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THE SETUP, USE AND EFFICACY OF SODIUM POLYTUNGSTATE SEPARATION METHODOLOGY WITH REPSECT TO MICROVERTEBRATE REMAINS

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ABSTRACT

Concentrated deposits of small remains from vertebrates, termed microvertebrate sites or vertebrate microsites, are a unique and detailed source of information about the history of life. Collecting fossils from these sites, however, presents unique challenges. The most time consuming, and thus most deterring, aspect by far is the separation of the fossils from the sediment. This study attempts to quantify to what extent the use of sodium polytungstate (=sodium metatungstate, $\mathbf{Na_6H_2W_{12}O_{40}}$, abbr. SPT) filtration increases fossil concentration, how quickly fossils sink in SPT solutions, and what is a good working density for SPT. We do this by generally following the methodology set out by previous authors, although with some substantial modifications, on an Upper Triassic deposit dominated by clay minerals and lithic fragments, as well as on a second, smaller quartz sand dominated microsite. We also provide a revised and detailed guide with our modifications to former practices and our recommendations to other workers interested in creating a SPT laboratory, including the strong advisory to work over thin plastic sheets, as SPT can react with metal and adheres strongly to glass when it crystallizes.

Our experiments have shown a significant improvement in fossil concentration (from ~2% of the clasts being fossils to ~19%) at the main site, with a sample from the other site showing the treated concentrate as 25% fossil. We have also found very few fossils in the float (<0.5%), but noticeable rates of fossil loss in SPT solutions above ~2.80 g/mL (up to 16%). Further, we have found that 2.75 g/mL is a good working density for several lithologies, as it is high enough to float most rock, low enough to sink most fossils, and low enough to be manageably maintained. SPT has, in processing one particularly rich site, saved many person-hours that otherwise would have been spent picking through less concentrated sediment.

RESUMO [in Portuguese]

As concentrações de depósitos de restos de pequenos vertebrados, chamados sites microvertebrate ou microsites vertebrados, são uma fonte única e detalhada de informações sobre a história da vida. A colheita de fósseis destes locais, no entanto, apresenta desafios únicos. O aspecto mais demorado é de longe a separação dos fósseis do sedimento. Este estudo pretende quantificar até que ponto o uso de politungstato de sódio (= metatungstato de sódio, Na6H2W12O40, abreviatura SPT) na filtração fósseis aumenta a sua concentração, a rapidez com que os fósseis temdem a afundar em soluções de SPT, e determinar qual é a densidade ideal para o uso de SPT. Seguimos em geral a metodologia estabelecida por autores anteriores, embora com algumas alterações substanciais quando aplicada num depósito Triásico Superior dominado por minerais de argila e fragmentos líticos, bem como um segundo depósito, dominado por areia de quartzo. Nós também fornecemos um guia revisto e detalhado com as nossas alterações de práticas e as recomendações para quem esteja interessado na criação de um laboratório de SPT. Aconselhamos a trabalhar em plástico fino, uma vez que o SPT pode reagir com o metal e adere fortemente ao vidro quando se cristaliza.

As nossas experiências mostraram uma melhora significativa na concentração de fósseis (de ~ 2% dos fragmentos fósseis sendo a ~ 19%) no primeiro depósito, e uma amostra do segundo depósito pode através desta metodologia concentrar-se com 25% de fósseis. Foram também encontrados muito poucos fósseis no flutuante (<0,5%), mas as taxas de perda perceptíveis de fósseis em soluções de SPT é acima dos ~ 2,80 g/ mL (até 16%). Além disso, verificámos que 2,75 g / mL é uma boa densidade de trabalho para litologias diversas, porque a densidade é alta o suficiente para flutuar mais rocha, e, por outro lado, baixa o suficiente para afundar mais fósseis, e baixa o suficiente para ser manejável. SPT, no tratamento de um local particularmente rico, poupou muitas horas-pessoa que de outra forma teriam sido gastas por triagem imediata do sedimento necessariamente menos concentrado.

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INTRODUCTION

Studies of microvertebrate fossils (or vertebrate microremains) are becoming increasingly common (Sankey and Baszio, 2008). Despite providing a wealth of information about past environments and ecosystems, microvertebrate studies are stymied by the difficulty of collection. The process of collecting and isolating large numbers of what are, by definition, tiny and potentially fragile fossils can be extremely time consuming and tedious. Methods have been developed to expedite this process (Cifelli et al 1996:18, Wilborn 2009), yet the fundamental methodology remains extremely similar to its original construction (Hibbard 1949). Further, no one to date has quantified the efficacy of these methods for vertebrate paleontology (though see Bolch, 1997 for dinoflagellates, Krukowski, 1988:315 for conodonts, Murray and Johnston, 1987:319 for heavy minerals in sediments, and Munsterman and Kerstholt, 1996 for palynological experiments). After a site has been located, it is typically surface collected, then excavated, with vast quantities of sediment being taken away. These bags of sediment are then screen-washed in an attempt to remove as much clay and fine silt, while simultaneously retaining as many fossils, as possible. After screen-washing, there typically remains a significant volume of concentrate, which is usually composed primarily of non-fossil clasts. After this step, a researcher, preparator or volunteer must go through the screen-washed concentrate one pinch of sediment at a time under a light microscope, isolating and removing individual fossils. These standard techniques for recouping micro-vertebrate remains from concentrate are extremely time intensive and often dependent on an extensive time investment by students or volunteers (Hibbard, 1949, Grady 1979).

Inevitably, there will be fossiliferous concentrate that needs to be hand picked. The advantage of heavy liquid separation techniques is that they reduce the amount of unnecessary (nonfossiliferous) sediment that needs to be picked through. Traditionally heavy liquid separation was often accomplished using bromide liquids, with their extremely toxic nature representing a significant drawback (Cifelli et al., 1996:17, Murray and Johnston, 1987:317, Murry and Lezak, 1977:17). Murray and Johnston (1987:319) compared SPT to tetrabromoethane (TBE) and found no significant difference for sedimentological applications in the final product, noting only cost and viscosity (concurrent with Cifelli et al., 1996:17-18, though see Jeppsson and Anehus, 1999:57 and below for explanations of this discrepancy) as drawbacks to SPT.

Heavy liquid concentration, regardless of the chemicals used, makes picking both easier and more enjoyable (finding lots of fossils instead of few fossils per unit volume). This also maximizes research time by speeding up fossil recovery. The heavy liquid discussed here, sodium polytungstate (=sodium metatungstate, $\mathbf{Na_6H_2W_{12}O_{40}}$, abbr. SPT) can be purchased dry and dissolved in deionized water to any desired density from 2.00 g/mL to 3.10 g/mL.

Tungsten compounds have been found to be safe in general (Kazantzis, 1979), and sodium polytungstate, unlike bromides and kerosene, is generally regarded as safe unless ingested or applied to the eye (Cifelli et al., 1996:17 and many references therein, also see the Material Safety Data Sheet [MSDS, linked in references] or equivalent safety documentation). Further, sodium polytungstate can be reused continually, assuming it is taken care of properly. It is however, quite expensive (>$200 per 0.1kg), and traditionally difficult to obtain (though the Internet has reduced that problem, as a simple Google® search will reveal). Further, we followed the recommendations of Callahan (1987:765) in using bleached coffee filters instead of filter paper (contra Murray and Johnston, 1987:318) as they appear to speed recovery, but they also seem to have allowed clays to enter and discolor the SPT (though no other side effects have been confirmed, they may have absorbed some of the SPT as a precipitate, see McCarty and Congleton, 1994:198). Six et al. (1999) describe a process of cleansing SPT of organic contents by percolation through a column of activated carbon, and similar methods may work for the removal of clay, though we did not test this, and Murray and Johnston (1987:317-319) and Callahan (1987:765) both argue that laboratory-grade filter paper is enough. Yet as a possible (though unlikely) consequence of clay contamination (clay from a previously separated site contaminating future sites' fossils) we advise caution in performing geochemical analyses on SPT separated fossils without heavily rinsing them until further studies on the solution's effects and the efficacy of clay removal are performed.

Despite these modest drawbacks, SPT still provides a powerful tool for the paleontologist/preparator's arsenal, as we found it easy to use, efficient, and very effective (see below). The ability to continually

reuse it, as well as its speed and efficacy, make it cost effective in the long run, albeit a rather large initial investment is required. Here we outline the materials we recommend for a sodium polytungstate separation laboratory, the methods of separation, and the efficacy of the system.

MATERIALS & SET UP

The primary site we chose for the study comes from the Upper Triassic Moncure locality (NCPALEO1904) in North Carolina. The site is a pedogenically altered deposit composed primarily of sand- to silt-sized clasts of clay minerals, and final estimates are that ~90% of the non-fossil clasts were removed. We also investigated, albeit to a lesser extent, a quartz-dominated sand deposit and a claystone rich in iron concretions. As the lithology of the sediment varies, the methods and results of this methodology vary, so our results should be viewed as a case study, rather than an absolute rule. However, our results are highly encouraging, and we recommend a starting density of 2.70-2.75 g/mL. See below for details on how to determine the ideal working density for a given locality.

Before the efficacy of SPT separation could be determined, a laboratory had to be set up. We followed most of the suggestions put forth by previous authors (Callahan 1987, Cifelli et al. 1996:18-22, Krukowski, 1988:314, McCarty and Congleton, 1994:190-201), though with many adaptations of our own. The following guide is thus adapted from Cifelli et al. (1996:18-22), previous work (Callahan 1987, Krukowski, 1988:314, McCarty and Congleton, 1994:195-201, Munsterman and Kerstholt, 1996, Murry and Lezak, 1977:16-18), and our own observations and experiments. The main points in which our guide is different from those of previous workers is in our use of plastic coverings, containers beneath the main containers, and within-container nets. These measures all serve to reduce downtime, make the separation process faster and easier, and maximize sodium polytungstate retention and recovery. Previous authors noted high viscosities and slow fall times for SPT solutions, but that was not our experience at all, and we found SPT to have extremely low viscosities and fast sink times at densities of 2.7-2.8 g/mL. Despite being relatively safe, caution should always be at the forefront, and as such we advocate the use of waterproof, disposable gloves (we use powder-free latex, from which SPT residue can be recovered) and that work is performed under a fume hood. We provide a list of recommended materials in Table 1. These items will all need to be purchased, and most of the laboratory set up, before any SPT is mixed. Some of the materials will have to be fabricated (e.g. the weights and nets) and others will have to be prepared. Here we present step-by-step instructions through the processing of fossiliferous material as though one has not yet set up the lab (see Table 2 for abridged version).

Materials List
Sodium polytungstate
Hotplate
Hydrometer calibrated to 2.0-3.0 g/mL
Deionized water (and plastic squeeze bottles)
Deep, sealable, plastic containers
Plastic graduated cylinders (250mL)
Plastic funnels (large)
Plastic stirring rods
Bleached coffee filters
Nylon mesh (opening size dependent on size of desired fossils)
Sealable plastic vials (such as centrifuge vials) and metal shot (steel or lead)
Large, flat containers (like baking trays)
Plastic ladle (preferably with a spout)
1L Beakers (plastic is preferable, but glass is acceptable for these)
Large (5 gallon) plastic buckets that can be nested

Table 1. A list of materials for setting up a sodium polytungstate laboratory.

First and foremost, as per Krukowski (1988:314), plastic tools and containers should be used. We cannot emphasize this enough. Glass is suitable, but plastic is by far and away preferable, as it does not react with the SPT (as does metal) and dried SPT residue flakes off of it easily, allowing for quick recovery (as opposed to glass, to which SPT adheres strongly). Because plastic is so convenient for recovery, we recommend covering the work area with plastic wrap or a waterproof tarp, to aid in the recovery of spills (if a drip falls upon the plastic wrap/tarp, merely let it evaporate

Laboratory Construction
Fill sealable plastic vials with metal shot to create weights.
Fashion nets to slightly larger than the base of your SPT containers out of waterproof (noncloth) mesh. Tie strings to the end, and attach weights to the bottom.
Cut more pieces of the mesh to fit inside your coffee filters. Again, make them larger than what they go into.
Wrap your basal containers in plastic (if they are not made of it), and put them in your workspace. Then cover the entire area you will be working on in plastic.
Fill a graduated cylinder with an appropriate amount of dry SPT (be conservative), and a graduated cylinder with an appropriate volume of DI water. Pour the water into the beaker, and then add the SPT slowly, stirring with a plastic rod.
Use the ladle to remove a sample of the SPT and test its density with a hydrometer in a graduated cylinder.
If too light, continue adding SPT until the desired density is reached. If too dense, add DI water to another beaker and pour the SPT solution into that.
When the desired density has been reached, place the deep container in the workspace on the basal tray and pour in the SPT. Insert the weighted net, and then begin processing.

Table 2**.** A quick guide to setting up a sodium polytungstate laboratory.

and then remove the SPT flake and place it back in a recovery solution). Further, setting the main SPT containers in baking trays (or other large flat containers with prominent lips) that have been covered in plastic (or are made of plastic) is strongly recommended. Plastic containers, and large plastic bins, can be readily attained from department or hardware stores (large Rubbermaid® containers work well for this). Working in plastic containers helps to contain any spills and further aids in recovery (Figure 1). Essentially, cover the work area with plastic wrap, and place plastic-covered flat containers on top of the plastic wrap for the initial setup. Now cut the mesh net to slightly larger dimensions than the bottom of the SPT containers (NOTE: Making the net larger than the bottom of the container allows it to adhere to the side in the SPT, preventing fossils from "missing" it, although floating material can adhere to the sides of a tall net) and tie plastic strings (or strips of netting) to each of the corners (Figure 2) and place small, sealable, plastic containers filled with metal shot on the mesh net (NOTE: tying the vials down to the corners and the center of the net is advisable). We used common centrifuge vials but any small plastic, watertight container will do. Also, cut squares of mesh net to go inside the coffee filters. Place the mesh-filter complex inside a plastic funnel, and the funnel onto a graduated cylinder. The two large (5 gallon/20 liter, or larger, depending on your needs) bins will be used for SPT recovery. One should be used to hold dilute SPT, while the other should have holes drilled in the bottom, near the center (NOTE: avoid the periphery, as water will pass through this bucket into the lower one, and keeping the holes near the center will lessen the chance of solution splashing out of the lower bucket) and be lined with the nylon mesh. This second bin will sit atop the dilute SPT bin, and post-treatment sediment and filters can be placed in here and rinsed (with DI water that will then percolate down, into the bottom bin).

Next place the deep plastic containers on the plastic-covered baking trays (see Figure 1). The sodium polytungstate powder can now be mixed with deionized water in the beakers to the values provided by the manufacturer. Krukowski (1988:315) advocated adjusting the solution's density only at the desired working temperature (to avoid temperature induced affects), and we concur. We found that a density of ~2.75 g/mL works best, and we do not recommend going above 3.00 g/mL, as the solution can quickly lose enough water to cause surface and edge crystallization. We found 2.65 g/mL to be too low for our two sites (too much quartz sank), but we did use a working density of 2.65-2.70 g/mL for a third site (in accord with Cifelli et al, 1996:18). We recommend filling two graduated cylinders to the appropriate level/weight, one with dry SPT and one with water, and then pouring the contents (first the water, and then the SPT) into the beaker. Always add the SPT to deionized water, never the reverse (Cifelli et al., 1996:18). This goes for solutions as well; always add more concentrated to less concentrated. If the SPT becomes too dense, adding DI water to graduated cylinders, then filling them with SPT, is an easy way to lower the density, though go slowly, as it is significantly easier to lower the density than it is to increase it. Adding water directly to SPT can result in density stratification. Use the hydrometer to confirm the solution's density, and to tweak it as desired by adding either SPT powder or water. Once the solution is made, fill the deep plastic containers about three-fourths (75%) of the way with the desired solution. To test a

density's efficacy, we recommend filling a graduated cylinder with the SPT, and placing representative samples of fossils (teeth, bones and scales, for most sites) as well as some sediment into the graduated cylinder, one subsample at a time (the scales, then the bones, then the teeth, for instance). Should an unacceptable amount of fossils float, lower the density, should too much sediment sink, raise the density. This allows for easy assessment of the SPT and quick recovery of the fossils and sediment when the assessment is complete.

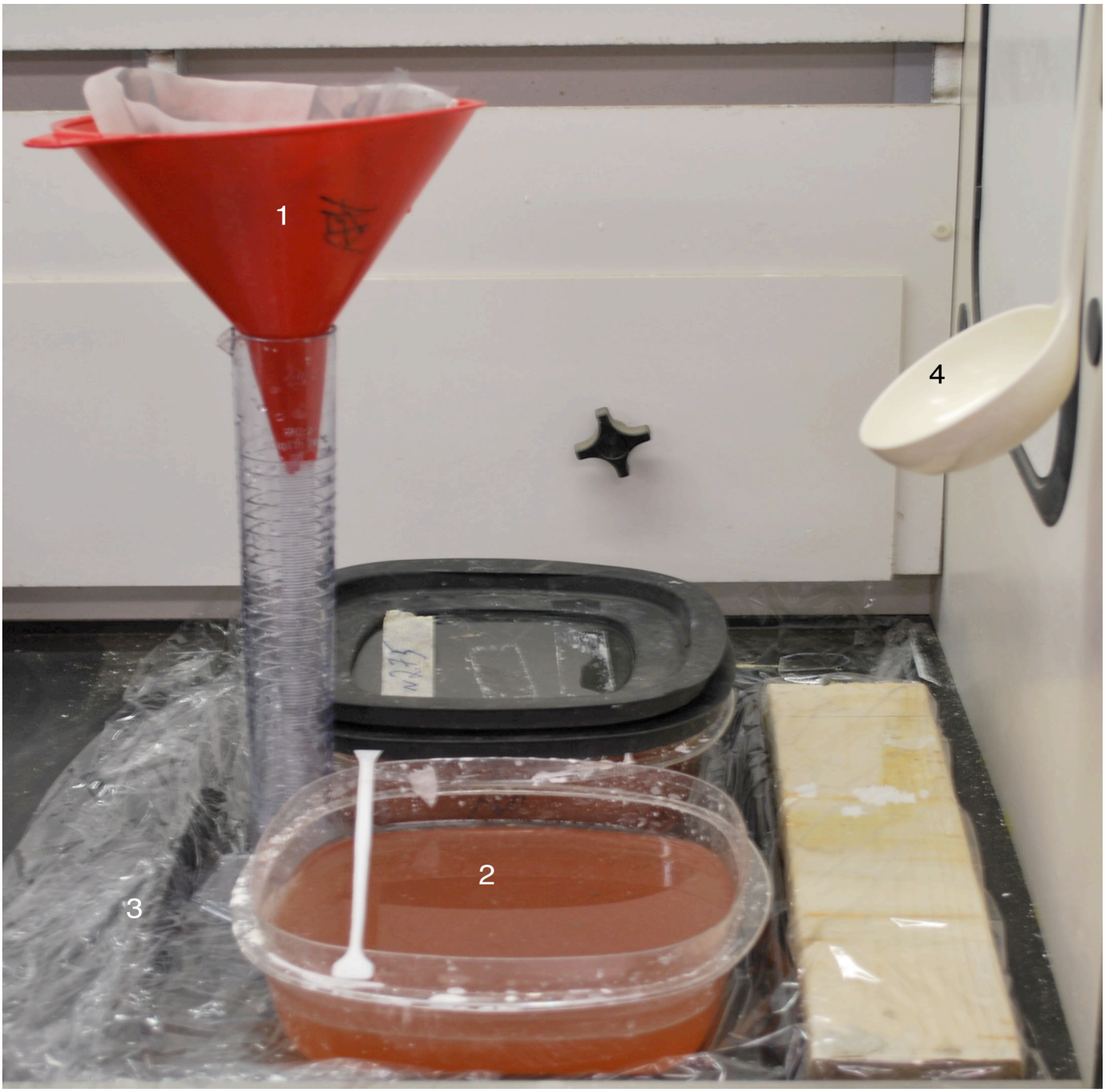

Figure 1. An example of a small SPT processing station. Note that this is highly reduced to emphasize the main components: the large funnel with nested filters and nets (1), the deep container (2) with clay-tinted SPT, the plastic covering over the basal tray holding the rest of the equipment (3), and the plastic ladle (4). In standard use there would be several graduated cylinders and multiple SPT-filled containers with nets in place, as well as a hydrometer (easily stored in a graduated cylinder of DI water).

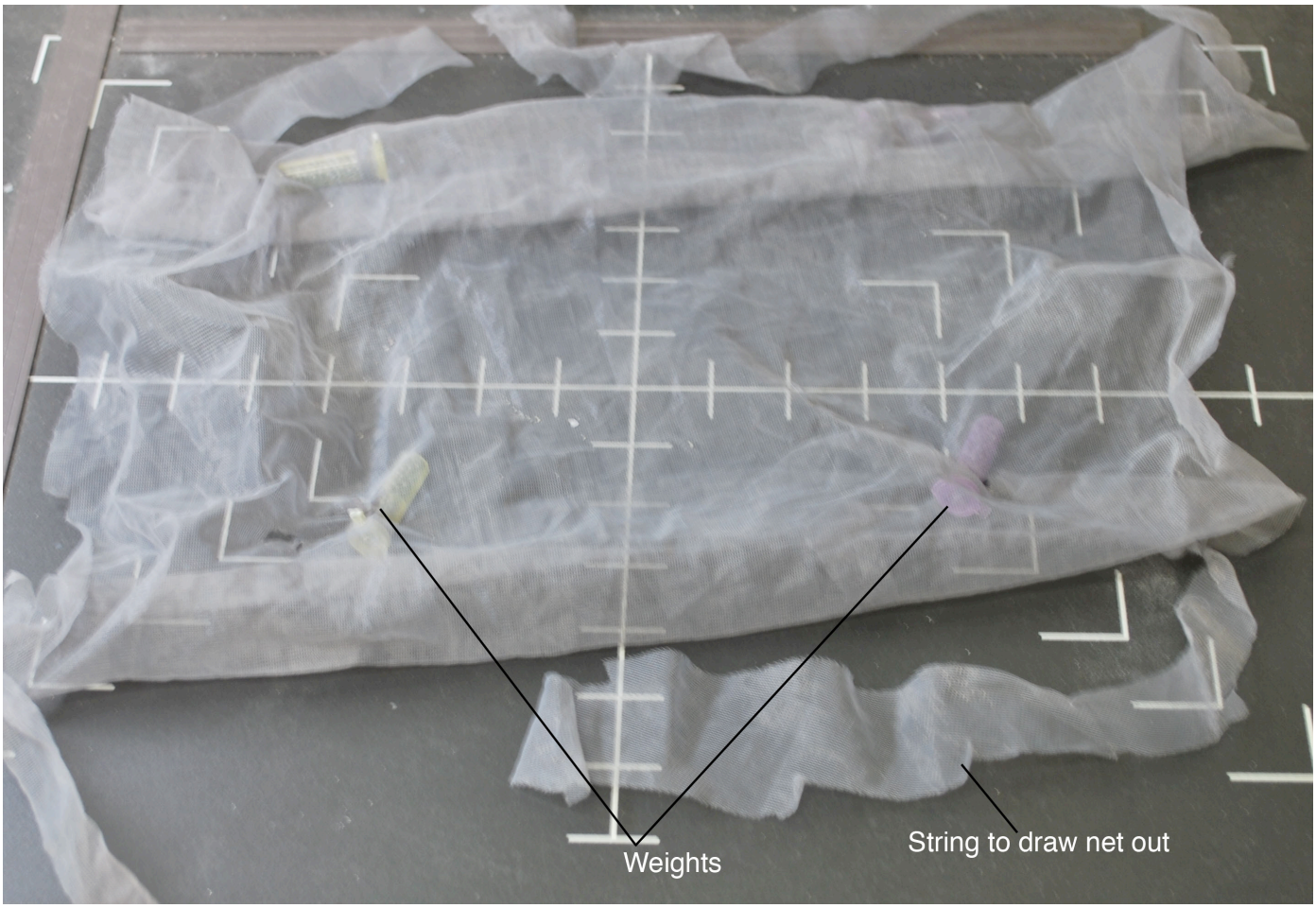

Figure 2. An example of a mesh net used for fossil extraction without needing to dump out SPT containers. Note the weights tied to the bottom and the strings to the corners.

Processing Guide
Slowly pour sediment into the deep container, mixing continually, until a thin layer of sediment is present across the top of the liquid.
Mix gently for a few seconds, and then allow the mixture to stand for several minutes, though the actual amount of time will vary with sediment differences.
Use a ladle to slowly skim off the sediment, submerging it only slightly to try and keep as much SPT in the container as possible.
Pour the ladle out into a graduated cylinder via a funnel lined with a coffee filter and a mesh net. Over the course of processing, periodically check the density of the SPT in the graduated cylinder.
Continue until 1) the sediment is gone, 2) the graduated cylinder is full (multiple cylinders are recommended) or 3) the SPT gets too low to continue without disturbing the fossils on the bottom (deep SPT containers/conservative sediment removal practices help prevent this).
When ready, add more sediment. We recommend adding the SPT from the graduated cylinder back into the main container after the next sediment sample, to facilitate mixing.
When either the sediment is depleted or you wish to see the fossils, skim all remaining sediment from the top, and remove the net. Place the net in a beaker of DI water, and turn it upside down. The fossils will sink instantly, and the net will be ready to return to the SPT.
Decant the water from the fossil-containing beaker into the large "Dilute SPT" bucket. Allow fossils to dry in beaker and, when dry, pour them into a processing tray for picking.
Place the sediment caught in the filters, and the filters themselves, into a large plastic bin with holes drilled in the bottom and a net covering the bottom. Nest this bin above the large, dilute SPT container and allow DI water to percolate through the sediment and filters, redissolving the SPT.
If sediment is still adhering to any tools, use a squirt bottle of DI water to rinse them off into the sediment bucket, otherwise, simply rinse all other tools off by submerging them in the dilute SPT and then rinsing them with DI water above the dilute SPT container.
Finally, seal all SPT containers up, and place the hydrometer in a graduated cylinder of DI water.

Table 3. A point-by-point guide to processing fossils in a sodium polytungstate laboratory.

SEPARATION METHODOLOGY

Here we present a detailed guide to processing sediment with SPT, greatly expanded from techniques outlined by Cifelli et al. (1996:17-22), but see Table 3 for an abridged version. With the laboratory set up, the solutions mixed, and the deep dishes 75% full of SPT solution that completely immerses the net, pour fossiliferous sediment (untreated concentrate) into the SPT-filled container. For best results, we recommend creating a thin, floating layer on the surface of the solution before stirring it gently with a plastic rod. After stirring, allow the concentrate to settle for approximately 5 minutes (NOTE: this time is based on the fossil sites we have investigated, other times may vary and we suggest experimenting with the solution to determine optimum time). After the "heavies" have settled, use the plastic ladle to skim off (remove) as much of the floating sediment as possible (and, with each ladle, try to leave as much SPT in the container as possible). Ladles with spouts are recommended, because they can be gently immersed so that the sediment flows into the ladle through the spout, allowing for most of the SPT to remain in the container. This is desirable to avoid disturbing the "heavies" if the SPT in the container gets too low and thus accidentally ladle them out (this is also why deep containers are preferred).

Figure 3. A close-up of the funnel apparatus with the outer plastic funnel (1) and inner mesh net (2).

Pour the ladle out into the funnel (the sediment will be caught by the mesh, and finer particles by the coffee filter, and the clean SPT will trickle down into the graduated cylinder, see Figure 3) and repeat until there is little or no floating sediment remaining in the deep container. There will be a small amount of sediment adhering to the ladle that can be ignored for now, as each ladle-full of floating sediment and SPT will remove/replace it. Continue processing until all of the floating sediment is removed (a small amount may continue to adhere to the sides of the net and container, but this amount is negligible and can be easily picked out from the fossils later). Once the floating sediment has been removed from the SPT, any tools with sediment adhering to them should be rinsed in a beaker of deionized water, which can then be decanted into the dilute SPT storage container.

We recommend checking the density of the SPT often (every couple of hours), and while the clean SPT is in the graduated cylinder is the ideal time to do so. Now, pour more concentrate into the deep container, and then pour the clean SPT from the graduated cylinder back into the deep container (this will help mix the new sediment batch). Repeat this process until there is no more concentrate, the nonfossiliferous "float" has been removed, and all desired microremains are on the net at the bottom. At this point, carefully remove the net and hold it above the SPT container until the SPT drainage reaches a slow drip (usually ~10sec), then gently squeeze the portion of the net above the fossils to force most of the remaining SPT out. Once the SPT is mostly gone, gently place the net upside-down in a beaker filled with deionized water. The fossils will sink to the bottom of the beaker, and the deionized water will clean the fossils and net. Decant the deionized water out of the beaker into a large container meant to hold dilute SPT, and then rinse the fossils again. Leave them in the beaker until they have dried, then pour them into a sampling tray and begin to pick through them. The floated sediment and leftover filters can be stored in a large plastic bin, with holes drilled in the bottom and a mesh filter at the base, which can then be rested on top of the dilute SPT container. Pour deionized water into this sediment bin to rinse the sediment, filters and left over mesh. Our recommended use of a net in the SPT container allows for the periodic recovery of micro-vertebrate fossils with minimal interruption or risk of spilling SPT. This is particularly useful in a small laboratory or when processing large samples over the course of many laboratory sessions.

During separation, the mesh-filter complex will fill up with SPT-coated sediment. As it fills up to the point where it can hold no more sediment, it must be changed. Place the filled mesh-filter complex in a funnel over an empty graduated cylinder, and flush it with DI water. Remove the sediment and coffee filter, and place them into the net-lined bin, then rinse the mesh net into the dilute SPT bin. Pour the dilute SPT from the graduated cylinder into the containment bin, then rest the net-lined bin above it, and flush with water to allow percolation through. Leaving the dilute SPT bin open allows for concen-tration via evaporation, though expedited concentration can be achieved through the use of a spare deep container and a hot plate. We recommend using low hotplate settings to evaporate the SPT solution. This is also the only time during the process where we use glass containers, and we only do so to avoid the complications of heating plastic. When the dilute solution on the hot plate gets low, refill it from the dilute SPT container (pouring low density into high density is acceptable here, as thermal convection and evaporation should prevent density segregation, but stirring is still recommended). Krukowski (1988:315) argued that such slow, low-heat recovery methods reduce the likelihood of SPT degenerating into sodium tungstate (Na_2WO_4). Lacking data on this phenomenon, we defer to that work. Also, given the low vaporization point of SPT (MSDS, Krukowski, 1988:315), low heat is further recommended.

Although SPT dries and coats the containers and implements used here, it readily redissolves and is recovered if the above protocols are followed. While we concur with Cifelli et al. (1996:17) that allowing the solution to crystallize is undesirable, we also can confirm Krukowski's (1988:314) observation that crystallized sodium polytungstate is easily dissolved if powdered, or if is present in isolated flakes (such as from a spill that dries on plastic). Time, not SPT, is the only lost commodity when the solution comes to crystallization, and even then, it does not take long to recover. Should spills occur, allow them to dry, collect the residue, powder it, and add it to the dilute SPT. If the dilute SPT is very dilute, and it is going to be awhile (>24 hours) before you attempt to concentrate it, not powdering the SPT is acceptable. We recommend using the waiting period ("down time") while the heavies are sinking to reconstitute SPT. We also recommend leaving dilute SPT containers (such as the large storage bin) open continually to allow reconcentration by evaporation.

QUANTIFICATION METHODOLOGY

We investigated two key questions regarding the use of SPT: to what extent fossils are lost to the float at different densities (due to imperfect separation), and how effective SPT is at concentrating fossils. Previous authors have noted that SPT is effective (see Krukowski, 1988 especially), but we were concerned with determining how much time was saved, and how concentrated the fossils became, to create an effective baseline for future workers to determine whether or not SPT use is cost-effective for their needs.

To quantify fossil loss, we performed controlled experiments on known numbers of fossils and sediment at known densities of SPT. To do this we filled 250 mL graduated cylinders with 131-245 mL of SPT solution and then timed how long it took for the first and last clast of each material type took to reach the bottom, and calculated the fall speed (cm/sec) with which they fell. We used two random samples (sets) of bone fragments, two sets of tooth fragments, one set of fish scale fragments and one set of nonfossil matrix. For each trial, we made note of how many of the clasts and fossils failed to sink, and the percentage of each type that sank at each density. These data are outlined in Table 4.

To quantify efficacy, we sampled our untreated concentrate and both fractions of our treated concentrate. We took random samples from the untreated concentrate, and fractions treated with sodium polytungstate at ~2.75 g/mL and examined the fractions under an Olympus SZX12 binocular microscope to count the number of fossils and the number of clasts. Fossils embedded in clasts were counted as fossils. The fossils and clasts were then each weighed on a digital scale. For two samples the size was so great that we used mean fossils per gram and clast per gram values determined from data collected prior to the analysis (by picking untreated concentrate) to extrapolate count estimates.

Material	Density (g/mL)	Number	Number that Sank	rate (cm/sec)	Percent Sank
Scales	2.82	100	88	0.4-0.03	88%
Bone Fragments Set 1	2.82	30	27	0.8-0.1	90%
Bone Fragments Set 2	2.81	100	84	0.5-0.02	84%
Bone Fragments Set 2	2.7	100	100	1.1-0.08	100%
Tooth Fragments Set 1	2.81	58	53	0.6-0.2	91%
Tooth Fragments Set 1	2.7	62	59	1.6-0.2	95%
Tooth Fragments Set 2	2.8	32	31	1.2-0.1	97%
Tooth Fragments Set 2	2.76	32	32	1.3-0.3	100%
Matrix	2.71	100	2	0.1-0.04	2%

Table 4. Data on sinking rates for the Moncure locality fossils. Note that, for other sites, fossil densities will vary dramatically, as will sinking rates. This data is summarized graphically in Figure 4.

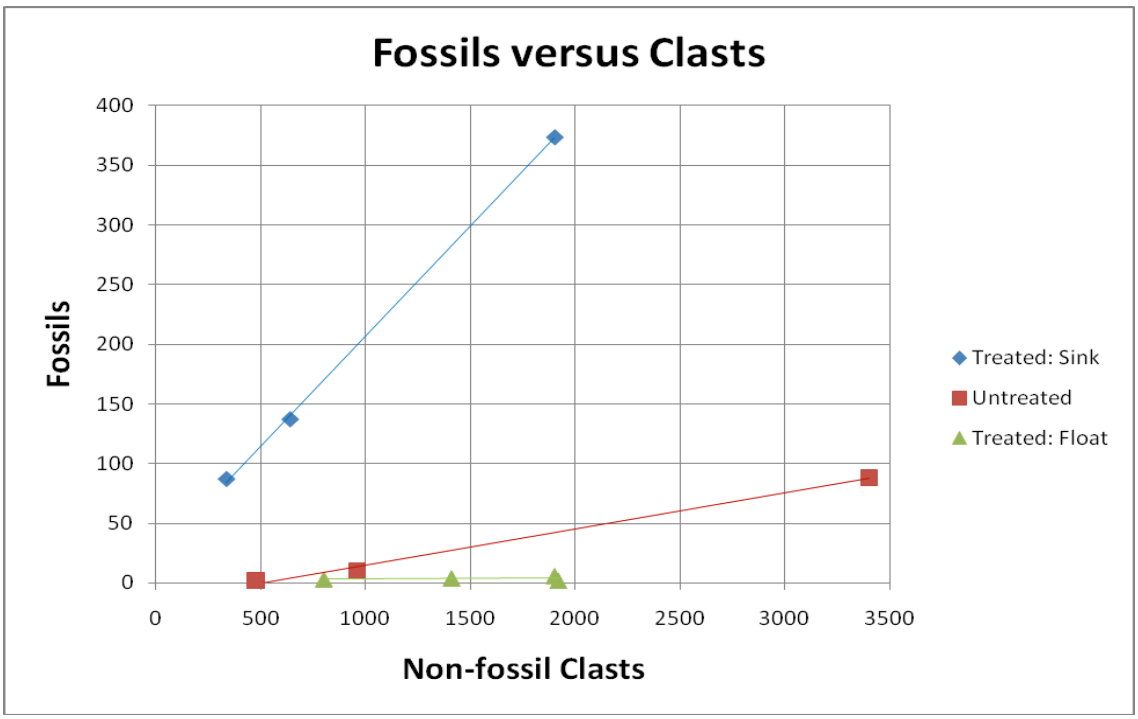

Figure 4. A graph of the number of fossils found versus the number of clasts found in several samples of the three datasets (SPT heavies, SPT float, and non-SPT control). Note that, for a sample of a given size, the Treated: Sink had significantly higher fossil content (see Results above), and that the Treated: Float practically lacked fossils (none of those values is above 10). Linear regression analysis was performed with Minitab v15 calculated adjusted R^2/p-values for the three groups as Treated: Sink 99.9%/0.012, Untreated: 99.0%/0.045, Treated: Float 0.00%/0.751, showing non-random trends for both the Treated and Untreated fractions.

RESULTS

In our controlled experiments, we found that only two out of 100 non-fossil clasts sank in 2.71 g/mL SPT, however by using the known starting mass of sediment (11kg), the known mass of the total sink (929g), and the estimated mass of the fossils present (157g), we estimate that approximately 12% of the total number of non-fossil clasts sank. This discrepency is likely due to the tendency of the non-fossil clasts from Moncure to fracture and fall apart (they are floculated clays, mostly) as well as grain size differences in lithology, resulting in more problematic counts and results than with fossils. Our results on fossil loss show that the percentage of fossils lost increases dramatically with density, with potential loss of as much as 16% for bone fragments in 2.81 g/mL (Table 4). However, these trials also show that the fossil loss can vary dramatically between fossil types and among densities. Given the variation in permineralization from site to site, we strongly recommend conducting similar trials for any new sites before attempting SPT separation.

From our main trial of the Moncure site, we gathered data on the number of fossils compared to the number of clasts for each fraction of interest: untreated, treated 'heavies', and treated float, with the data summarized in Table 5, and presented graphically in Figure 4. Although we took few samples, each sample was large in and of itself (337-1900 individual clasts examined for each sample). We tested the hypothesis that the concentration of fossils was greater in the fraction of the treated sediment that sank as compared to the untreated sediment with a one-tailed Wilcoxon test (a nonparametric test similar to the Mann-Whitney *U* test, as there were too few data points to assume normality) and found a significant difference (p-value <0.05), supporting the established notion that SPT separation does increase fossil concentration (as expected from Cifelli et al., 1996, Krukowski, 1988). Our investigations into the floated material revealed that it was less than 1% fossil, with all of the documented floated fossils from the Moncure site, and most (12/16) of the Moenave site fossils being fish scales embedded in much larger clasts (see Table 5 and Figure 4). This value (0.25% fossil) should not be confused with the above data on fossil loss (up to 16%), as the former refers to the prevalence of fossils found in the floated fraction, and the latter to the chance that fossils will be lost to the float. In other words, if 16% of fossils float while 90% of clasts float, the fossil concentration in the float will be significantly less than 16%, assuming there are more clasts in the untreated concentrate than fossils in the first place. Both values are important in assessing the utility of SPT treatment as one (16%) relates to the maximum documented risk, and the other (0.25%) relates to the difficulty of recovering lost fossils.

Fraction Type of Sample	# of Fossils in Sample	# of Clasts in Sample	% Fossils in Sample
Treated-Float	3	803	0.37%
Treated-Float	2	1918	0.10%
Treated-Float	4	1410	0.28%
Treated-Sink	373	1900*	16.41%
Treated-Sink	87	337	20.52%
Treated-Sink	137	640	17.63%
Untreated	88	3400*	2.52%
Untreated	2	475	0.42%
Untreated	10	960	1.03%

Table 5. Raw data comparing the number of fossils versus the number of clasts in several random samples taken from the different fractions examined. * denotes a value calculated by taking the mass, and multiplying by the average number of clasts per gram (~1100 clasts/gram for fine Moncure sediments). Average percentage of fossil clasts are: Float: 0.25%, Sink: 18.18%, Untreated: 1.32%.

We examined a smaller sample from a hematite-cemented quartz sand (from the Moenave Formation). We found fossil concentrations of ~24.90% (129 fossils for 389 nonfossils) in the sink and 0.66% (17 fossils for 2563 nonfossils) in the float from this locality.

Our results indicate that, below 2.80 g/mL, the fossil loss is minimal (<10%), and that at about 2.70 g/mL the nonfossil clasts are largely floated (~88%). The float was overwhelmingly nonfossiliferous (0.25% fossil), though considering there was a high volume of it, there could be a significant number of fossils there. We were encouraged, however, that in 6000 examined grains, every fossil located from the Moncure locality, and the overwhelming majority from the Moenave site (12/16) were fish scales embedded in larger clasts. Different fossil types showed different sinking rates, as well as different sinking percentages, at similar densities. Ultimately, SPT worked well and was easy to use, taking us only 24 work-hours to set up a lab, run experiments and process 11kg of screenwashed concentrate, and at no point during processing did we run so low on SPT (as a result of reconcentration lag) that we had to

stop processing, and have since processed several more small (<10kg of concentrate) sites.

CONCLUSIONS

Sodium polytungstate is an expensive, albeit extremely effective, method of microvertebrate fossil separation for certain lithologies. Apart from the SPT, only the hydrometer and deionized water are potentially difficult to obtain. It has been shown to increase, at three sites, the fossil concentration from ~1 in 100 (1.3%) to ~1 in 5 (19%). After operating the laboratory (using 5kg of dry SPT powder) for 24 working hours (including setup and experimental time) we managed to process all 11kg of concentrate without running so low on SPT as to ever need to stop processing (though there was a significant amount of dilute SPT from tool cleanings at the end). The lab remains in operation and, although we are recovering some SPT residue, functionally we have as much SPT as when we started, and have processed more than outlined here. The SPT process undoubtedly saved time in processing the Moncure locality, even when accounting for time taken to process the materials and set up the lab. This and the comparable fossil concentration improvement seen in the Moenave Formation site (~25% fossil in SPT-concentrated fraction) provides evidence that SPT treatment could be useful to other workers. We recommend a starting density of 2.70-2.75 g/mL, as this is low enough that the density increase during prolonged work will not result in significant fossil loss, but is also high enough to float most of the non-fossil clasts. Though every site varies, and for some sites it may be necessary to float bone and sink the rock (see McCarty and Congleton, 1994:189 for Table 8.1, showing different mineral and biological densities).

The fossils found in the float were few (see Table 5 and Figure 4) and were typically small scales embedded in larger clasts. Some fossils, such as teeth embedded in larger clasts, still sank, and after processing (picking) over 6000 grains of the float the only fossils found were scales, all of which we embedded in larger clay clasts, even though controlled experiments showed significant fossil loss (Table 4) at densities over 2.80 g/mL. Testing individual sites, and especially individual fossil types (as there is variation between them) is vital before beginning to use SPT separation.

Unlike others (Murray and Johnston, 1987:317; Cifelli et al., 1996:17), we found no noticeable viscosity increase in the SPT solution over time or with increased density. Jeppsson and Anehus (1999:57) argue that calcium carbonate and dolomite, when present in a sample, can cause an increase in viscosity, yet our main sample bore pedogenic carbonate and the viscosity was not noticeably different during the processing of it, than during the processing of the carbonate-poor Moenave Formation site. It is worth noting that calcium ions (as present in calcite, aragonite, dolomite and tap water) may cause an insoluble precipitate to form (Cifelli et al., 1996:18), and we did encounter the infrequent formation of a precipitate, though have failed to determine whether it is an SPT reaction or associated with the clays (McCarty and Congleton, 1994:198 note that clays can absorb SPT, which may cause this phenomenon). Soaking samples in dilute acetic, formic or a similar, weak acid and then rinsing, as well as screen-washing thoroughly (or even using kerosene) to remove clays, before running through SPT may be advisable, if only to avoid both the reported viscosity and precipitate issues (McCarty and Congleton, 1994:198).

Sodium polytungstate provides an efficient and reusable, albeit at a high initial investment, means to greatly improve fossil concentrations in microvertebrate samples. These fossil-dense samples are more quickly processed on the whole, and thus greatly facilitate research goals. There is a risk associated with the use of sodium polytungstate, in that many fossils (up to 16% in our trials) may be lost, and the resulting float is extremely nonfossiliferous (0.25% fossil) as to make reprocessing time-consuming and extremely difficult. As such, workers will need to evaluate the utility of sodium polytungstate on a site-by-site basis, with considerations as to the relative frequency of different fossil type, relative importance of total sampling, and total sample size (if enough sediment is collected, then the fossil loss may be outweighed by the research time gained). We hope that this contribution will help future workers make informed decisions about whether or not to use heavy liquid separation, and guide those that do through laboratory setup and processing.

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