Performance of wetland forbs transplanted into marshes amended with oil sands processed water

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Abstract Companies mining oil sands in Alberta (Canada) face the challenge of reclaiming wetlands under water use restrictions. Wetland reclamation after mining will generate marshes characterized by elevated salinity and residual hydrocarbons. Oil sands wetlands are also impoverished in forbs, suggesting that their establishment may be constrained by water chemistry. We transplanted skullcap, mint, and smartweed plants into experimental trenches that simulated two possible reclamation scenarios: wetlands amended with on-site freshwater or with oil sands processed water (OSPW). The main scientific question was is OSPW a suitable water amendment as freshwater for reclaiming wetland forb habitat? As a surrogate of plant health, we studied

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plant ecophysiology (gas exchange, leaf fluorescence), leaf chemistry, and plant growth. Results showed that there were no differences in skullcap mineral contents under either treatment; however, mint and smartweed plants subjected to OSPW had a significantly higher Na content than those under freshwater. Smartweed darkadapted leaf fluorescence showed a reduced photochemistry in OSPW relative to plants in freshwater. Mint leaves exhibited lower stomatal conductance in OSPW than in freshwater, a condition that negatively affected transpiration and carboxylation. Skullcap plants grown in OSPW had lower net CO2 assimilation rates than those in freshwater but did not show any other ecophysiological difference between treatments. Mint plants experienced growth reductions (i.e., shoot height) in OSPW. Our results show, for the first time in the literature, that plants photosynthetic capacity was negatively affected by OSPW. Conditions in OSPW proved to be suitable for establishment as transplanted forbs showed 100 % survival after the first growing season. However, impaired physiological functions in plants subjected to OSPW indicated that OSPW amendment created a less hospitable habitat for wetland forbs than freshwater.

 $\begin{tabular}{ll} \textbf{Keywords} & \textbf{Bitumen} \cdot \textbf{Effluent} \cdot \textbf{Freshwateramendment} \cdot \\ \textbf{Salinity} \cdot \textbf{Wetland reclamation} \\ \end{tabular}$

Introduction

Oil sands energy developments in Northern Alberta, Canada, have raised both scientific and public concern



due to their substantial areal extent and the suite of environmental changes they perpetuate at the regional level (Foote 2012; Rooney et al. 2012). A total of 4800 km² of boreal forests are predicted to be profoundly disturbed by mining (Canadian Association of Petroleum Producers 2014), of which 64 % support wetland vegetation (Rooney et al. 2012). Wetlands, especially peatlands, might not be restored due to both geomorphic constraints and the sensitivity of peatland vegetation to post-mining hydrologic and environmental conditions (Johnson and Miyanishi 2008; Rooney et al. 2012). In such a scenario, graminoid marshes will replace peatlands and become a significant land cover in the reclaimed landscape (Rooney et al. 2012).

Oil sands industries use large volumes of freshwater to recover oil from bituminous sands. Under a zerodischarge policy and freshwater-use limitations, oil sands processed water (OSPW) must be recycled (Canadian Association of Petroleum Producers 2014). During recycling, industrial water becomes enriched in salts, heavy metals, and miscellaneous hydrocarbons such as naphthenic acids, polycyclic aromatic hydrocarbons (PAHs), and residual bitumen (Allen 2008). OSPW is stored in expansive tailings ponds (some >20 km²) and is used as an amendment for the reclamation of subsaline wetlands. In a situation where oil sand industries face public pressure to decrease current freshwater footprints, amendment of industrial wetlands with freshwater to dilute chemicals is a considerable cost and must be supported by a sound scientific rationale before being implemented.

The net effect of oil sands mining on postdisturbance biodiversity has not been assessed (Rooney et al. 2012), yet it is apparent that plant diversity has been negatively affected as oil sands marshes are impoverished in native wetland forbs (Trites and Bayley 2009; Raab and Bayley 2012, 2013; MC Roy PhD thesis). There are many cumulative reasons leading to the underrepresentation of forbs in the reclaimed landscapes: lack of viable seeds in the soil bank of salvaged materials (Mackenzie and Naeth 2010), barriers for natural propagule dispersal due to habitat fragmentation and isolation of created marshes in the postmined landscape, paucity of positive biological interactions and beneficial organisms (e.g., pollinators), absent or inadequate hydrologic control necessary for wetland colonization and zonation, increased asymmetrical competition with tolerant and thriving graminoids (e.g., cattail), or the presence of physicochemical constraints (i.e., salinity, residual hydrocarbons, heavy metals) which exceed tolerance limits of forbs species. Physiological tolerance of forbs to OSPW has not yet been assessed, precluding prediction of forb establishment and representation in the closure landscape.

Three studies carried out in the oil sands by Trites and Bayley (2009), Raab and Bayley (2013), and Roy (MC Roy PhD thesis) have shown that smartweed (*Polygonum amphibium* L.), mint (*Mentha arvensis* L.), and skullcap (*Scutellaria galericulata* L.), although commonly present in shoreline habitats of natural marshes of the Fort-McMurray region, are essentially absent in the oil sands industrial wetlands investigated. We selected skullcap, mint, and smartweed as sensitive, widely distributed, and representative indicator forbs. We evaluated their ecophysiological performance in two possible reclamation scenarios: wetlands amended with freshwater or, alternatively, with OSPW.

The primary question of both scientific interest and operational significance for management was is OSPW a water amendment equally suitable as freshwater for reclaiming wetland forb habitat? We focused our research on three responses to water amendments: (1) plant leaf ionic contents; (2) plant ecophysiological performance through the study of water relations, leaf fluorescence, and photosynthesis rates; and (3) plant growth response.

Materials and methods

The experiment was conducted on an area under ongoing reclamation in Suncor Energy Ltd. property (56° 58' 50" N, 111° 30′ 20" W). There, six parallel trenches of 40 m long by 4 m wide with a north-south orientation were constructed in 1992 to study the effect of processed waters on wetland vegetation in semi-controlled conditions. Trenches were constructed, sealed with an impermeable liner, and then a 30-cm thick deposit of organic sediments was placed to provide a plant growth medium. Trenches were assigned to the following water treatments: (a) freshwater (amendment with freshwater taken from nearby Loon Lake, an on-site remnant water body, (56° 58′ 42″ N, 111° 28′ 03″ W) or b) OSPW (amendment with oil sands processed tailings ponds water). These treatments are characteristic of the environmental conditions to which plants would be exposed to in the oil sands created wetlands. The oil company periodically filled the trenches with their respective



water treatments through the growing season to compensate for water losses and to adjust conductivity to appropriate levels. Wetland macrophytes spontaneously colonized the trenches; dominant species were cattail (*Typha latifolia*), water sedge (*Carex aquatilis*), and shrubby willows (*Salix* spp.). The established vegetation was not disturbed to ensure that the site resembled the natural variation evident in an early successional stage of oil sands wetlands.

In late August 2010, a total of 25 plants having from three to five ramets and well-developed rhizomes and root systems were carefully excavated from a natural marsh in the region (56° 56′ 19.08″ N, 111° 39′ 41.85″ W) and transplanted into 1-gal pots filled with commercial peat potting soil. The pots, fitted with drainage holes, were held in a freshwater trench to acclimate from autumn 2010 through early spring 2011. Pots mimicked optimal natural growing conditions by being partially submerged to keep substrate and roots in a saturated medium. More than 90 % of all transplants survived, and of these, 18 of the most vigorous ones were chosen then randomly assigned to the different experimental water treatments on May 24, 2011. Three plants per species were transplanted in each of the six trenches. Two plants were randomly assigned to each extreme end and one to the center of each trench as checks on unforeseen gradients in an attempt to deal with the interdependent replication in each of the trenches (sacrificial pseudoreplication, Hurlbert 1984) by covering a wider range of environmental variation (i.e., vegetation cover, sun exposure, shoreline slope aspect, and point of water input).

Water chemistry (ion and naphthenic acids concentrations) was sent for analysis to an independent registered analytical laboratory by the oil company. We made routine surface water quality measurements; electrical conductivity and salinity were measured with a handheld YSI Model 85 (YSI Inc., Yellow Springs, OH, USA), and pH was measured with a portable Hanna HI98121 (Hanna Instruments, Mauritius). Totally expanded leaves along the top half of shoots were harvested from three plants per trench/species combination near peak standing crop in late August 2011. Leaf chemistry was analyzed in the NRAL lab of the University of Alberta (Natural Resources Analytical Laboratory 2014).

Plant ecophysiological measurements were carried out in late August 2011 on a clear day. Ecophysiological measurements were taken near midday when photosynthetic photon flux density (PPFD)

was above 1500 μmol m⁻² s⁻¹. Gas exchange rates (net CO₂ exchange rate, net transpiration rate) and stomatal conductance were measured in mature, fully exposed leaves in a similar position on each plant species by using an infrared gas analyzer (LCi, ADC Bioscientific Ltd., Herts, UK). Chlorophyll a fluorescence transients were simultaneously measured in leaves in the same plants with a Hansatech Pocket PEA fluorometer (Hansatech Instruments Ltd., King's Lynn, Norfolk, UK). Measured leaves were dark-adapted with leaf clips for 20 min. The transients were induced by a light pulse of 3500 µmol m⁻² s⁻¹ with a peak wavelength of 627 nm provided by a high intensity LED. Leaf fluorescence parameter measured was F_v/F_m , the maximum quantum yield of photosystem II, and PI, the performance index of chlorophyll a fluorescence, and parameters which summarize the health of the photochemical reactions of photosynthesis (Strasser et al. 2000). Shoot height, i.e., the height of reproductive shoots taken from the rooted sediment level to the apex, was measured in the tallest three shoots per plant. Leaf length and width were measured in the larger leaves in each of the three shoots.

Plant performance was analyzed using sample t tests to compare performance in plants transplanted to trenches filled with either OSPW or freshwater. All datasets were checked to ensure normality and homoscedasticity. Datasets were transformed where data were not normal, or variances were significantly different. All results are presented as non-transformed means \pm S.E. from nine replications per species and water amendment type. Water chemistry data is presented as non-transformed means \pm S.E. of three replications. In this case, a principal component analysis (PCA) coupled with a t test was performed to determine which water chemistry components were significantly different between water types (OSPW and Freshwater). The PCA was used to express the covariation of the nine water chemistry components as a single composite variable. Prior to the PCA analysis, data were log-transformed and standardized. The trench scores in the reduced space were then used to perform a t test. The analyses were performed using R v. 3.0.3.

Results and discussion

A PCA analysis of the water chemistry revealed that the first axis (PC1) explained 94 % of the total variation,



while the second axis (PC2) only explained 4 %. All the analyzed water chemistry components were highly correlated with the first axis (Table 1). The PCA-coupled *t* test revealed significant chemical differences between trenches depending on whether they were amended with OSPW or freshwater (*t*=-11.8, df=4, *p*<0.001). OSPW-filled trenches were richer in Na, K, and Cl but lower in Ca than trenches filled with freshwater (Table 1). Differences in individual element concentration between treatments were reflected in K/Na and Ca/Na ratios with OSPW trenches significantly lower than those of OSF treatments (Table 1). OSPW trenches had also higher electrical conductivity (EC), pH, and naphthenic acid (NA) levels than freshwater trenches (Table 1).

Amendment differences were further reflected in the leaf mineral content differences for two of the three species (Table 2). For instance, there was significantly higher leaf Na content in mint and smartweed plants transplanted into OSPW than in those transplanted into freshwater (p=0.020, Table 2). There were no significant differences in skullcap leaf mineral contents between plants transplanted into each type of water amendment (p>0.05, Table 2). Sodium exclusion from leaves is a key adaptive strategy to avoid toxicity (Teakle et al. 2007; Munns and Tester 2008); our data indicate that skullcap exhibits a greater ability to exclude Na from leaves with respect to mint and smartweed, species that may be susceptible to ion toxicity due to Na

Table 1 Water chemistry and covariation of water chemistry components with PCA 1 (94 % explained variation) of wetlands amended with oil sands processed water (OSPW) or freshwater

	Trenches				
	OSPW	Freshwater	PCA1		
Na ⁺ (mg l ⁻¹)	621.6±63.8	47.6±2.7	-0.859		
$K^+ (mg l^{-1})$	15.0 ± 1.1	3.9 ± 0.5	-0.844		
$Ca^{2+} (mg l^{-1})$	31.6 ± 2.8	61.1 ± 0.8	0.822		
$Cl^- (mg l^{-1})$	382.6 ± 32.4	61.3 ± 6.5	-0.860		
EC (us cm ⁻¹)	3116 ± 274	$707\!\pm\!11$	-0.858		
pH	8.41 ± 0.02	8.12 ± 0.11	-0.708		
K ⁺ /Na ⁺ (mol mol ⁻¹)	$0.043\!\pm\!0.002$	0.136 ± 0.012	0.846		
Ca ²⁺ /Na ⁺ (mol mol ⁻¹)	0.052 ± 0.007	1.261 ± 0.073	0.860		
NA (mg l^{-1})	18.6±3.9	1.3 ± 0.1	-0.860		

Values represent means \pm S.E of three trenches per treatment *EC* electrical conductivity, *NA* naphthenic acids



accumulation. The capacity of plants to maintain high tissue macronutrients to Na ratios is another key determinant of plant salt tolerance (Maathuis and Amtmann 1999). The ability to selectively incorporate or exclude ions was apparent in the macronutrients to Na ratios as K/Na and Ca/Na ratios were tangibly higher in plants than in water (cfr. Tables 1 and 2). Mint and smartweed showed more optimal macronutrients to ion ratios in trenches amended with freshwater relative to trenches amended with OSPW (Table 2). Both mint and smartweed leaves had higher K/Na ratios in freshwater than in OSPW (p < 0.05, Table 2). It is generally accepted that maintenance of cytosolic K/Na above 0.5-1 is an important aspect of salt tolerance for glycophytes (Shabala and Cuin 2008; Munns et al. 2011). Our data indicate that leaf K/Na ratios of the three species were well above the mentioned range, suggesting that leaf injuries due to K/Na imbalances are not taking place in mesophyll cells of plants exposed to OSPW. On the other hand, calcium imbalances are associated with deeper detrimental effects of Na on K/Na homeostasis due to poorer membrane selectivity (Zhong and Läuchli 1994; Shabala et al. 2005). Smartweed demonstrated a lower Ca/Na ratio in OSPW with respect to freshwater (p=0.002, Table 2) suggesting that this species may be especially susceptible to sodium toxicity. None of the species displayed differences in N nutrition between treatments (p>0.05, Table 2), indicating that forbs growing in the OSPW treatment were not being fertilized by ammonia pollution, a soluble by-product of bitumen extraction that is dissolved in OSPW (Allen 2008).

The assessment of photosystem 2 (PSII) activity through chlorophyll a fluorescence has proved to be a useful indicator of both environmental stress in cattail plants that dominate impacted oil sands wetlands (Mollard et al. 2013a) and of positive hormetic-like responses of graminoids and microalgae due to the exposure to nutrient enriched OSPW (Debenest et al. 2012; Mollard et al. 2012; Roy et al. 2014) thus indicating latent nutritional stress in freshwater conditions. Skullcap and mint did not show differences on PSII function when transplanted to OSPW or freshwater (Table 3) indicating that OSPW and freshwater amendments are equally suitable for these species with respect to both the light harvesting and energy transfer reactions of photosynthesis. However, smartweed, the species which proved to be more affected by ion imbalances, showed an impaired functionality of the PSII in OSPW as seen by reductions in maximum quantum yield of

Table 2 Mineral contents of leaves on a dry weight basis and results of the *t* tests for plants transplanted to wetlands amended with oil sands processed water (OSPW) or freshwater

	Skullcap		Mint		Smartweed				
	OSPW	Freshwater	t value	OSPW	Freshwater	t value	OSPW	Freshwater	t value
Na ⁺ (μmol g ⁻¹ DW)	137±42	54.2±7.54	2.05	82.2±12	47.0±6.6	2.42*	218±59	45.6±2.5	6.90***
K ⁺ (% DW)	1.53 ± 0.10	1.71 ± 0.10	1.22	1.38 ± 0.10	1.45 ± 0.08	0.50	1.30 ± 0.16	1.22 ± 0.24	0.27
Ca ²⁺ (% DW)	1.21 ± 0.10	1.38 ± 0.27	0.69	1.89 ± 0.30	1.65 ± 0.25	0.01	0.73 ± 0.07	0.92 ± 0.14	1.21
K ⁺ /Na ⁺ (mol mol ⁻¹)	5.15 ± 1.28	9.16±1.83	1.67	4.74 ± 0.74	8.87 ± 1.46	2.52*	2.23 ± 0.61	6.69 ± 1.00	3.79**
Ca ²⁺ /Na ⁺ (mol mol ⁻¹)	3.78 ± 0.81	6.78 ± 1.25	1.87	6.19 ± 1.96	9.78 ± 2.20	1.21	1.34 ± 0.47	$5.14 \pm .81$	4.05**
TKN (% DW)	1.23 ± 0.09	1.19 ± 0.07	0.34	1.39 ± 0.15	1.23 ± 0.15	0.77	1.36 ± 0.15	1.50 ± 0.34	0.08

Values represent means \pm S.E. calculated from plants transplanted to three different positions in each of three OSPW or freshwater trenches TKN total Kjeldahl nitrogen

Test significance: *p<0.05; **p<0.01; ***p<0.001

photochemical efficiency $(F_{\rm v}/F_{\rm m})$ and photochemistry performance index (PI) with respect to plants transplanted to freshwater (p<0.05 both cases, Table 3). The abovementioned results indicate that leaf fluorescence, which integrates multiple environmental and internal signals related to stress (Maxwell and Johnson 2000), was negatively affected by conditions encountered in the OSPW wetlands.

In wetlands, where water is normally readily available to roots, any stress impairing water uptake by affecting either root growth or root water permeability

Table 3 Chlorophyll *a* fluorescence results of leaves

	Trenches					
	OSPW	freshwater	t value	p value		
Skullcap						
$F_{\rm v}/F_{\rm m}$	0.77 ± 0.01	0.77 ± 0.02	0.05	0.962		
PI	0.73 ± 0.21	0.74 ± 0.18	0.02	0.985		
Mint						
$F_{\rm v}/F_{\rm m}$	$0.84 {\pm} 0.00$	$0.84 {\pm} 0.00$	1.08	0.294		
PI	4.13 ± 0.47	4.25 ± 0.41	0.18	0.857		
Smartweed						
$F_{\rm v}/F_{\rm m}$	0.79 ± 0.01	0.81 ± 0.00	2.60	0.019		
PI	1.16 ± 0.17	2.03 ± 0.33	2.29	0.035		

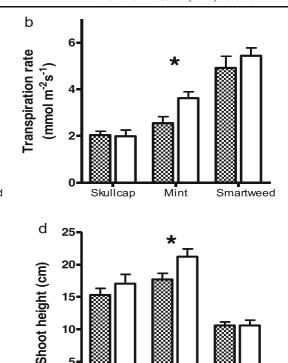
Values represent means \pm S.E. calculated from plants transplanted to three different positions in each of three OSPW or freshwater trenches

 F_v/F_m maximum quantum yield of primary photochemistry, PI photochemical performance index, OSPW oil sands processed water

can unbalance the whole plant water relations and lead to water deficits compounded from multiple causative factors (Pezeshki 2001). In addition to salinity reaching ranges with toxic or osmotic detrimental effects, the higher naphthenic acids contents in OSPW with respect to freshwater trenches (Table 1) could potentially impair root activity and contribute to water deficits and measurable changes in leaf water relations (Kamaluddin and Zwiazek 2002). The interaction of simultaneously elevated salinity and naphthenic acids could lead to synergistic effects on forbs. Mint plants demonstrated significantly lower stomatal conductance in OSPW compared to freshwater (p=0.028, Fig. 1a). These reductions led to differences in transpiration rates (p=0.020, Fig. 1b) suggesting that, for mint, the diurnal balance between transpiration and water uptake is less favorable in OSPW than in freshwater.

The diffusion of CO_2 to the reaction site of RuBisCO, the enzyme involved in the first carbon assimilation reaction, is one of the main processes influencing photosynthetic performance in stressed plants (Flexas et al. 2004; Chaves et al. 2009). Thereby, carboxylation limitations due to the previously mentioned lower stomatal conductance seem to explain the reduced net photosynthetic rates of mint plants transplanted to OSPW trenches relative to those growing on freshwater trenches (p= 0.038, Fig. 1c). Despite the fact that skullcap did not show statistically different photochemical efficiencies and stomatal conductance between treatments (Table 3, Fig. 1a), plants transplanted to OSPW trenches showed a lower net photosynthesis rate than those growing on freshwater trenches (p=0.025, Fig. 1c). This result





5

0

Skullcap

Fig. 1 Performance parameters measured on plants transplanted to trenches filled with oil sands processed water (OSPW) or oil sands freshwater (OSF). a Stomatal conductance, b transpiration

Skullcap

rate, c net photosynthesis rate, and d shoot height. Asterisks indicate significant differences after t tests (p < 0.05)

Mint

Smartweed

suggests that some dissipative process may be acting downstream of PSII or, alternatively, mesophyll diffusive resistances to CO₂ movement may be affecting skullcap assimilation capacity of leaves on OSPWgrown plants. Finally, in spite of abovementioned lower photochemical activity in smartweed subjected to OSPW (Table 3), plants did not show statistical differences in net assimilation rates between treatments (p>0.05, Fig. 1c). This indicates that OSPW detrimental effects on photochemical activity were not strong enough to compromise smartweed carboxylation. Previous literature indicated either a facilitative effect of effluents on cattail photosynthesis rate of plants exposed to OSPW and potentially caused by ammonium enrichment (Bendell-Young et al. 2000; Crowe et al. 2001), or a neutral effect on cattail and water sedge plants dominating oil sands wetlands with respect to conspecific individuals established in natural wetlands (Mollard et al. 2012, 2013a). Our results contribute, for the first time, evidence that shows that plants' photosynthetic capacity was negatively affected by OSPW. The toxicity causes of OSPW with respect to photosynthesis are clear for mint as it was in line with its lower stomatal conductance. On the other hand, despite water relations sensitivity of skullcap plants along a gradient of environmental stress (Mollard et al. 2013b), we did not find the causes for the lower assimilation rates observed in skullcap plants exposed to OSPW.

In controlled experiments, OSPW proved to have few or no deleterious effects on wetland graminoids growth and whole performance (Bendell-Young et al. 2000; Crowe et al. 2001; Pouliot et al. 2012; Roy et al. 2014). Our expectation was to find dramatic reductions in plant performance in our study as forbs are usually more sensitive to stress than graminoids in restoration scenarios (Pywell et al. 2003). However, in spite of the abovementioned leaf chemical and physiological alterations, which are a clear indication of physiological stress in forbs transplanted to OSPW trenches, plants showed 100 % survival at the end of the growing season and no apparent differences in vigor or stress symptoms such as pronounced chlorosis or necrosis in leaves of



OSPW-exposed compared to freshwater plants. Furthermore, none of the species showed differences in leaf dimensions between treatments (p>0.05, data not shown). On the other hand, mint, the species that displayed the poorest ecophysiological performance in OSPW, showed significantly shorter shoots in this treatment with respect to freshwater (p<0.05, Fig. 1). Altogether, our results suggest that while OSPW is slightly or non-toxic for most of the vascular plants studied so far (Bendell-Young et al. 2000; Crowe et al. 2001; Pouliot et al. 2012; Roy et al. 2014), wetland forbs may show a range of tolerances from little affected (e.g., skullcap, smartweed) to sensitive species (i.e., mint).

Conclusions

Oil sands companies face the challenge of creating habitats for wetland vegetation in the novel conditions of reclaimed oil sands landscapes. The functionality and diversity of those habitats might be limited by high levels of salts and residual hydrocarbons. Conditions in both OSPW and freshwater treatments proved to be mild for the survival of all tested wetland forbs during the first growing season as seen by small or undetectable reductions in growth. However, sublethal effects of OSPW on forbs were seen in their impaired ion selectivity and limited net CO₂ assimilation rates in two of the three species studied. While photosynthetic differences at the leaf level do not necessarily scale up to plant abundance in a community, the reduction in net carbon assimilation rates in OSPW amended trenches may lead to tighter trade-offs with respect to energy budgets and a narrower margin for recovery in the case of limiting resources or compounding or cumulative stress factors (e.g., grazing, drought). In this way, freshwater created a better habitat for wetland forbs than OSPW, indicating that water used as an amendment may have an important role in structuring forb assemblages and affecting oil sands wetlands functional integrity. The successful forbs transplants and growth in trenches suggest that manually introducing these species may be a viable reclamation strategy to increase richness in the post-mined landscape.

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Compliance with ethical standards

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Conflict of interest The authors declare that they do not have conflicts of interest.

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