Risks of Mixtures of Oil Sands Contaminants to a Sensitive Mayfly Sentinel, *Hexagenia*

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Abstract: Tailings ponds in northeastern Alberta, Canada contain massive amounts of oil sands process water (OSPW) that cannot currently be released due to the toxicity of some components. Limited space and the need for reclamation of oil sands operation sites will necessitate the release of OSPW in the near future. Knowledge of the composition and toxicity of OSPW is lacking yet is crucial for both risk assessment and management planning. This study examines chronic toxicity of a mixture of OSPW components sodium naphthenate and naphthenic acid (NA) to nymphs of the mayfly *Hexagenia* spp. in control and polycyclic aromatic hydrocarbons (PAH)-spiked sediment treatments. The objective of this study was to determine whether the addition of the PAH-spiked sediment significantly contributed to or masked responses of these sensitive mayflies to mixtures of NA. Mean survival in nymphs exposed to NA and PAH-spiked sediment treatments was reduced by 48% compared to those exposed to the NA mixture alone. Lethal responses were observed in all of the PAH-spiked sediment treatments. However, within PAH-spiked and control sediment treatments, there was no significant difference in nymph survival due to NA concentration, indicating that changes in survivorship were predominantly a reflection of increased mortality associated with sediment PAHs and not to the NA mixture treatment. Sublethal effects on body segment ratios suggest that mayflies exposed to NA and PAH-spiked sediment, as well as those exposed to the highest NA concentration tested (1 mg/L) and control sediment, made developmental trade-offs in order to emerge faster and escape a stressful environment. These results reveal that the release of OSPW to the surrounding environment could cause a reduction in mayfly populations. Mayflies provide ecosystem services and are an important food source for higher trophic levels in both the aquatic and terrestrial communities.

Keywords: oil sands; contaminants; risk assessment; guidance

1. Introduction

Canada’s oil sands in northeastern Alberta are among the largest crude oil deposits in the world [1]. Oil sands are a complex mixture of bitumen, sand, clay, and water that must be extracted and upgraded, often using hot water and proprietary surfactants [2]. The hot water extraction process used for Canada’s oil sands requires the use of substantial amounts of freshwater, much of which is recycled but is often originally sourced from the Athabasca River or its tributaries. After use in extraction, the residual water contains a complex mixture of left-over bitumen, dissolved salts, trace metals, and organic compounds such as surfactants. The aqueous and solid components of this mixture, collectively referred to as tailings, is then pumped to on-site tailings ponds, where the solid components...
settle [2]. The aqueous portion, commonly referred to as oil sands process water (OSPW), can be recycled but tends to accumulate chemicals and become increasingly saline over time [3].

At present, there is a zero-discharge policy for oil sands tailings in Canada [2]. However, due to limited storage capacity, the treatment and release of treated OSPW to the surrounding environment may be required in the future [4,5]. Unfortunately, understanding of the composition and toxicity of OSPW is lacking, yet is crucial for both risk assessment and management planning [6]. OSPW varies between tailings ponds due to differences in mining practices, for example, the application of different surfactants such as metallic naphthenates [6]. Assessing risk is further challenged by the considerable uncertainty concerning the ecological effects of OSPW on aquatic environments, in part due to the complexity of these process water mixtures. There is a lack of environmental guidelines even for many of the more common chemicals used in the extraction process such as sodium naphthenate.

Naphthenic acids have been identified as important components of OSPW and high concentrations have been correlated with toxicity [7]. These carboxylic acids are the organic components of many common surfactants used in the extraction of oil from bitumen. Naphthenic acids are toxic to aquatic species and are of concern due to their solubility and widespread persistence in waterways [8,9]. In a prior study, mixtures of naphthenic acids and a common metallic surfactant, sodium naphthenate, were found to be acutely toxic to sensitive aquatic communities, and mayflies in particular [10]. This study further examines this trend by investigating the same naphthenic acid mixtures in addition to the presence of a suite of sediment-borne PAHs.

Polycyclic aromatic hydrocarbons (PAHs) are another common contaminant in the Athabasca oil sands region and the levels of the compounds have increased in the lower Athabasca watershed since the industry boom of the 1960s [11]. PAHs are known toxins to both fish and invertebrates [12]. Effects of naphthenic acid mixtures in combination with other contaminants present in OSPW are largely unknown but are assumed to be additively toxic [13,14]. Many tributaries of the lower Athabasca River are prone to slumping such that a significant influx of sediments and contaminants is not uncommon [15]. A recent study on PAH signatures in sediment found significant contributions of naphthalenes, phenanthrenes, fluoranthenes, pyrenes, as well as benzo(a)pyrenes in sediments from two tributaries of the lower Athabasca River [16]. These environmentally-relevant PAHs were among those examined in the sediment testing portion in this study.

The objective of this study was to explore the combined effects of mixtures of three overlapping types of contaminants commonly found in the oil sands region (naphthenic acids, sodium naphthenate, and 17 PAHs) for an environmentally-relevant aquatic insect species (mayfly nymphs). Various levels of naphthenic acid mixtures were tested on Hexagenia spp. mayflies both with and without the added stress of PAH-spiked sediment. Endpoints examined included a combination of toxicological (e.g., lethal) and functional (e.g., growth) responses. We hypothesized that co-exposure to aqueous naphthenic acid and sodium naphthenate mixtures and PAH-spiked sediments would be more toxic than either substance individually.

2. Materials and Methods

2.1. Study Species

Hexagenia spp. were chosen for this study because, in aquatic ecosystems, sediment can accumulate contaminants. Consequently, sensitive invertebrates such as burrowing mayflies are at higher risk of exposure to contaminants in both sediment and water, which has the potential to produce additive or even synergistic toxic effects [17]. Fertilized Hexagenia spp. eggs were collected in June 2017 from Lake St. Clair in Windsor, Ontario using the method of Hanes and Ciborowski [18]. Culturing protocols were adapted from methods of Fremling [19], Friesen [20], and Giberson and Rosenberg [21]. In brief, hatched nymphs were transferred to glass culture tanks held in the growth chambers at 22 ± 3 °C with a 16:8 light to dark cycle. Tanks contained approximately 5 cm of sediment topped with deionized water and all received constant aeration and feeding twice per week. See Howland et al. [10] for more detail.
2.2. Establishment of Treatments

In this study, two sediment treatments (control, PAH-spiked) were examined in a 21 day chronic exposure experiment (10/4/18 to 3/5/18). In each sediment treatment, 5 concentrations of a 10:1 mixture of naphthenic acids and sodium naphthenate were reproduced (see Table 1 for concentrations). Each treatment contained 3 replicates which received ten late instar *Hexagenia* spp. nymphs (average total length of 6.7 mm). Mixture concentrations were prepared using a commercial preparation (lot # DLODE of NO397, TCI America, Portland, OR) that had previously been chemically characterized as similar to tailings pond water and acutely toxic to mayflies with a 48 h LC50 of 0.43 ± 0.34 mg sodium naphthenate/L (4.3 mg naphthenic acid/L) (see Howland et al. [10]). The aqueous 10:1 naphthenic acid/sodium naphthenate solution will be referred to as the NA mixture hereafter. Sediment treatments included a PAH-spiked sediment from Sigma Aldrich (CAS# CRM104-50G, lot# LRAB5247) (Table 2), and an unspiked natural (control) sediment of similar <1 mm particle size collected from Magaguadavic Lake, New Brunswick (45°47.62’ N × 67°13.48’ W, see [9]). Wet sediment was finely sieved to 1 mm and 30 g of control (unspiked), wet sediment was added to each replicate 300 mL beaker (Kimble, KIMAX™ tall form beakers, Fisher Sci., Hampton, NH, USA). For PAH treatments, 10 g of PAH-spiked sediment was also added. Each replicate then received 0.15 g of ground NutraFin Bug Bites fish food (Rolf C. Hagen Inc., Baie d’Urfé, QC, Canada). Beakers were then filled to the 300 mL mark with the appropriate NA solution and gently mixed to ensure integration of NA and ground NutraFin into the sediment pore water. Plastic lids with a hole drilled in the center for aeration were placed on top and sealed to prevent evaporation (Figure 1). The beakers were stored in Percival® (Percival Scientific, Perry, IA, USA) environmental chambers at 22 ± 3 °C with a light-dark cycle of 16:8 h. Over the course of the 21-day experiment, each replicate was fed 0.15 g of NutraFin twice per week and at which time solutions were also statically renewed. Constant aeration was provided using an air pump, tubing, and glass pipettes fed through plastic lids sealed to the top of each beaker (Figure 1). The beakers were allowed to settle for 24 h before mayflies were added to the treatments.

**Table 1.** Comparison of the actual (mean ± standard error [SE]) versus nominal concentrations of 10:1 mixture of total naphthenic acids and sodium naphthenate as determined by scanning synchronous fluorescence spectroscopy analysis. All solutions were prepared using a serial dilution of stock concentrations of a commercial source representative of oil sands process water (OSPW).

| OSPW | Naphthenic Acids | Sodium Naphthenate |
|------|------------------|-------------------|
| %    | Nominal (mg/L)   | Actual (mg/L) ±SE | Actual (µM) ±SE | Nominal (mg/L) | Actual (mg/L) ±SE | Actual (µM) ±SE |
| 0.01 | 0.01             | 0.01 <0.01         | 0.059 <0.059     | 0.01           | <0.01 <0.01        | <0.052 <0.052 |
| 0.1  | 0.1              | 0.17 <0.01         | 0.999 <0.059     | 0.01           | 0.01 <0.01         | 0.052 <0.052 |
| 1    | 1                | 1.45 0.02          | 8.517 0.117      | 0.1            | 0.12 <0.01         | 0.624 <0.052 |
| 14   | 10               | 14.47 0.25         | 84.991 1.468     | 1              | 1.16 0.02          | 6.034 0.104 |

2.3. Chemical Analyses

Comparison of the commercial NA compound with OSPW samples from Syncrude and Suncor tailings ponds revealed analogous compositions. The chemical analyses are described in detail elsewhere (see Howland et al. [10]). In brief, NA mixture concentrations were prepared using a commercial 10:1 preparation of naphthenic acids and sodium naphthenate. Two approaches were used to analyze and characterize the composition of the NA commercial preparation. First, the analysis was conducted using the scanning synchronous fluorescence spectroscopy (SFS) method of Kavanaugh et al. [22] on an Aquamate spectrophotometer with deuterium and tungsten lamp attachments (Thermo Spectronic, Rochester, NY), which revealed a similar fluorescence signature between our commercial NA mixture and the tailings pond samples. Second, to fully characterize the naphthenic acids from the commercial preparation, samples from the same lot number of the commercially prepared stock were also analyzed at the Environment and Climate Change Canada laboratory in Saskatoon, SK by
high resolution mass spectrometry (Orbitrap) with electrospray ionization (see Howland et al. [10]). This analysis revealed that the naphthenic acid composition in the commercial samples were 99.8% \( O_2 \) species and that the range of carbon numbers (8 to 22, \( z = 1 \) to \( z = 6 \) \( O_2 \) DBE) found in our commercial NA mixture was also typical of oil sands process water extracts.

Table 2. Comparison of estimated actual (mean ± standard error [SE]) concentrations of 17 polycyclic aromatic hydrocarbons (PAH) present in spiked sediment tests used in this study versus field levels reported by Droppo et al. [16].

| PAH                        | This Study AVG (ng/mg) | ± SE  | Droppo Study [16] AVG (ng/mg) |
|----------------------------|------------------------|-------|------------------------------|
| Acenaphthene               | 0.174                  | 0.009 |                              |
| Acenaphthylene             | 0.118                  | 0.006 |                              |
| Anthracene                 | 0.16                   | 0.008 |                              |
| Benzo(a)anthracene         | 0.034                  | 0.002 |                              |
| Benzo(a)pyrene             | 0.08                   | 0.004 | 0.3322                       |
| Benzo(b)fluoranthene       | 0.102                  | 0.005 |                              |
| Benzo(b+k)fluoranthene     | 0.052                  | 0.008 |                              |
| Benzo(g,h,i)perylene       | 0.044                  | 0.002 |                              |
| Benzo(k)fluoranthene       | 0.063                  | 0.003 |                              |
| Chrysene                   | 0.046                  | 0.002 |                              |
| Dibenzo(a,h)anthracene     | 0.025                  | 0.001 |                              |
| Fluoranthene               | 0.108                  | 0.005 | 0.2714                       |
| Fluorene                   | 0.075                  | 0.004 |                              |
| Indeno(1,2,3-cd) pyrene     | 0.076                  | 0.004 |                              |
| Naphthalene                | 0.094                  | 0.005 | 0.4072                       |
| Phenanthrene               | 0.116                  | 0.006 | 0.077                        |
| Pyrene                     | 0.064                  | 0.003 | 1.7156                       |

Figure 1. Laboratory setup for 21 day exposure to NA mixture treatments (0, 0.001, 0.1, 1 mg/L) and control sediment or polycyclic aromatic hydrocarbons (PAH)-spiked sediment: (a) individual replicate and (b) treatment set up in an environmental chamber.

PAH-spiked sediments were prepared using a certified reference material. Sediments were weighed on an OHAUS® Navigator™ scale to the closest 0.01 g and then added to the un-spiked sediment as described previously. These reference sediments are certified for round-robin testing of laboratories seeking International Organization for Standardization (ISO) 8100, 8270, 8310 or equivalent certification. Sediment quantity in each beaker was unfortunately insufficient for individual analytical testing, however, a series of reference materials and 300 g composite samples prepared using the
same methods as those in the test beakers were also sent to a local commercial laboratory with ISO certification for validation (RPC Fredericton). All results from the whole and composite samples were found to be within acceptable ISO certification limits. Actual concentrations for these standard materials are reported in Table 2.

2.4. Biological Response Variables

At the end of the 21-day experiment, replicate beakers were dismantled, and the contents collected. Sediment was sieved to remove nymphs and the number surviving from each beaker was recorded. Surviving mayfly nymphs were then collected and preserved in 95% ethanol for subsequent measurement and weighing—decomposition made this impossible for the mortalities. Total body length, thorax length, thorax width, head length, and head width were measured using the Auto-Montage® imaging system (Syncroscopy, Synoptics Inc., Frederick, ND, USA) with a Leica© digital camera and dissecting microscope (Leica© Microsystems Ltd., Cambridge, UK). Wing pad development was noted and each nymph was also subsequently weighed. Finally, nymphs were dried for 48 h in a 60 °C drying oven and re-weighed to determine dry weight. At the onset of the experiment, a subsample of the same-sized nymphs was also collected, measured, and weighed in order to determine the size at the onset of the experiment (time zero).

2.5. Statistical Analyses

A split-plot ANOVA design [23] was used to analyze the data. The predictor variables were NA concentration and sediment type (blocking variable) and the response variables were the nymph measurements (e.g., length, wet weight). A principal component analysis (PCA) was used to visualize and independently evaluate the relative importance of naphthenic acids, sodium naphthenate, and the 17 PAHs tested on Hexagenia body size measurements. PCA is a multivariate technique that can be used to confirm the strength of relationships among different factors (principal components). Nymph survival between sediment types was compared using Welch’s t-test, as the assumption of homogeneity of variances was not met when tested by Levene’s Test for Homogeneity of Variance (p = 0.008). Nymph survival between NA solution treatments was compared using one-way ANOVA. Shapiro-Wilks test was used to test normality. All tests were conducted using R (v. 3.1).

3. Results

3.1. Lethal Responses to Treatment

Mayfly survival in the combined NA solution and PAH-spiked sediment treatments was reduced by 48%, on average, compared to responses in nymphs exposed to the NA mixture alone (t = 3.28, df = 14.44, p = 0.005) (Figure 2). Lethal responses to the treatments were observed in all NA mixture concentrations within the PAH-spiked sediment treatments. However, within PAH-spiked as well as within the control sediment treatments, there was no significant difference in nymph survival due to NA mixture concentration (PAH-spiked sediment: p = 0.36, F = 1.22, F-crit = 4.07; control sediment: p = 0.70, F = 0.49, F-crit = 4.07). Therefore, changes in survivorship were predominantly a reflection of increased mortality associated with sediment PAHs and not to the aqueous NA mixture (Figure S1).

3.2. Sublethal Responses to Treatment

The PCA biplot highlighted the two distinct groupings dependent on exposure to PAH or control sediment that cumulatively accounted for 91.3% of the variation in the nymph body size dataset (Figure 3). Factor 1 explained 66.3% of the variation (x-axis, Figure 3), was strongly positively correlated to the presence of PAHs in the spiked sediment. Factor 2 explained 25.0% of the variation and was modestly positively correlated to NA mixture concentration and strongly negatively correlated to the body size measurements (Figure 3).
PAH mixture concentrations within spiked sediments significantly (F(4, 2019) = 3.28, df = 14.44, p = 0.005) (Figure 2). Lethal responses to the NA mixture and control sediments exhibited significant (p < 0.001) growth over the course of the 21-day experiment with the exception of head length (Figure 4). Thorax length (43.1% ± 1.9 SE; Figure 4a), thorax width (25.5% ± 2.0 SE; Figure 4b), and wet weight (105.5% ± 9.8 SE; Figure 4d) were uniformly larger (34.7% ± 1.7 SE) irrespective of NA treatment by the end of the experiment. In contrast, the head length was reduced in all NA treatments compared to the control (p < 0.001). Head length was particularly reduced in the 0.001 and 0.1 mg/L NA treatments, which were 13.3% and 17.1% lower than the control head lengths respectively. In contrast, treatments with PAH-spiked sediments significantly (p < 0.05) reduced the size of some body segments in the mayflies (Figure 4). For instance, thorax length (p = 0.004) and thorax width (p = 0.031) were both

**Figure 2.** Mean nymph survival with standard error, grouped by sediment treatment. Survival was significantly lower in the PAH-spiked sediment treatment group in comparison to the control sediment treatment group (denoted by “*”).

**Figure 3.** Principal components analysis (PCA) explaining 91.3% (66.3 + 25% EV) of the total variation in the body size measurements of *Hexagenia* nymphs exposed to an NA mixture gradient (0–1mg/L) and sediment with or without PAHs (see Table 2 for PAH concentrations).
reduced 7.7% and 10.2%, respectively, in mayfly survivors exposed to PAH-spiked sediment treatments compared to the control (Figure 4a,b). Although not significant at the $p < 0.05$ level, wet weight of nymphs in PAH-spiked sediment treatments were also reduced by 14.2% ($p = 0.103$, Figure 4d), while the total length was reduced by 3.8% compared to the controls ($p = 0.0942$, see Figure S2).

![Boxplot comparison of sublethal changes](image_url)

**Figure 4.** Boxplot comparison of sublethal changes (showing the maximum, 75th percentile, median, 25th percentile, and minimum) in thorax length (a), thorax width (b), head length (c), and wet weight (d) in *Hexagenia* mayfly nymphs exposed to an NA mixture in either control (white, left) or PAH-spiked sediment treatments for a 21-day period. Significant differences from the control (0 mg/L NA and control sediment, far left bar) are indicated by “*” and time zero measurements are indicated by grey horizontal lines for comparison.

Further, in PAH-spiked sediments, surviving mayflies typically grew 33.7% ± 2.6 in thorax length over the course of the 21-day experiment compared to time zero (Figure 4a). However, growth pattern responses within PAH-spiked sediment treatments were NA mixture concentration dependent (Figure 4). Notably, the thorax (Figure 4a), head length (Figure 4c), and wet weight (Figure 4d) of the mayflies were unchanged ($p > 0.05$) compared to time zero in the combined PAH-spiked sediment and
lowest concentration of NA mixture tested (0.001 mg/L). A similar pattern was also observed for the mayflies in the combined PAH-spiked sediment and NA treatments at the 0.1 mg/L level for the thorax width (Figure 4b) and wet weight, which was particularly variable (e.g., CV = 72.3%; Figure 4d).

As above, the total length to thorax length ratios also significantly differed due to the sediment treatment type but seldom responded to changes in NA concentration (Figure 5). In particular, total length to thorax length ratios were significantly different (p = 0.0006) between the PAH-spiked sediment and control sediment groups. Total length was favored (ratios <0.3) in the mayflies in the control sediment and thorax length was favored (ratios >0.3) in mayflies in the PAH-spiked sediment (see Figure S3). Thorax length favored ratios were also apparent (p = 0.072) in the highest concentration (1 mg/L) NA mixture control sediment treatment compared to the 0 mg/L NA mixture control sediment treatment (Figure 5).

![Figure 5](image-url)

**Figure 5.** Boxplot comparison of total length to thorax length ratios (showing the maximum, 75th percentile, median, 25th percentile, and minimum) in total length to thorax length ratio in *Hexagenia* mayfly nymphs exposed to an NA mixture in either control (white, left) or PAH-spiked sediment treatments for a 21-day period. Significant differences from the control (0 mg/L NA and control sediment, far left bar) are indicated by “*” and the time zero measurements are indicated by a grey horizontal line for comparison.

### 4. Discussion

In the event of a planned release of OSPW into the Athabasca River or other freshwater rivers, mayflies and other aquatic macroinvertebrates may be chronically exposed to low concentrations of tailings contaminants. Chronic exposure to even very low concentrations of toxins can cause both lethal and sublethal effects in mayflies [24,25]. Sublethal effects on growth and development in response to stress can have an impact on the reproductive success of adult mayflies [26,27]. This study documents both lethal and sublethal response of *Hexagenia* nymphs due to exposure to environmentally-relevant concentrations of aqueous NA mixtures with control versus PAH spiked-sediments.

#### 4.1. Lethal Responses to Treatment

The reduction in mayfly survival was predominantly due to exposure to the PAH-spiked sediment treatment (Figures 2 and 3). This effect is of concern because there has been a striking increase in PAH levels in the Athabasca area sediment since the oil boom of the 1970s [11,28,29]. Total sediment PAH concentrations of over 6000 ng/g have been measured in the Athabasca region, with higher concentrations associated with samples closer to development sites [28]. Levels of PAHs tested in this study were 50 times lower than concentrations measured near oil sands developments (Table 1), and significant effects were observed even at these levels. However, it is possible that local populations of *Hexagenia* have adapted tolerance to PAHs.
In this study, no differences in survival were detected between control sediment treatments due to the addition of NA treatment. However, it is worth noting that the greatest concentration of the NA mixture tested was but 1 mg/L, which is two orders of magnitude lower than concentrations reported in tailings ponds (128 mg/L, [4]). At present, even the most effective methods of removing naphthenic acids, such as constructed wetlands, remove only up to 80% of the types of substances tested in this study [30]. Thus, the concentrations of NA could easily remain in the 8–24 mg/L range. In a previous study, acute toxicity of the same mixture of NA was also tested and found that NA could be acutely toxic with a 48 h LC50 of 0.43 ± 0.34 mg sodium naphthenate/L (4.3 mg naphthenic acid/L) [10]. It seems plausible that even a 100-fold dilution of concentrations of naphthenic acids and their metallic salts in contemporary tailings ponds could be toxic to sensitive aquatic insects like mayflies. The combination of highly toxic responses to environmentally-relevant concentrations of PAH-contaminated sediment irrespective of NA concentration suggests that even low concentrations of NA could be toxic to aquatic communities when faced with cumulative stressors.

Responses to sediments with trace metal concentrations are, at present, under studied and may offer further insights. Trace metal levels in the Athabasca region are naturally elevated and can be further amplified by industrial activity [31,32]. These effects paired with other stressors such as oil production emissions, and/or natural occurring PAHs and hydrocarbons, as well as climate change [10], could have a devastating effect on Hexagenia populations. Aquatic macroinvertebrates such as Hexagenia provide significant contributions to ecosystem services in freshwater rivers that could create a cascading effect on the ecosystem if populations are diminished [33]. Aquatic invertebrates provide prey for aquatic predators and are also consumed by terrestrial predators such as birds, reptiles, and insects. Adult insects also transport aquatic material to terrestrial habitats upon emerging, which is an important energy transfer between ecosystems [34].

4.2. Sublethal Responses to Treatment

In control sediment treatments, a significant reduction in head length was observed in all NA mixture treatments in comparison to the 0 mg/L control. This indicates that developmental trade-offs were made in order to reduce time to emergence in response to NA exposure. Although differences were not significant, the average wet weight displayed a similar pattern, excluding the 0.001 mg/L treatment, which was slightly larger than the 0 mg/L treatment. This may be due to a common sublethal stress response called ‘hormesis’—a biphasic dose response to toxins that represents an apparently favorable response to low dose treatment [35]. Rather, the ability for organisms to compensate for exposure may be an important indicator of plasticity to these stressors and may also be useful for estimating the no observable effect concentration.

There were no significant differences in thorax width among treatments and there was less growth over time in comparison to thorax length. This again indicates that resources were used to develop the thorax and wings to expedite emergence, rather than reach an optimal body size for reproduction. Similar patterns have also been reported in other studies conducted by Scrimgeour et al. [27] and Peckarsky et al. [36] who have tended to find that mayflies can escape a stressful aquatic environment by favoring development over size. Similarly, in the PAH-spiked sediment treatments, there was an interesting pattern among thorax width, head length, and wet weight responses: Mayflies exposed to 0.001 mg/L NA did not show significant growth compared to time zero, while almost all other treatments did—those that did not still grew, but there was more variation leading to a non-significant result. This again supports the theory that development trade-offs are made in order to escape exposure to stressors by emerging earlier.

All NA mixture treatments in the PAH-spiked sediment group, as well as the highest concentration NA mixture within the control sediment group favored a longer thorax in relation to the total length. This again indicates advancing thorax and wing development as a trade-off for reaching larger size overall in response to stress.
When analyzing sublethal effects, it is imperative to remember that we are only measuring responses of the surviving nymphs. Therefore, it is possible that the survivors in the higher NA mixture concentration treatments were larger and/or more fit than the nymphs that died in those treatments. In the treatments with PAH-spiked sediment, there was a lot of variation in survival, indicating possible differences in size and/or fitness between individuals within the population.

5. Conclusions

In aquatic systems such as the Athabasca River, it is important to consider other possible stressors when working on a management plan for OSPW release. Sediment and nutrient load, flow velocity and volume, pollution from emissions and groundwater, and the presence of other contaminants can all affect community responses. Survival in treatments with both NA mixture and PAH-spiked sediment was reduced by 48%. This highlights the importance of considering additive effects from multiple stressors. Sublethal effects also indicate body size trade-offs to enable earlier emergence. While small changes may seem insignificant, long term effects on adult fecundity and/or survival could be detrimental to the ecosystem. In colder climates such as northern Alberta, Hexagenia spends from two to four years in their aquatic stage [37], making them susceptible to multiple exposures depending on the proposed OSPW release plan. Release of OSPW to the surrounding environment is not advised until current technologies can further reduce toxicity.

Future Directions

Continued study is warranted—a chronic mesocosm study that exposes a field-collected community to low concentrations of NA or diluted OSPW would prove useful to help determine long term effects of OSPW release on community structure. Use of field-collected invertebrates from the Athabasca region would also account for differences in sensitivity among populations. In the event of a planned OSPW release, there would also likely be other contaminants as well as suspended sediment depending on the treatment process. All of these factors should be taken into account when creating a management plan for the release of OSPW in the Athabasca region.

Supplementary Materials: Are available online at http://www.mdpi.com/1424-2818/11/8/118/s1, Figure S1: Comparison of mean survival in Hexagenia, Figure S2: Comparison of sublethal changes in total length in Hexagenia, Figure S3: Representation of total length-favored and thorax length-favored body segments in Hexagenia.

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