider FOV; Selected specimens highlighted for comparison



Untreated slurry-smear slide; organic matter particularly evident in PPL



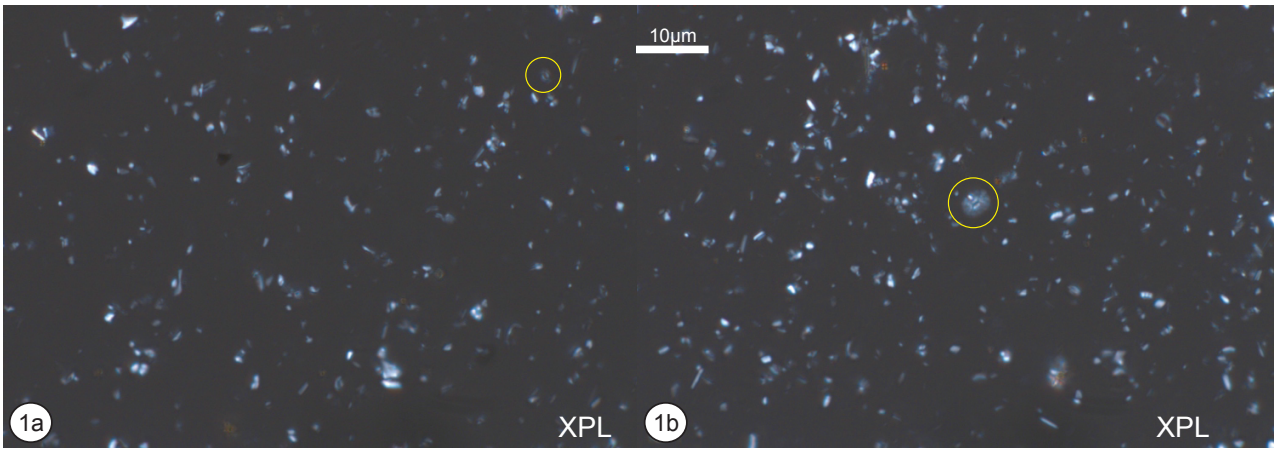
Pretreated with one exposure to NaClO solution



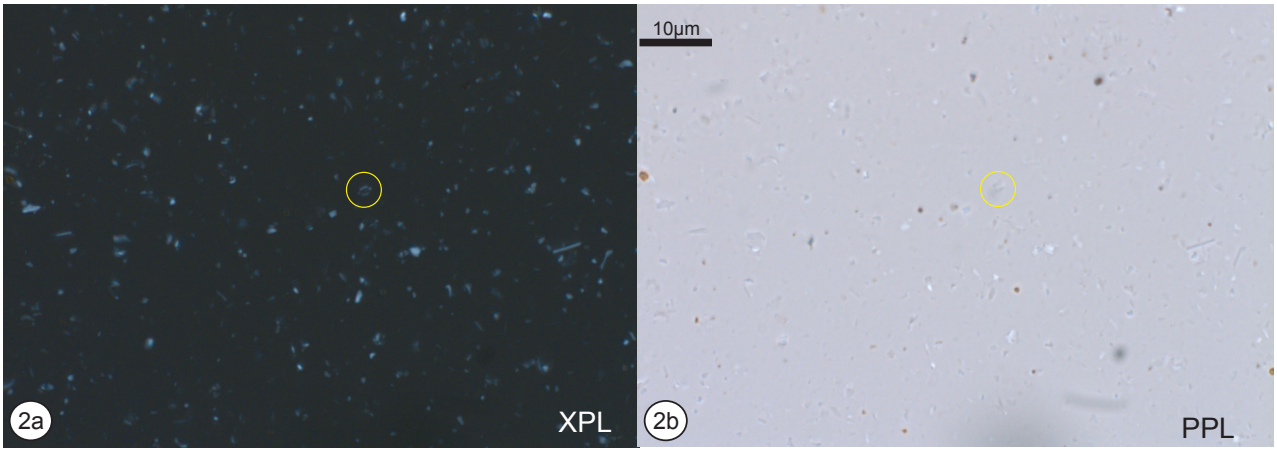
Pretreated with two exposures to NaClO solution

Plate 4

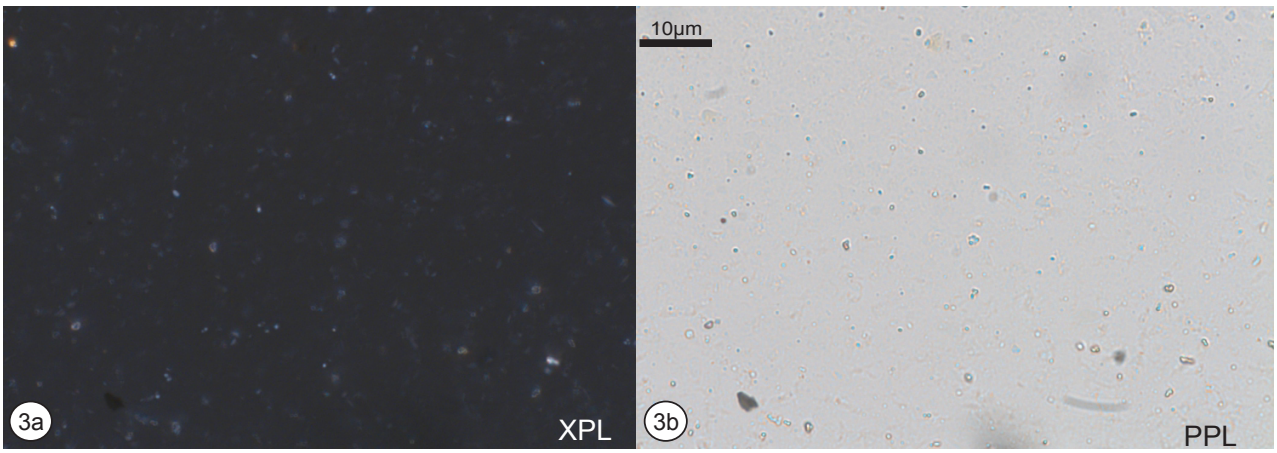
Photomicrographs of supernatant residues, taken at 630x. Decanted residues consist primarily of clay-sized (2-3µm) particles reflecting dominant sediment mineralogy (1, 2: $CaCO_3$, 3: SiO_2), with rare small nannofossil specimens, highlighted for comparison.



Sample CFR-BL 23.0



Sample CFR-ML 6.0



Sample HC 369.0