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Research Article

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Species Composition and Morphological Variation of Crayfish in the Gault Nature Reserve

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Abstract

Crayfish play a crucial role as ecosystem engineers in freshwater ecosystems, yet they remain poorly represented in ecological studies. Integrating aquatic invertebrates into biodiversity monitoring efforts is urgently needed, particularly given their important ecological role as indicators of freshwater ecosystem health. The purpose of this pilot study was to record crayfish presence in the Gault Nature Reserve and to characterize species identity, morphology, and dorsal colouration of individuals from two habitats sampled (Lake Hertel and a nearby stream), with colouration assessed for substrate matching. This study records the occurrence of two crayfish species in the Gault Nature Reserve of McGill University and examines their morphological and colouration differences. Two species of crayfish were identified: the Virile crayfish (*Faxonius virilis*) in Lake Hertel and the Appalachian Brook crayfish (*Cambarus bartonii*) in a nearby stream. A key outcome of this pilot survey is the identification of different crayfish species living in different habitat types within the reserve. This finding demonstrates the necessity of broader biodiversity surveys of freshwater species in Gault. Morphometric analyses showed significant size differences between species aligning with ecological differences observed between the two site-specific species, though these disparities likely reflect species–specific traits more than habitat effects alone, which could not be isolated in this study design. Colouration analyses did not support the hypothesis that crayfish colouration matches substrate colours for camouflage. The analysis of colouration is considered exploratory and must not be considered a conclusive test of substrate matching because of the failure of photographic colour extraction to match colouration and the absence of consideration of body size, sex, and developmental stages. Future research should incorporate more controlled colour assessments, expand the survey area, and conduct genetic analyses and transplant experiments to clarify whether colouration and morphology are determined by environmental or genetic factors.

Introduction

Fully documenting all components of an ecosystem is often limited by the scale of resources needed. However, failing to sufficiently study all the components of an ecosystem can result in blind spots that compromise restoration efforts, species protection[1], and invasive species management. The Gault Nature Reserve of McGill University located in Mont St–Hilaire, Quebec, Canada, is a protected reserve of 1,000-hectare of old-growth forest within Canada's first UNESCO Biosphere Reserve, where a sugar maple-hickory forest matrix provides a variety of terrestrial and freshwater habitats. Although a significant body of work has been conducted, freshwater ecosystems are understudied relative to terrestrial habitats at the reserve, a trend observed worldwide [2]. Freshwater biodiversity is declining much faster than most terrestrial ecosystems, yet it remains significantly underrepresented in research and conservation efforts[1,3]. As the quantity of research projects and conservation efforts increases at the Gault Reserve, it is essential to broaden the research focus to develop a comprehensive understanding of the mountain's ecology. Additionally, when engaging in invasive species management, recreational activities, scientific research, and trail maintenance that can affect aquatic life, there is a critical need to have a full account of the organisms living on the mountain[4,5]. Furthermore, as the reserve increases its efforts in invasive species management, currently with terrestrial plants, it is crucial to ensure invasive species, like the Rusty crayfish (*Faxonius rusticus*), which can cause major disruptions in freshwater ecosystems[6], are detected early in aquatic habitats. Historical gaps in surveillance mean that no mollusks or crustaceans have been officially recorded by the reserve, although past anecdotal reports suggest the presence of both. In contrast, over 200 species of birds and more than 800 species of microlepidoptera have been officially recorded.

Crayfish are critical components of freshwater systems because they are omnivorous, serve as prey to many fishes, and function as ecosystem engineers[7,8]. Crayfish alter their habitats by changing detrital processing rates, causing bioturbation of sediments, and altering algal cover on substrates[7,9]. Moreover, crayfish are considered keystone controllers of trophic webs, bioindicators of ecosystem health, and indicator species of environmental conditions such as pollution and/or water temperature changes[8]. In headwater streams, crayfish can increase the rate of leaf litter decomposition, as well as affect the amounts and distributions of fine particulate matter, thus affecting benthic habitat conditions[9]. Crayfish can also cause bioturbation of stream beds, thus inhibiting particle consolidation, affecting algal cover, as well as sand-gravel surface composition[7].

Crayfish traits vary across habitats, and both morphology and colouration can reflect interacting biological and environmental factors[8,10]. Body size and shape can covary with flow conditions and substrate structure[10,11]. Research has demonstrated habitat-size and shape variation in crayfish, including Perry et al. (2013), which showed variation in rusty crayfish morphology based on lake and stream habitat with differing flow rates, and Clark et al. (2008), which showed size-dependent habitat use among lotic crayfish, with larger individuals tending to occur in deeper, slower pools and smaller individuals tending to be found in riffles with coarser substrate. Colour phenotypes may correlate with habitat background, and colouration can also be influenced by diet and the breeding season; however the functional significance of colouration in freshwater crayfish remains unclear[12, 13].

This study aims to address this knowledge gap by providing the first evaluation of crayfish presence, identification, and morphology in the Gault Nature Reserve. Specifically, we aim to: [1] confirm the presence of crayfish in Lake Hertel and a nearby stream and, if present, identify them to species and [2] compare their morphology and colouration differences in relation to their distinct habitats. We hypothesized that crayfish occur in the reserve and that individuals from different habitats will differ in morphology. We expect that morphological characteristics such as carapace, abdomen, and claw length or width, as well as total length, will be smaller in crayfish from stream habitats compared to lake habitats due to potential adaptations to distinct environmental conditions related to habitat types, such as substrate type and colour, water flow, depth, and predator and prey types. In particular, crayfish from faster-flowing habitats may exhibit smaller morphologies than crayfish from slower or lentic habitats. Additionally, we expect that both groups' dorsal exoskeleton colouration will match their respective habitat substrate colour to improve camouflage ability.

Since each species is represented at only one sampling site, habitat type and species are confounded in this pilot study. We therefore regard all morphology and colour data as descriptive data associated with each site rather than as tests for habitat effect. Our main goal is to record the occurrence and species identity of crayfish at two freshwater habitats in the reserve, as well as the associated morphometrics and dorsal colour patterns for the individuals collected.

Methodology

As, to our knowledge, the presence of crayfish in the Gault Nature Reserve of McGill University has never been officially documented, we relied on anecdotal reports to identify potential site locations. A pilot study was done on July 10, 2025, to determine our capacity to find and catch crayfish with different capture techniques in 3 locations (2 streams and Hertel lake). For this study, 2 of the 3 sites identified in the pilot study were retained for this study and were sampled for crayfish on July 11 and 12, 2025, in the Gault Nature Reserve of McGill University, located in Mont St–Hilaire, Quebec, Canada.

We captured crayfish by visually locating the specimens and catching them manually. Each site was sampled three times over a two–day period. A total of 41 crayfish were retained for experimentation. The sample size from the lake site was 28 crayfish (*Faxonius virilis*) (18 males, 9 females, 1 individual of unidentifiable sex) and 13 from the stream site (*Cambarus bartonii*) (1 male, 9 females, 3 individuals of unidentifiable sex). Life stages were not recorded. Crayfish were sedated with clove oil and ethanol then transferred to a shallow beige container to provide a uniform background. Photographic lights and a tripod holding an iPhone were used to take dorsal and ventral photographs. Crayfish were marked with nail polish before release to avoid recapture.

Photographs and observations were taken using a WILD M3C microscope (Heerbrugg, Switzerland) to locate unique features that can be used for species identification, including the presence of hairs and/or spines on specific body parts, rostrum shape, etc. The crayfish we found in the lake were identified as *Faxonius virilis* (The Virile crayfish). We identified the stream crayfish as *Cambarus bartonii* (Appalachian Brook crayfish). Crayfish morphometrics (carapace length [CL], carapace width [CW], abdomen length [AL], and total length [TTL]) were analyzed in R, testing site/species–associated differences with MANOVA, then ANOVA. Photos were processed to extract HEX colours, converted to CIE Lab, and compared with substrate reference colours using ΔE (CIEDE2000); since the extracted colours were generally darker than the photographed appearance, the colour analysis was considered preliminary.

A more thorough description of the methodology is presented in the supplementary materials (S1). The dataset (S2) and R scripts (S3–5) are available as supplementary materials.

Results

Two species of crayfish were found and identified at the Gault Nature Reserve of McGill University. We identified the stream crayfish as the Appalachian Brook crayfish (*Cambarus bartonii*) and the Lake Hertel crayfish as the Virile crayfish (*Faxonius virilis).*

Lake crayfish (*Faxonius virilis*) were significantly larger than stream crayfish (*Cambarus bartonii*) across all four body measurements combined (MANOVA: Pillai=0.649, F=17.59, df=4, 38, $p<3.02\times10^{-8}$). Univariate ANOVAs confirmed Lake crayfish (*Faxonius virilis*) were significantly larger than stream crayfish (*Cambarus bartonii*) for carapace length (ANOVA: F=32.13, df=1, 41, $p<1.28\times10^{-6}$), carapace width (F=17.81, df=1, 41, $p<1.32\times10^{-4}$), abdomen length (F=19.43, df=1, 41, $p<7.36\times10^{-5}$), and total length (F=32.26, df=1, 41, $p<1.24\times10^{-6}$) (Figure 1A). Because species and site are confounded, this difference reflects species/site differences rather than a tested habitat effect. Univariate ANOVAs were interpreted conditional on a significant MANOVA; p–values were additionally adjusted using the Holm procedure to correct for multiple comparisons. All univariate effects remained significant when Holm correction was applied. Effect sizes and 95% CI for all traits and Holm–adjusted p–values are presented in Supplementary Material S1. All data met the assumptions required for MANOVA and ANOVA

Carapace length was 24.00 ± 0.73 mm in the lake group and 16.24 ± 1.27 mm in the stream group; carapace width was 10.52 ± 0.32 mm and 7.81 ± 0.64 mm; abdomen length was 15.74 ± 0.53 mm and 10.96 ± 1.12 mm; and total length was 43.35 ± 1.16 mm and 30.41 ± 2.24 mm, respectively.

Although no statistical test was conducted, box plots suggest that males tended to be larger than females across all four body measurements (Figure 1B). We note that the lake crayfish group included mostly males, while the stream group was mostly female, which may influence size comparisons. Because life stage and sex were not included as covariates, the observed patterns should be interpreted cautiously.

Lake crayfish (*Faxonius virilis*) had a significantly smaller colour difference (ΔE) from the other habitat substrate compared to their own (ANOVA: F=5.258, df=1, 56, p=0.0256) (Figure 2). Based on extracted colours, Lake crayfish (*Faxonius virilis*) had a dorsal exoskeleton colouration more similar to the stream substrate than to the lake substrate. Stream crayfish (*Cambarus bartonii*) showed no significant difference in colour similarity between their own and the other habitat substrate (F=0.3061, df=1, 24, p=0.585) (Figure 2). However, since the extracted HEX values failed to correspond with the photographed appearance, the colour results are seen as exploratory and are not taken as a definitive test of substrate matching.

Discussion

In this study, we sampled one location in a lake habitat, Lake Hertel, and one stream location to confirm crayfish presence, identify the species, and compare their morphology and colouration differences in relation to their distinct habitats. We hypothesized that if crayfish are present in the Gault Nature Reserve, individuals from different habitats would differ, and all mor-

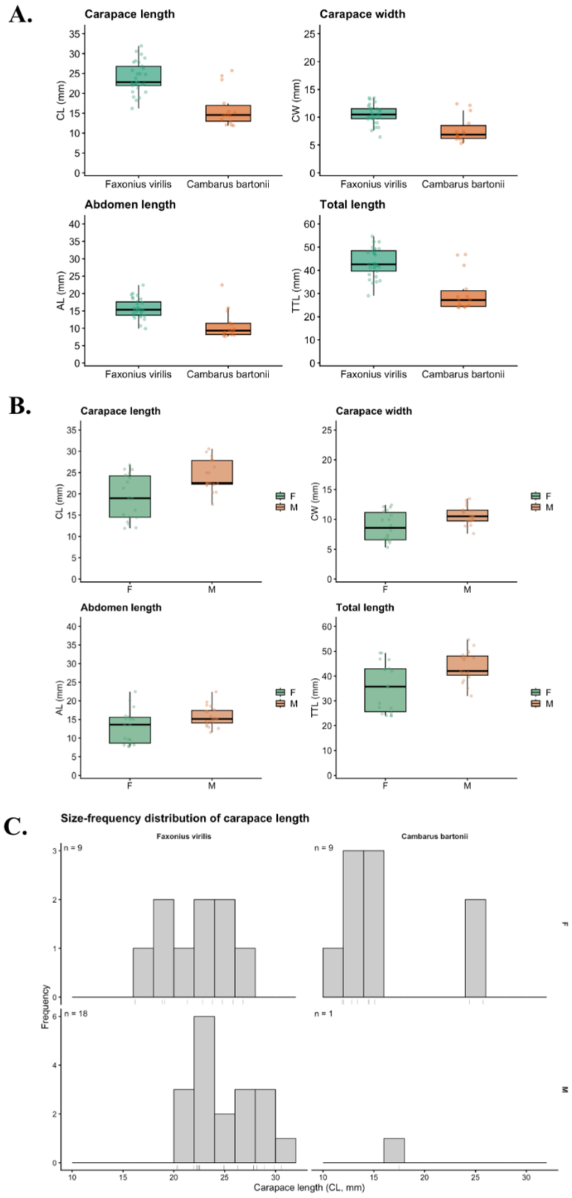

Figure 1. Boxplots comparing body measurements (carapace length, width, abdomen length, and total length) (A) Between Lake Hertel and stream crayfish. Lake specimens (Faxonius virilis) were significantly larger across all metrics than stream crayfish (Cambarus bartonii) (MANOVA: Pillai = 0.649, F = 17.59, df = 4.38, $p < 3.02 \times 10^{-8}$). (B) Body measurements of Lake Hertel and stream crayfish grouped by sex. Boxplots show carapace length, width, abdomen length, and total length, illustrating size variation by sex and between the two site-specific species. Because habitate and species are confounded, differences cannot be attributed to habitat alone. (C) Size-frequency histograms of carapace length (CL, mm) shown by species and sex, with sample sizes indicated in each panel, provided to visualize the observed size distributions and potential size class structure. Histograms are descriptive only because sex ratios were unbalanced and life stage was not recorded.

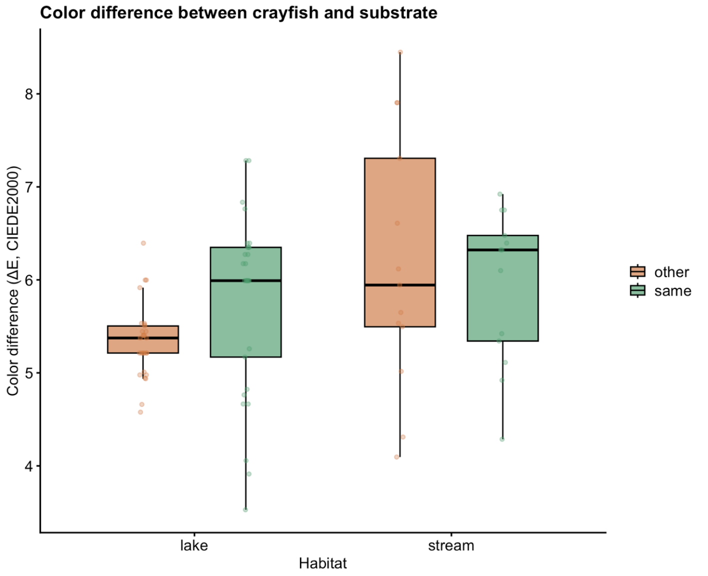

Figure 2. Color difference (ΔE, CIEDE2000) between crayfish dorsal exoskeleton and substrate from both habitats. Lake crayfish (Faxonius virilis) showed a significantly smaller ΔE when compared to the stream substrate than to their own (ANOVA: F = 5.258, df = 1, 56, p = 0.0256). Stream crayfish (Cambarus bartonii) showed no significant difference between substrates. However, this finding is considered preliminary because of the discrepancy between the hex values obtained and what was photographed.

phology metrics of stream crayfish (*Cambarus bartonii*) would be smaller than those of lake crayfish (*Faxonius virilis*) and that both groups' dorsal exoskeleton colouration would more closely match their respective habitat substrates.

Insufficient inventories of freshwater species create critical blind spots for conservation, invasive species management, and early detecting of ecological change [1, 3]. Species identification revealed not one but two different species in the two sites sampled at the Gault Nature Reserve: the Appalachian Brook crayfish in the stream habitat and the Virile crayfish in the lake habitat. When this study was initiated, variations in morphology were expected to be driven by habitat differences as we only expected to find one species of crayfish based on anecdotal reports. Species identification fundamentally changes the interpretation of the morphometric analyses. Since each species of crayfish was represented at only one site, species identity and habitat are completely confounded in this experiment. Therefore, habitat effects cannot be separated from baseline interspecific differences without replication of habitats within species. These results can be considered a good baseline with regards to detection records from two sampled locations. However, it must be noted that the results must be considered as presence records rather than absence records for both species in other habitats within the reserve.

The most significant finding of this investigation is not the preliminary colour result, but the discovery of two crayfish species in different habitat types in the Gault Reserve. This indicates habitat filtering, habitat specificity of the two crayfish species, or biotic interactions (such as competition and predator-mediated habitat use) as possible explanations for the observed segregation of the two crayfish species. Since each of the two crayfish species was collected in only one habitat type in this preliminary investigation, future investigations in the reserve and seasonally replicated investigations must verify whether this segregation is consistent through time and whether the two crayfish species co-occur in other areas of the reserve.

Adult Appalachian Brook crayfish can range from 18.5 mm to 36.7 mm in carapace length, while the Virile crayfish can range from 23.2 to 55.0 mm [14, 15]. In our study, the Appalachian Brook crayfish (maximum carapace length was 25.74 mm) from the stream were smaller by all metrics compared to the Virile crayfish (maximum carapace length 29.86 mm), although our data was not separated by developmental stages or sex (Figure S3B). Since juveniles were not identified and excluded, size and color estimates may reflect a mixture of life stages, which may cause bias within and between sites. Although size difference likely reflects species–related differences, results still align partially with our hypothesis that stream crayfish (*Cambarus bartonii*) would be smaller than lake crayfish (*Faxonius virilis*), consistent with ecological patterns observed in other systems where body size correlates with habitat constraints such as water depth, flow, and resource availability. Perry et al. (2013) demonstrated this intraspecific trend in Rusty crayfish (*Faxonius rusticus*), which were significantly smaller in high–velocity streams compared to low–velocity streams and lakes[11]. Furthermore, Clark et al. (2008) found that the Allegheny crayfish (*Faxonius obscurus*) of larger size tend to occupy deeper pools with slower current velocity and smaller grain sizes, while smaller individuals preferred riffle habitats with substrates made of coarser grain size[10]. For a direct comparison, reciprocal transplant experiments can be conducted in the future by rearing juvenile stream crayfish (*Cambarus bartonii*), under lake-like conditions and juvenile lake crayfish (*Faxonius virilis*), under stream-like conditions to assess whether the size differences persist under different environmental conditions. This type of experiment would be possible as, although both species were found at the Gault Nature Reserve only in their preferred environment, both species are known to live in both lake and stream environments[14]. Body size is ecologically significant in crayfish because it affects resource usage, food and shelter competition, species interaction, and susceptibility to variable predation[8]. The pronounced differences observed between species at Gault suggest strong species-habitat segregation (or habitat association) within the reserve, which could reduce interspecific crayfish competition and stabilize the community structure. Recognizing these species–specific patterns is essential for conservation, in order to ensure that conservation efforts are not compromised by the failure to identify or monitor all species present, which could result in ineffective or potentially harmful management strategies.

The survival and evolutionary success of many organisms largely depend on their capacity to avoid detection by predators[16]. The second part of our hypothesis predicted that crayfish dorsal colouration would closely match the substrate of their native habitat, reducing predation risk, an ability many crustaceans have evolved[16, 17]. To our surprise, the statistical analysis did not support this prediction: neither species showed a statistically significant match to their own substrate compared to the substrate from the alternate habitat. In fact, the Virile crayfish matched the colour of the stream substrate better than its own substrate according to our data. This result did not support the camouflage hypothesis, and the colour findings are considered preliminary due to the limitations in the image–based colour extraction. Given that the substrate reference colours were based on a habitat average and that the crayfish colours were based on an extraction from all non–background pixels without glare masking, the colour analysis is interpreted as a preliminary analysis rather than an actual test of camouflage. Although multiple mechanisms could contribute to colouration, the current data do not allow strong inference about selection or signaling, so the colour component is interpreted as exploratory. It is possible that background matching is not a dominant selective pressure for these species in the habitats we studied, as both the studied species used shelter, mostly rocks, to hide during capture, which may indicate a greater reliance on physical refuge than on colouration for predator avoidance. However, given the preliminary nature of the obtained results, interpretations remain speculative. Freshwater crayfish show variations in colour within species, populations, and even intra–individual differences, but the function behind dorsal colouration is poorly understood[18]. The colour discrepancy between the substrate and the dorsal exoskeleton could be explained by colour variation that occurs throughout their life stage. Indeed, colour can vary depending on developmental and reproductive stage in some crayfish species [15, 17, 19]. Additionally, some studies suggest that crayfish colouration can also be influenced by diet [19, 20]. Our results underscore that colouration in crayfish may result from multiple interacting factors, including ontogeny, diet, and reproductive strategies, rather than camouflage alone, if it has any function at all although, our results being preliminary, may not accurately capture true colouration patterns

However, it is possible that the results from this report may not accurately represent true crayfish colouration of the studied population. The obtained HEX colour values represent mostly shades of black when visualized which contradicts our observations, where the crayfish collected were lighter and showed more inter–individual colour variation (Figure S2D–E, Figure S2I–J, Figure S3A–C). This was especially true for the Virile crayfish that produced very dark values while in the laboratory the Appalachian Brook crayfish adults were the darkest (Figure S2D–E, Figure S2I–J, Figure S3C–IDS6). All team members noted that captured crayfish appeared to better match the substrate from their own habitat, with the stream's rocky substrate and associated crayfish having more reddish tones, while the lake crayfish had more neutral colours and spots that blended better into the mix of sand, pebbles, and rocks of the lake (Figure S4). The lake crayfish (*Faxonius virilis*) had more neutral colours and spots that blended better in the mix of sand, pebbles, and rocks of the lake (Figure S4). These colour values may be due to suboptimal lighting and image standardization that compressed the colour values and tended to favor darker colours. It is possible that the Virile crayfish matched the colour of the stream substrate better than its own lake substrate is due to overlapping colour ranges between the two habitats. It may also be a function of the crayfish sample size being too small, rather than representing an adaptive response to a substrate these crayfish do not inhabit. While methodological issues such as those described above cannot be excluded, there is some support in the literature for our initial substrate colour matching hypothesis. Nevertheless, this support from the literature does not address the discrepancy in our own results. While Mathews (2025) demonstrated that ontogenetic changes in the Virile crayfish are strongly correlated with colour, this pattern of strong inter– and intra–individual colour variation occurred in the fall, not the summer[17]. According to Mathews (2025), during the fall reproductive season, inter–individual colour variation is high, with some crayfish developing bright green or blue chelae while others remain brown, and intra–individual variation is also high as chelae differed in colour from the carapace and abdomen, both of which did not correlate with sex or body size[17]. In contrast, during the summer when our study took place, most individuals displayed uniformly brown colouration (Mathews, 2025) [17], meaning these seasonal reproductive color changes were less likely to be the primary source of variation in our summer samples. In light of the above, we believe that the most likely explanation for the discrepancy between observed color and extracted color values in this dataset is methodological limitations in image standardization and photographic color extraction, although biological color variation cannot be ruled out.

Despite these methodological limitations, previous studies on Virile crayfish provide valuable context that can help interpret our findings. Using laboratory and field experiments, Thacker et al. (1993) found that the colour of Virile crayfish strongly correlated with its habitat substrate colour, but diet did not, which is consistent with our hypothesis. Additionally, field transfer experiments demonstrated the ability of adults to change colour[12]. Light penetration measurements suggest that light wavelengths available in a habitat are related to the types of colour morphs present[12]. Moreover, Thacker et al. (1993) found that crayfish color changed in a laboratory rearing experiment in which juvenile *Orconectes virilis* were held in separate lake waters, indicating that habitat-related water conditions may play a role in crayfish color expression[12]. However, various experiments showed no difference in survival rates of colour morphs regardless of whether they matched their substrate or not, suggesting that it is still unclear whether colour serves any function to the Virile crayfish. Seasonal and body region colour variation has been reported for *Faxonius virilis*, but the data do not allow inference regarding signaling versus camouflage because of the limitations of photographic colour extraction.

The literature review for Appalachian Brook Crayfish yielded limited and sometimes inconsistent information on coloration. Fewer studies were found that directly address dorsal exoskeleton colour for this crayfish species, potentially reflecting the confusion and disagreement in the literature regarding this species' taxonomic ID. Some experts have proposed subspecies (*C. b. bartonii, C. b. cavatus, C. b. carinirostris*), but this level of taxonomic resolution is beyond the scope of this paper [21, 22]. The broad spread in ΔE values from this study suggests that the Appalachian Brook Crayfish in the stream had a wider range of colours than the Virile Crayfish from Hertel Lake. As we did not separate crayfish by developmental stage for our colour analysis, ontogenetic differences in colour may explain this broad spread, but to date no correlation between ontogenetic changes and colour has been found elsewhere in the Appalachian Brook crayfish, indicating that intraspecific colour variation is likely due to other factors[23]. However, the Massachusetts Division of Fisheries and Wildlife (2025) states that younger individuals of Appalachian Brook crayfish are lighter, which is also what we observed in the field (Figure S2I–J)[24]. No study was found addressing whether this species tends to colour match its substrate, which points to a gap in the literature.

The Bartlett's test revealed a highly significant difference in variance for colour difference values ($p \approx 1.02 \times 10^{-7}$), due mostly to the Appalachian Brook stream crayfish having a much higher colour variance (Figure 2). This broad spread in ΔE values suggests that the Appalachian Brook crayfish had a wider range of colours than the Virile crayfish from Hertel Lake. Several explanations could explain this pattern. One possibility would be ontogenetic variation, as we did not separate crayfish by developmental stage for our colour analysis, but our review revealed disagreement among sources regarding the correlation between ontogeny and Appalachian Brook crayfish colouration[23, 24].

Another explanation is hybridization, as the Appalachian Brook crayfish is known to form hybrid zones with related species, potentially introducing colour polymorphisms[25], explaining the wider colour range reflected in our results. Although less likely, as we only detected one species of crayfish in this stream (*Cambarus bartonii*), larger surveys and DNA analyses would be needed to exclude this theory. Alternatively, this variance in dorsal colour may reflect deeper evolutionary forces. Graham (2023) proposed that crayfish colours may be a neutral trait caused by genetic drift or pleiotropic effects rather than adaptations for camouflage[13]. Under this hypothesis, high colour variability could occur because it offers neither advantage nor disadvantage in terms of fitness. This would explain the high variance from our results and why our data showed no clear substrate–matching pattern despite observed camouflage during fieldwork. Ultimately, the pronounced colour variability observed in stream crayfish (*Cambarus bartonii*) may result from factors such as hybridization or evolutionary processes unrelated to strict substrate matching, though adaptive background–matching should not yet be ruled out.

Future studies should aim to address limitations of this study by separating individuals by sex and developmental stage for morphometric and colour analyses, as variation might be at least partly due to ontogeny. In addition, replicating this study with a higher quality camera, superior lighting, stronger sedative to keep crayfish still, standardized conditions, with consistent diet and at different reproductive seasons would help clarify the biological significance of colouration in the Virile crayfish and the Appalachian Brook crayfish. Transplant experiments between lake and stream habitats could reveal whether observed body size differences are environmentally induced or species–specific and whether dorsal colour would change to match the substrate. Expanding surveys across all water bodies in the reserve is needed, as they were not covered by this study, combined with genetic testing (e.g., DNA barcoding) to confirm species identification and rule out potential hybridization. This is especially important for conservation, with invasive crayfish species being a threat in Quebec and some even being able to hybridize with native species in some cases [25, 26, 27]. Future studies should also test what accounts for the segregation of these two species into the lake and stream, respectively, by investigating if they differ in habitat preference, seasonal movement, or competitive interactions that restrict co-occurrence. Ultimately, understanding species identity, morphology, and colouration has real implications for conservation planning. Crayfish are important prey, omnivores, and ecosystem engineers; changes in their traits or abundance can affect detritus processing, food web structure, and habitat condition[7, 8]. Moreover, because crayfish are sensitive to environmental disturbances such as acidification, metal contamination, and low pH[14], monitoring their populations could serve as an early warning for freshwater degradation, especially with the high number of visitors at the reserve possibly being a source of contamination and disturbances and with streams near trails being less monitored than Hertel Lake. Our findings lay the groundwork for future monitoring at Gault Nature Reserve and highlight the need to integrate aquatic invertebrates into research and conservation efforts.

Conclusion

This study provides the first documented record, to our knowledge, of crayfish at the Gault Nature Reserve of McGill University, with two different species occupying different habitats: the Virile crayfish in Lake Hertel and the Appalachian Brook crayfish in a nearby stream. This species/habitat distinction is the key result of this study, as it indicates new, previously unrecorded freshwater biodiversity and raises interesting ecological questions about habitat preference and community structure at the reserve. Morphometric tests also indicated that there were size differences between the two groups, although due to the confounding of species and habitat, these differences should be considered more as species/site differences than habitat differences in isolation. The colouration analyses did not support the substrate–matching hypothesis as proposed and are considered preliminary because of issues with image standardization and referencing the colour of the substrate.

Future work should include better–controlled colour measurements, seasonal sampling, and both sex– and age–specific analyses. Future research should clearly identify life stage and either limit analysis to adults or include size/developmental stage as a covariate. In addition, a reserve–wide survey alongside DNA genetic identification should be done to rule out hybridization and explore the occurrence of crayfish in other water bodies. Doing so, the initial hypothesis of this paper could be tested by comparing morphology within the same species across different habitats to identify environment–specific traits and confirm whether other species of crayfish inhabit the reserve. The use of transplant experiments could also clarify whether observed patterns are species–specific or environmentally driven.

Our findings address a major research gap in the reserve's freshwater ecosystem and underscore that even very well monitored, protected, and studied areas such as the Gault Nature Reserve can harbor omissions in its biodiversity monitoring coverage. Crustaceans and other freshwater organisms are among the only taxonomic groups without a species list at the reserve. Unfortunately, freshwater ecosystems are often the ones suffering from inferior research and conservation attention[1, 3]. Freshwater ecosystems cover a small fraction of Earth's surface, yet they contain a disproportionately large share of global diversity and are especially fragile and vulnerable to contamination from chemicals and invasive species[1, 2]. Crayfish are important components in these ecosystems; they are keystone players that shape community dynamics and ecosystem function[7, 8]. Through their feeding, burrowing, and sediment–disturbing activities, they accelerate leaf litter decomposition, redistribute fine sediments, and modify habitat complexity[8, 9]. These changes ripple across trophic levels, affecting fishes, algae, invertebrates, and even terrestrial species. Changes in crayfish populations would alter nutrient flow and resilience of freshwater systems[8, 9], as well as make them more vulnerable to the introduction of invasive species such as the keystone invader Rusty Crayfish (*Faxonius rusticus*), which causes devastating damage to Canadian ecosystems[6]. Furthermore, they serve as bioindicators of water quality and habitat health, making them a key species to monitor as an indicator of broader ecological health important for conservation planning.

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