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TECHNICAL MEMORANDUM

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TO Megan Lord-Hoyle, Vice-President, Sustainable Development
Baffinland Iron Mines Corp.

CC

FROM Marina Winterbottom **EMAIL marina_winterbottom@golder.com**

UPDATE ON THE STATUS OF *MARENZELLERIA* SPECIES ON BAFFIN ISLAND

This technical memorandum provides an update on the status of specimens from the genus *Marenzelleria*, collected in various locations within Milne Port as part of Baffinland Iron Mine Corporation's (Baffinland) Non-Indigenous Species and Aquatic Invasive Species (NIS/AIS) monitoring program. These specimens were initially identified as *M. viridis*, a species documented as invasive to European waters and placed on invasive species watchlists and flagged as High Risk for the Project area. **This update is provided** subsequent to an independent review of the specimens by a global expert on Spionidae (the order of marine worms that includes *Marenzelleria*), which resulted in the reclassification of the specimens as *M. arctia*. As suggested by its name, *M. arctia* is an arctic species, originally described from collections in North American arctic waters (Chamberlin 1920); accordingly, ***M. arctia* is not considered a potential NIS/AIS in the Regional Study Area including Milne Port.** Baffinland will continue to monitor for the presence of any *Marenzelleria* species in the Milne Port area and, as a precaution, Baffinland will treat all identified *Marenzelleria* specimens as having the potential to be invasive until the classification of *M. arctia* is confirmed through molecular methods. **This corrected classification confirms that, to date, no Project-related invasive species have been found through the AIS/NIS monitoring program.**

1.0 BACKGROUND

1.1 Taxonomic Identification

Conclusive taxonomic identification of collected specimens to species level and determination of NIS/AIS status in the Project area is challenging for a variety of reasons, including:

- Marine fauna in the Canadian Arctic are not thoroughly described in the existing literature and marine species inventories have not been undertaken in the Eastern Canadian Arctic as frequently as other Arctic regions, particularly in comparison to surveys in Northern Europe. This lowers the confidence in the species ranges on record, particularly for less common or more recently described species that may be cryptogenic to a broader area and not yet been described outside the range on record.
- Species ranges on record are not complete for all taxa. Recently described and/or rare taxa often have a limited range description, with a broader range inferred based on biological characteristics and tolerances.

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- Redescriptions and reclassifications of species and taxonomic groups lead to difficulties in determining the historic range of a species. The range on record may be linked to a previous name or description and existing species inventories and databases are not always updated as new species descriptions are accepted. Archived samples are often no longer available for review following redescription, leading to uncertainty in the species identification prior to the redescription or reclassification.
- Many available species descriptions are based on adult samples, with limited description of immature, juvenile or larval characteristics. Non-adult specimens may lack features present in adults that allow for specific identification (Steinerstauch 2019, pers. comm.).
- Fragmented samples, or samples damaged during collection, may also be missing key features that would be used to determine species.
- Availability of publications may impact descriptions. More recently published works may not be readily available or accepted by the wider taxonomic community, and updates may not be reflected in the identification keys used by the taxonomy labs.

Due to these uncertainties, Baffinland has developed an AIS/NIS monitoring protocol aimed to assess risk of Project-related introductions. The initial stages of the protocol involve independent verification of the specimens by outside laboratories or experts in the relevant taxonomic groups to confirm or clarify the initial identification, where required.

1.2 Genus *Marenzelleria* – Species Description and Known Geographic Distributions

The genus *Marenzelleria* contains five recognized species, of which *M. bastropi* and *M. neglecta* are the most recently described (Bick 2005; Sikorski and Bick 2004). *Marenzelleria* sp. can be difficult to distinguish based simply on morphology due to a combination of limited descriptions, overlapping morphological traits, lack of differentiating features in immature specimens, and hybridization between species (Sikorski and Bick 2004; Bick 2005; Blank et al. 2005). *M. viridis*, *M. neglecta*, and *M. arctia,* in particular, are morphologically similar, resulting in the three species being part of a cryptic sibling species complex (Sikorski and Bick 2004; Bick 2005; Green 2015). The recent redescriptions of the genus, descriptions of new species based on historical collections (*M. bastropi* and *M. neglecta*), incorrect species denomination in reporting, and synonymization of the former description of *M. jonesi* with *M. viridis* lead to uncertainty in the historical specimen records, particularly where distributions overlap (Blank et al. 2008; Sikorski and Bick 2004). As many historically collected specimens are no longer available, there is an inherent uncertainty in the actual species that may be represented by these original collections. Despite morphological similarities between species, there are notable behavioral and ecological differences that may aid in species differentiation (Renz and Forster 2013; Sikorski and Bick 2004).

At present, recognized species in the genus include:

- ***M. arctia*** – an Arctic Basin species, first described in the Beaufort Sea, Alaska, USA (Chamberlin 1920). Generally found at depths from 0 to 30 m, with an apparent preference of depths between 20-30 m (Sikorski and Bick 2004; Green 2015). Tolerant of large fluctuations in temperature and salinity, with salinities of 3-16‰ being the most favourable range (5-7‰ for reproduction) (Sikorski and Bick 2004). Phylogenetic analysis of *Marenzelleria* suggests *M. arctia* is the most basal taxon in the genus and may represent the ancestral species (Blank and Bastrop 2008).

- ***M. bastropi* (*M.* sp. A, *M.* Type III[1])** – Most recently described species in genus. Current known distribution is limited to Currituck Sound, North Carolina, where it occurs sympatrically with *M. neglecta*. Closely related (morphologically) to *M. neglecta* and *M. viridis*.
- ***M. neglecta* (*M.* Type II)** – Indications of a broad range, including the Atlantic Ocean, the Baltic Sea, and the Arctic Ocean (Bastrop et al. 1997; Sikorski and Bick 2004). Morphologically similar to *M. viridis*, and having overlapping habitats, differentiation between *M. viridis* and *M. neglecta* may be made based on *M. neglecta* generally preferring lower salinities (0.5-10‰ compared to 16‰ for *M. viridis*) (Sikorski and Bick 2004).
- ***M*. *viridis* (*M.* Type I)** – natural range presumed to be the western coast of the north Atlantic – described as native to east coast North America from Nova Scotia to Delaware, with a probable native range that includes waters around Newfoundland to Chesapeake Bay (Fofonoff et al. 2021). *M. viridis* is apparently more sensitive to low salinities compared to other species of *Marenzelleria*, typically found in eulittoral habitats with brackish waters where salinities do not fall below 16‰ (Sikorski and Bick 2004; Bastrop and Blank 2006).
- ***M*. *wireni*** – distributed in arctic waters. Found in a range of depths between 1 m and 55 m, where salinities are not below 30‰ (Sikorski and Bick 2004).

1.2.1 History on the Classification of *Marenzelleria as Invasive*

The genus *Marenzelleria* (presumably *M. viridis*) was first detected in waters outside of its natural ranges in 1979 (Bastrop and Blank 2006; O'Reilly and Nowacki 2019). The initial introductions in the North Sea led to rapid expansion, with detections occurring in most North Sea estuaries in the following years, and the first detection in the Baltic Sea occurring in 1985 (Bastrop and Blank 2006). Since the genus was first confirmed in the North Sea, invasions of one or more species (including *M. viridis, M. neglecta* and *M. arctia*) have been confirmed in the Pacific Ocean, North Sea, Baltic Sea, Barents Sea, White Sea and Sea of Azov (Bastrop and Blank 2006; ICES 2016; Fofonoff et al. 2021). *Marenzelleria* spp. are considered to be among the most successful invasive species in the Baltic Sea (ICES 2016).

Native species and functional diversity are generally naturally low in areas where *Marenzelleria* spp. have successful invaded (Kauppi et al. 2015; Maximov et al. 2014). In particular, these areas are characterized by very low abundances of marine polychaete worms, especially larger burrowing forms functionally similar to *Marenzelleria* (Maximov 2015; Quintana et al. 2018). These ecosystems are generally adapted to an absence of bioturbators and, due to a lack of competition, *Marenzelleria* spp. were able to fill that void, disrupting the existing environment by changing sediment characteristics through burrowing behaviour. In addition to low diversity, increasing eutrophication has also caused these areas to be vulnerable to successful invasion by *Marenzelleria* and other invasive species (Kauppi et al. 2015; Maximov et al. 2014).

Accordingly, *M. viridis* and *M. neglecta* are listed in the Database of Global Marine Invasive Species Threats as 'invasive to areas outside of East Coast North America' (Molnar et al. 2008). They are also listed in the National Risk Assessment as a potential invader to Canadian waters, including the Arctic region (Casas-Monroy et al. 2014).

[1] Type I, II, and II, and sp. A were names assigned to specimens with features that differentiated them from currently described species. While descriptions now exist, these names are still used in some literature, or were used in literature relevant to this memo.

However, collections of *M. viridis* in Canadian waters, including the Canadian Arctic, may indicate the currently listed range or taxonomic record is incomplete (Stewart et al. 1985; Cusson 2018; Brown et al. 2011; GBIF 2021; Miller et al. 2014). A review of the literature indicates that while the known documented range of the species does not include the Canadian Arctic, available evidence via historical collections suggested the genus was present in the area prior to Project operations and that *M. viridis* may be cryptogenic, if not indigenous to the Canadian Arctic (Stewart et al. 1985; Cusson 2018; Brown et al. 2011; GBIF 2021; Miller et al. 2014; Golder 2021a). However, due to the morphological similarities between species and the lack of available specimens for review, these may represent instances of *M. arctia*, or possibly *M. wireni* or *M. neglecta* (Radashevsky 2021, pers. comm.)

The primary invasion vector is considered to be transport through ballast water and sediments and once established, locally by currents (Bastrop et al. 1997; Molnar et al. 2008). In locations where *Marenzelleria* has been introduced, it may reach high densities, in some locations replacing native infauna and altering sediment characteristics (Molnar et al. 2008; Fofonoff et al. 2021). Once established, management is considered highly difficult or impossible (Molnar et al. 2008).

1.3 History of *Marenzelleria* at Milne Port

Specimens from the genus *Marenzelleria* were identified in benthic samples collected in Milne Port between 2016 and 2020 (Figure 1, Table 1). These samples were all collected shallow waters (3 m to 35 m) along the southern shore, primarily in locations known to have considerable freshwater influence, either from Phillips Creek, or from other smaller watercourses flowing into Milne Port. In 2016, 2017 and 2018, the collected specimens were unable to be identified to species level. In 2019 and 2020, specimens were initially identified as *Marenzelleria viridis*. The specimens were flagged for further review due to this species' invasive status in Europe and their inclusion on several invasive species watchlists. At the recommendation of Fisheries and Oceans Canada (DFO), these specimens were sent to the Benthic Ecology Lab at Université Laval for independent verification, which agreed with the identification of *M. viridis*.

Subsequent to this initial identification, in 2020, locations where *M. viridis* was found in 2019 were targeted for resampling with a focus on collection of specimens for DNA barcoding to resolve the species identification. Despite targeted sampling in areas previously found to have the species, no potential *M. viridis* specimens were found in benthic samples collected for DNA (Golder 2021a). Most stations where *Marenzelleria* sp. were collected in 2016, 2017 and 2018 have not been directly resampled, due to changes in the design of the program when station numbers were increased to provide better coverage of the marine environment. However, the expanded program has stations in close proximity to the old locations, allowing for the general locations to be resampled in 2019 and 2020 (Table 1). Of the historic stations with updated locations, only SW-2 (equivalent to BM-7) has had *Marenzelleria* collected in more than one sample year, with no new collections in 2020, indicating that *Marenzelleria* is not displaying invasive behaviours in Milne Port (Table 1).

LEGEND

- *Marenzelleria sp.* (2017)
- *Marenzelleria sp.* (2018)
- *Marenzelleria viridis* (2019)
- *Marenzelleria viridis* (2020)

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1:15,000 METRES

REFERENCE(S)

MILNE PORT IMAGERY CAPTURED AUGUST 2020 © 2020 DIGITAL GLOBE. ADDITIONAL IMAGERY COPYRIGHT © 20190802 ESRI AND ITS LICENSORS. SOURCE: MAXAR VIVID. USED UNDER LICENSE, ALL RIGHTS RESERVED.
PROJECTION: UTM ZONE 17 DATUM: NAD 83

CLIENT
BAFFINLAND IRON MINES CORPORATION

PROJECT
MARY RIVER PROJECT

TITLE
***Marenzelleria* OBSERVATIONS IN MILNE PORT OVER TIME**

CONSULTANT
GOLDER

YYYY-MM-DD	2021-08-17
DESIGNED	CB
PREPARED	AJA
REVIEWED	MW
APPROVED	MW

PROJECT NO.	CONTROL	REV.	FIGURE
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Table 1: History of Marenzelleria Specimen Collection in Milne Port

Initial Identification	Year	Collected/ Identified By[1]	Locations	Collection Depth	Site Conditions	Comments
Marenzelleria sp.	2016	SEM/ Envirosphere	Not reported	Not Reported	Not reported	Single specimen identified in benthic samples, specific location not reported.
Marenzelleria sp.	2017	Golder/ Biologica	BM-7	3-15 m*	Estuarine	2 specimens collected at former station, close to current station SW-2 (sampled 2019, 2020)
Marenzelleria sp.	2018	Golder/ Biologica	BM-1, BM-7, BM-9, BM-10, BW-4, BW-5	3-35 m*	Fresh/ estuarine	Locations near or equivalent to current stations SW-5, SW-2, SE18-1, SE18-2 (sampled 2019, 2020), SW-10, SW-15 (sampled 2020)
Marenzelleria viridis	2019	Golder/ Biologica	SE-1, SW-2	12 m, 21 m	Salt/ estuarine	Targeted again for sampling in 2020, with no new *Marenzelleria* collections
Marenzelleria viridis	2020	Golder /Biologica	SW-11, SW-12, SW-13, SW-14	17.2 m, 17.7 m, 13.5 m, 18.2 m	Fresh/ estuarine	New stations, but within 50 m of some historic sampling locations

[1]SEM: Sikumiut Environmental Management Ltd, Envirosphere: Envirosphere Consultants Ltd., Biologica: Biologica Environmental Services Ltd.

*Notes: depth range reported only, specific depth at collection not available

2.0 INDEPENDENT VERIFICATION

Specimens collected in 2020 that were identified and independently verified as *M. viridis* by both Biologica and Laval were sent to Dr. Vasily Radashevsky of the Russian National Scientific Center of Marine Biology in Vladivostok. Biologica recommended Dr. Radashevsky review the identification due to his expertise on Spionidae, the order of marine worms that contains *Marenzelleria*, as well as his familiarity with Canadian spionids through collaborative research with the Canadian Museum of Nature in Ottawa. Dr. Radashevsky examined four specimens fixed in formalin. All specimens were determined to be in very good condition at the time of receipt (Radashevsky 2021, pers. comm.). At present, *M. arctia* and *M. viridis* can only be distinguished morphologically by their maximal size and number of branchiate chaetigers[2]. Based on morphological examination, Dr. Radashevsky concluded that all specimens sent to him from Milne Port matched features described for *M. arctia*. Further, while not a conclusive distinguishing trait, pigmentation in the head of the specimens closely matched specimens of *M. arctia* from the White Sea (Radashevsky 2021, pers. comm.). **For these reasons, Dr. Radashevsky was confident in the identification of the specimens as *M. arctia*, as opposed to the previously assumed *M. viridis* based on his experience in specific identification for this genus.**

The *M. arctia* specimens identified by Dr. Radashevsky were deposited in the public polychaete collection at the National Scientific Center of Marine Biology in Vladivostok, Russia and will be reported on in conjunction with other *Marenzelleria* specimens in upcoming review of the genus (Radashevsky et al. under review) and a description of *M. arctia* currently in preparation (Radashevsky et al. in prep).

[2] If a specimen has fewer than 120 chaetigers in total, and up to 40 branchiate chaetigers, it is classified as *M. arctia* whereas if a specimen has up to 250 chaetigers in total and up to 130 branchiate chaetigers, it is classified as *M. viridis* (Radashevsky 2021, pers. comm.)

2.1.1 Supporting Evidence to Independent Verification

As described in detail in Section 8.5.4.1.8 of the 2020 MEEMP report, a Multiple Lines of Evidence (MLE) test was performed for *M. viridis* to evaluate supporting information related to its potential invasiveness at Milne Port. Increased sampling effort in Milne Port indicated no warning signs of invasion such as a decrease in benthic community indicators (e.g., diversity, richness, evenness) in conjunction with an increase in the relative abundance of *M. viridis*. Rather, benthic infaunal communities were shown to be diverse and well established throughout Milne Port (Golder 2021a).

Benthic sampling (Golder 2021a) in Milne Port demonstrated a high abundance and diversity of polychaetes in this area. The local receiving environment is not subject to the degree of disturbance from eutrophication as observed in other areas such as the Baltic Sea. *Marenzelleria* spp. are not expected to have a competitive advantage in Milne Port as was observed during invasions in Europe. Should a non-indigenous species of the genus *Marenzelleria* be introduced to Milne Port, the risks of an invasion similar in scale to what has been observed in European waters is therefore not expected.

This corrected species identification is further supported by the environmental conditions at Milne Port. Oceanographic data collected at Milne Port indicates that the nearshore environment is subject to a wide range of salinity (from near zero to 30 PSU - approximately equivalent to 0-30‰),) and water temperature (0°C to 12°C) due to distinct water masses moving with tides, presumed to be influenced by freshwater input from Phillips Creek and melting sea ice in Milne Port (Golder 2021b). *Marenzelleria* specimens identified between 2016-2020 were collected in similar locations to the Ore Dock tide gauge and the mouth of Phillips Creek. The range of temperatures and salinities observed in the area support the identification of M. arctia, which is more tolerant of large fluctuations in temperature and salinity, and generally found in lower salinities compared to M. viridis, which is not typically found in areas where salinity falls below 16‰ (Sikorski and Bick 2004; Green 2015; Quintana et al. 2018).

3.0 NEXT STEPS

Due to the initial species level identification of *Marenzelleria* specimens as *M. viridis* and the difficulties in conclusively identifying *Marenzelleria* species by non-molecular methods, outlined in Section 1.1, Baffinland will continue to monitor for the presence of any *Marenzelleria* species in the Milne Port area. As a precaution, Baffinland will treat all identified *Marenzelleria* specimens as having the potential to be invasive until the classification of *M. arctia* is confirmed through molecular methods. *Marenzelleria* species are distinguishable through COI[3] sequences, and conclusive determination of species will be based on DNA barcoding of future *Marenzelleria* specimens collected in Milne Port (Radashevsky 2021, pers. comm.).

Baffinland will continue to undertake targeted monitoring for this genus in Milne Port, will send any specimens collected in summer 2021 for DNA analysis, and will continue to collaborate with federal and global specialists to resolve species. In addition, Baffinland is currently working with Biologica to retrieve and send all archived *Marenzelleria* samples from the NIS/AIS monitoring program to Dr. Radashevsky for taxonomic review

[3] COI refers to the mitochondiral cytochrome c oxidase subunit I gene. DNA barcoding involves sequencing a short fragment of the COI gene (which act as “DNA barcodes”) from taxonomically unknown specimens and performing comparisons with a library of DNA barcodes of known taxonomy.

4.0 CLOSURE

We trust this information is sufficient for your needs at this time. Should you have any questions or concerns, please do not hesitate to contact Marina Winterbottom, on behalf of the undersigned, at 604-296-7312.

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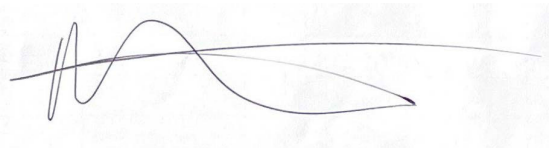
Christine Bylenga, PhD
Marine Scientist



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