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The amphipod *Orchomenella pinguis* — A potential bioindicator for contamination in the Arctic

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Abstract

Indigenous organisms can be used as bioindicators for effects of contaminants, but no such bioindicator has been established for Arctic areas. *Orchomenella pinguis* is a benthic amphipod, ubiquitous in the Arctic and can be found in high numbers. We collected *O. pinguis* at sites with different contamination levels. Population characteristics (body length distribution, average dry weight and amphipod organic content) were related to sediment contaminant concentrations, in order to identify suitable endpoints for using this species as a bioindicator. We show that *O. pinguis* was prevalent in both clean and contaminated areas, easy to sample and that its population characteristics could be linked to both contamination and sediment organic content. We suggest that *O. pinguis* is a suitable bioindicator for the Arctic, but that endpoints such as reproductive effects and phenotypic and genotypic responses are needed together with population characteristics to assess impacts of contamination.

Keywords: field study, benthic amphipod, contaminated sediment, Greenland, multivariate statistics

1. Introduction

Bioindicators are used to assess effects of contaminants in the environment in monitoring programmes around the world. However, there is no established bioindicator of effects for Arctic marine areas, although the Arctic ecosystem has been suggested to be more vulnerable to contaminants than temperate ones since a disturbance of one species may cascade more easily through the food web due to the lower biodiversity and less complex food webs with fewer trophic levels (Chapman and Ridle, 2005).

It has furthermore been demonstrated that contaminants are deposited in the Arctic through long-range transport (Lockhart, 1995; Riget et al., 2004), and during the last 50 years there has been a structural change in Greenland, leading to a centralized consumer and industrial society resulting in a larger and more concentrated discharge of contaminants. An increasing number of studies has examined possible impacts of such contaminants on Arctic marine organisms from laboratory studies (e.g., Olsen et al., 2007; Petersen and Dahllöf, 2007; Camus and Olsen, 2008; Petersen et al., 2008), but only few field studies on effects in the Arctic marine environment have been published (Jorgensen et al., 2006, 2008).

Greenland, as most Arctic areas, is highly reliant on marine ecosystem services given that a large part of the population hunts and fishes locally and since the main part of Greenland's export is based on fisheries. The maintenance of the economy

and export is therefore highly dependent on the protection of the local marine environment.

One way to assess effects of contamination is through the use of indicator organisms, which are any biological species or group of species whose function, population or status can be used to determine the state of the ecosystem of which they are a part (Adams, 2005). For organisms to be valuable bioindicators it has been suggested that they occupy important trophic positions or niches, are keystone species, or contribute significantly to the biomass and energy flow in the system (Adams, 2005). Furthermore they should be easy to sample, stationary, have a short generation time, low rate of dispersal and be reasonably tolerant to biotic changes and contamination (Thomas, 1993).

A potential indicator organism for Arctic marine environments is the small benthic amphipod *Orchomenella pinguis* (Boeck), Lysiannasidae, Gammaridea. In the Arctic food web *O. pinguis* constitutes an important group as a scavenger and forms a major link to the pelagic system as a component of prey items for fish, birds and benthic-feeding mammals such as seals (Conlan, 1994). In general, amphipods conform to the criteria of being relatively sedentary, highly abundant, have a relatively high stress threshold (Duquesne et al., 2000) and have long been applied as sensitive environmental bioindicators (Thomas, 1993). *O. pinguis* has been shown to be more or less ubiquitous within the Arctic (Horner and Murphy, 1985; Sainte-Marie, 1986a, 1986b, 1991; Legezynska et al., 2000), is found in large numbers (1535 m⁻²; Sainte-Marie, 1986b), and is relatively sta-

tionary within a site (Sainte-Marie, 1986b). It is also likely to be among the first organisms to be affected by contaminants originating from land due to its occurrence in shallow coastal waters. Furthermore, *O. pinguis* feeds partly on detritus (Sainte-Marie, 1986b), and lives in close association with sediments as a surface burrower. Since sediments are sinks for contaminants, this species is likely to be highly exposed to contaminants not only from water and food sources, but also via contaminants bound to sediment particles. Thus *O. pinguis* fulfils at least some of the criteria for a suitable bioindicator, however nothing has been reported of its occurrence in relation to contaminants and sediment organic content, or its potential to adapt to such habitats. We therefore collected different populations of *O. pinguis* at sites with different sediment contaminant concentrations and organic content, and this is the first paper describing effects on population characteristics of this species in Greenland. This information is needed to assess the species' potential as a bioindicator and to identify suitable endpoints for measurement.

The study area, Ulkebugten, is a larger bay on the west coast adjacent to Sisimiut, the second largest town in Greenland. Already in the 1920s and 1930s a fish factory and shipyard were established here, and from the 1960s the town grew rapidly. There is no sewage treatment, so all wastewater from the ~5500 inhabitants and industry, including a fish factory and a hospital, is discharged, untreated, directly into the sea. Furthermore, Ulkebugten is heavily trafficked by boating, with a marina used for motorboats and motorized dinghies, and a larger harbor with motorized dinghies as well as larger fishing boats, and trawlers. Ferries and cruise ships also frequently anchor in Ulkebugten during the summer season.

The objective of our work was to determine if *O. pinguis* has the potential to serve as a bioindicator for contamination in the Arctic by (i) investigating its occurrence in contaminated areas; (ii) assessing whether living in contaminated areas has any effect in terms of population characteristics of the species; and (iii) suggesting suitable endpoints for detecting contaminant effects.

2. Materials and methods

2.1. Locations and sampling

Sampling was conducted over three years on four occasions: October 2006, August 2007, May and August 2008 in Ulkebugten, an open fjord in Sisimiut in West Greenland at 5–15 m depth (Table 1). Sampling locations were selected at different sites presumed to have different contamination levels based on previous pilot studies and knowledge of local contamination sources (Figure 1). However, only sites 1–3 were sampled in October 2006.

Amphipod populations were collected using simple baited traps (cylindrical closed netting of about 70 cm in height and 40 cm in diameter). The traps were constructed in a way that the trap was pressed flat when being sunk down to the sea floor and after a given collection time (overnight), the trap unfolded when ropes tied to a buoy at the water surface were pulled, so that the organisms were trapped when the netting was pulled to the surface. Bait consisted of 2–3 day old fish mounted directly in the traps. After sampling, the amphipods were kept in

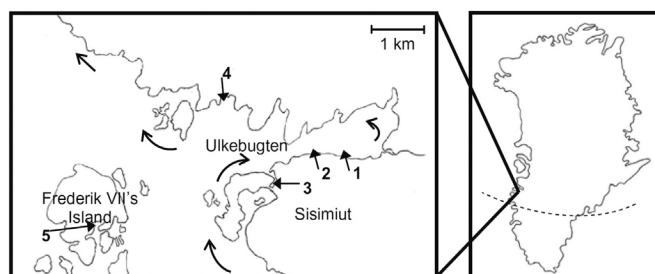


Figure 1. Sampling locations in Ulkebugten, Sisimiut, Greenland. The sampling locations are, from the inner part of the fjord to open waters the three sites closest to point sources 1: hospital wastewater outlet, 2: marina, and 3: harbor; one site 4: outer bay (outside town, but close to the small airport), and finally a site characterized by the open water masses, 5: Frederik VII's Island.

large buckets (10 l) at 5 °C in aerated seawater provided with a handful of sediment and a bit of macroalgae until processing.

Sediment was collected using a Van Veen grab at each site on each sampling occasion. After sampling the sediment was drained of overlying water and stored at –20 °C until analysis.

2.2. Sediment analyses

Analyses included measurements of organic content, 19 PAHs and six heavy metals. The organic content of the sediment samples were determined as loss on ignition at 550 °C after 6 h.

PAH analysis was performed according to the method described by Boll et al. (2008) with minor modifications. Mixtures of 18 individual PAHs (PAH mix 2, Supelco); i.e., the 16 PAHs suggested by the US-EPA as priority pollutants, and 1- and 2-methylnaphthalene were used as quantification standards. An internal standard consisting of a mixture of six different deuterated standards in toluene (Cambridge Isotope Laboratories, UK) was used as recovery standard. For estimation of extraction efficiency, replicate reference sediment samples with known PAH-concentrations were included. Briefly, extraction of sediment (approximately 5 g) was performed by pressurized liquid extraction with a Dionex ASE 200 accelerated solvent extractor using dichloromethane as solvent under a pressure of 1500 ψ and a temperature of 100 °C; the oven heat-up time was 5 min and the program had two extraction cycles with 15 min static time and a flush volume of 35%. After extraction the samples were concentrated at 75 °C to approximately 5 ml and transferred to a volumetric flask and filled with dichloromethane to 10 ml. Extracts were stored in dark vials at –20 °C until analysis. PAHs were analyzed by GC/MS using a Thermo Finnigan TRACE Ultra GC-DSQ MS equipped with a J&W PH-5MS capillary column (60 m, 0.25 mm i.d., 0.25 μ m film thickness). Samples were analyzed using selected ion monitoring, the dwell time was 25 ms/ion and ion source temperature was 250 °C. The GC temperature program used was as follows: isothermal at 35 °C for 2 min, 20 °C/min to 100 °C, 5 °C/min to 315 °C for 15 min. The GC used a PTV injector in splitless mode. Concentrations of the PAHs were classified as not detected/under detection limit when peak detection was less than peaks for the lowest standard (i.e., 1.28 ng/ml). Recovery of reference material ($n = 4$) was 83–135%, except

Table 1. Sampling sites, GPS positions and sampling occasions.

		2006/October	2007/August	2008/May	2008/August
(1) Hospital outlet	66°N 56.584–53°W 39.169	+	+	+	+
(2) Marina	66°N 56.611–53°W 39.528	+	+	+	+
(3) Harbor	66°N 56.000–53°W 40.093	+	+	+	+
(4) Outer Bay	66°N 56.529–53°W 41.229	–	+	+	+
(5) Frederik VII's	66°N 55.857–55°W 45.447	–	+	+	+

for acenaphthylene which showed a recovery of 374% and was therefore left out of the analysis of contaminant levels.

The concentrations of heavy metals in sediments were analyzed after pre-treatment as described in Danish Standard 259 "Determination of metals in water, sludge and sediments—General guidelines for determination by atomic absorption spectrophotometry." Laboratory procedures and quality assurance followed accredited methods and included microwave digestion followed by analysis on an Agilent 7500ce inductively coupled plasma mass spectrometer (ICP-MS) operated according to the USEPA (1994) and Perkin-Elmer 5100PC with FIMS atomic absorption techniques for Zn, Cu (air/acetylene flame AA), Cd (graphite furnace AA) and Hg (flow injection cold vapor AA), all according to Perkin-Elmer standard setup. All methods used external standard curves. Detection limits for metals ranged from 0.01 to 10 mg/kg dry weight. For every 10 samples, a quality control sample of freeze-dried sediment (MESS-3 from National Research Council, Canada) was run. Recoveries of reference material were 85–115% and relative standard deviations (RSD) 1–5%, except for Pb with a low recovery (77%) and high RSD for As (12%) and Cd (18%) for six digests.

2.3. Population analyses

Amphipods from each site were analyzed for body length, dry weight, and organic content. Newly hatched juveniles and egg/juvenile carrying females were removed, which made the analyzed populations consist of older juveniles (body length > 2.5 mm), non-gravid females and males. Although the sexes were not separated in the analyses, females appeared larger (personal observation). Size was measured on each individual ($n = 200$) whereas weight and organic content were measured on amphipods placed in groups of five ($n = 40$). A picture of each sample group was taken using a stereomicroscope with a camera attached for later measurements of individual body length (L, mm) using an image analysis program (SigmaScan Pro vers. 5.0.0). Length of individuals was measured along their dorsal surface from the rostrum to the telson. The samples were then placed on a small piece of pre-weighed foil and left to dry at 55 °C overnight for dry weight measurements, after which they were left at 550 °C for 6 h for measurements of organic content as loss on ignition.

2.4. Statistics

Differences between sites and sampling occasions for amphipod length, dry weight and organic content were tested by the non-parametric Kruskal-Wallis test since assumptions of normality and homogeneity of variances could not be met. Differentiation between sites with respect to population characteristics and level of contamination was described by Partial Least Square (PLS) analysis. Population characteristics were normalized to organic content in the sediment (Y-variables), while contaminant concentrations were log-transformed (X-variables). All data were standardized by $1/\text{std}$ to account for different units and scales. Martens' uncertainty analysis was used to determine significant differences between sites (Martens and Martens, 2000). All statistical analyses were conducted using Statistica 8, SYSTAT 11.0 and Unscrambler 8.0.

3. Results

3.1. Site characterization

The sediment contamination levels at the sites were estimated by measurements of 18 PAHs and six heavy metals (Table 2), though acknowledging that other groups of contaminants also

may occur at the sites. For example pharmaceuticals and household chemicals are likely to be discharged at the wastewater outlet by the hospital site, and anti-fouling paint products are likely to occur to a higher extent in the marina and harbor.

The harbor site contained PAHs at a magnitude 10 times higher than the second most contaminated site with a PAH sum of ~13,000 µg/kg at the harbor, and ~1000 µg/kg at the hospital outlet, respectively. The marina was less contaminated (PAH sum of ~300 µg/kg), whereas both the outer bay and Frederik VII's Island were contaminated with PAHs to a much lower degree with PAH sums of 20 and 8 µg/kg, respectively. The harbor site was mostly contaminated with the larger PAHs (>4 ringed), with highest concentrations of fluoranthene, pyrene and benzo[*b* + *j* + *k*]fluoranthene, whereas PAH contamination at the outer bay to a great extent was caused by naphthalene and to a lesser extent by 1- and 2-methylnaphthalene. These compounds are 2-ringed PAHs and are most likely a result of jet fuel runoff from the runway at the airport close by.

The harbor was also the site most contaminated with heavy metals at ~400 µg/kg. A large fraction of the metal contamination was by zinc and copper, which made up 50% and 30% of the total metal levels at this site. The hospital was the second most contaminated site with regard to heavy metals, followed by the marina, Frederik VII's Island and the outer bay. However, at the two latter sites Zn made up 71% and 60% of the heavy metals. In general the differences in metal concentrations between the sites were not as great as the differences in PAH-concentrations.

The sediment organic content (Table 2) varied to a small extent within sites between sampling occasions, but to a greater extent between the sites, where the harbor and hospital sites had the highest organic content. The organic content of the sediment samples was reflected in the color and grain size, where the sites with the higher organic content had the smaller grain size, higher silt content and darker sediment (Table 2).

3.2. Population characterization

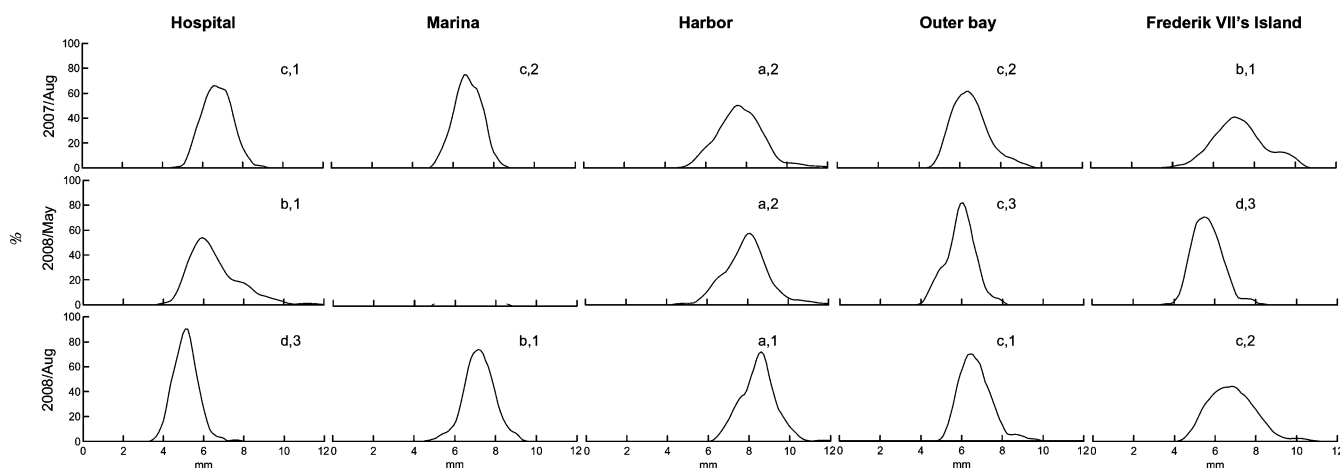
The amphipod species *O. pinguis* responded at most sampling occasions rapidly and in large numbers (5–10,000 individuals) to the bait placed on the seafloor, except for May 2008 where we did not succeed in sampling *O. pinguis* at the marina. Each catch was occupied by this species only, except at the Frederik VII's Island site where amphipods of the species *Onisimus* sp. co-occurred in the traps. The great number of amphipods collected in each trap is assumed to be a good reflection of the actual population and reflected a high density of the species. With a single box-core sample taken in the middle of Ulkebugten the density was determined to be as high as 9000 m⁻² (own observation August 2008).

In October 2006 amphipods were only sampled at the hospital, marina and harbor sites. A comparison of the individual body length of these three populations showed that the populations in the marina and in the harbor consisted of larger juveniles (4 and 4.5 mm, respectively), whereas the population at the hospital outlet consisted of two cohorts i.e., both juveniles (4.5 mm) and larger adults (9 mm). This is in agreement with observations made on juveniles being released from the brood plates in May 2008, August 2007 and 2008.

The length distribution of amphipods (Figure 2) for the three occasions when all sites were sampled differed both between sites and between sampling occasions (Kruskal-Wallis: $p < 0.05$). It should be noted that *O. pinguis* could not be caught in enough numbers at the marina in May 2008, despite a large sampling effort. The harbor population was the largest on all three occasions, while the two cleaner sites, Frederik VII's Island and the outer bay, had different length distributions on all occasions, which was not the case for the two moderately contaminated sites (hospital and marina).

Table 2. Sediment characteristics. Average measurements of three samplings (October 2006, August 2007 and 2008): organic content (%), PAHs ($\mu\text{g}/\text{kg}$), metals (mg/kg) and sums of these at the five sites.

	Hospital	Marina	Harbor	Outer bay	Frederik VII's Island
Sediment grain size	Silty	Small grained	Silty	Grained	Grained
Sediment color	Dark/black	Dark grey	Black	Grey	Grey
Organic content	4.0	1.7	5.6	0.8	1.1
Naphthalene	9.9	7.8	179.0	10.4	1.5
2-Methylnaphthalene	20.8	6.7	171.4	3.2	1.0
1-Methylnaphthalene	9.3	4.1	88.3	3.0	0.7
Acenaphthene	16.3	2.7	206.9	0.0	0.3
Fluorene	4.3	3.7	197.3	0.5	0.4
Phenanthrene	82.5	21.3	1362.4	1.4	1.0
Anthracene	77.0	8.8	498.7	0.1	0.3
Fluoranthene	156.2	35.8	2378.9	0.5	1.0
Pyrene	116.7	28.2	1872.0	0.5	0.8
Benzo[a]anthracene	50.2	13.3	883.4	0.0	0.0
Chrysene	58.7	22.8	891.0	0.0	0.1
Benzo[b + j + k]fluoranthene	156.1	57.3	1805.4	0.0	0.6
Benzo[a]pyrene	86.9	30.0	871.9	0.0	0.3
Indeno[1,2,3-c,d]pyrene	97.2	9.8	570.7	0.0	0.1
Dibenzo[a,h]anthracene	58.0	5.3	312.2	0.0	0.0
Benzo[g,h,i]perylene	49.6	28.1	577.1	0.0	0.1
Sum PAHs	1049.4	285.7	12866.3	19.6	8.2
Copper	22.9	12.1	131.0	5.7	6.0
Zink	41.7	26.0	197.5	21.7	32.3
Arsenic	17.4	9.0	13.8	7.2	5.8
Cadmium	0.4	0.1	0.6	0.1	0.1
Mercury	0.2	0.0	0.4	0.0	0.0
Lead	6.3	3.2	33.1	1.3	1.3
Sum metals	88.9	50.4	376.4	35.7	45.5

**Figure 2.** Size distribution (individual body length; mm) of *O. pinguis* in populations collected at five sites and on three sampling occasions ($n = 200$). Different letters indicate significant differences ($p < 0.05$, Kruskal–Wallis) between sites within the sampling occasion (horizontal), whereas different numbers indicate significant differences between years within a site (vertical).

Therefore, when relating population characteristics to contamination level, which is more constant over time compared to population dynamics; length, weight, organic content and density of the different years were pooled within the populations, thus giving a time-integrated description of the population including several possible cohorts (Figure 3).

The harbor population was significantly larger for all integrated population characteristics (Figure 4a–d; Kruskal–Wallis: $p < 0.05$). The populations from the most and second most contaminated sites, the harbor and the hospital, differed markedly in most population characteristics, where the population from the harbor was the largest; the one from the hospital was the smallest, with the marina and the two reference sites in between (outer bay and Fredrik VII's Island).

In order to investigate whether these differences in population characteristics could be linked to contamination levels, a PLS analysis was performed where the X-matrix consisted of log-transformed sediment contaminant concentrations and the population characteristics were normalized to 1% sediment organic matter, thereby correcting for differences in food availability among sites. The model could explain 87% of the variation in population characteristics based on the contamination matrix, and there was clear separation of the contaminated sites, as well as between the contaminated (hospital, marina and harbor) and the reference sites (Frederik VII's Island and the outer bay) (Figure 4a). The contaminated sites were more characterized by maximum and minimum values for the population characteristics compared to the reference sites (Figure 4b). The harbor was by far the most

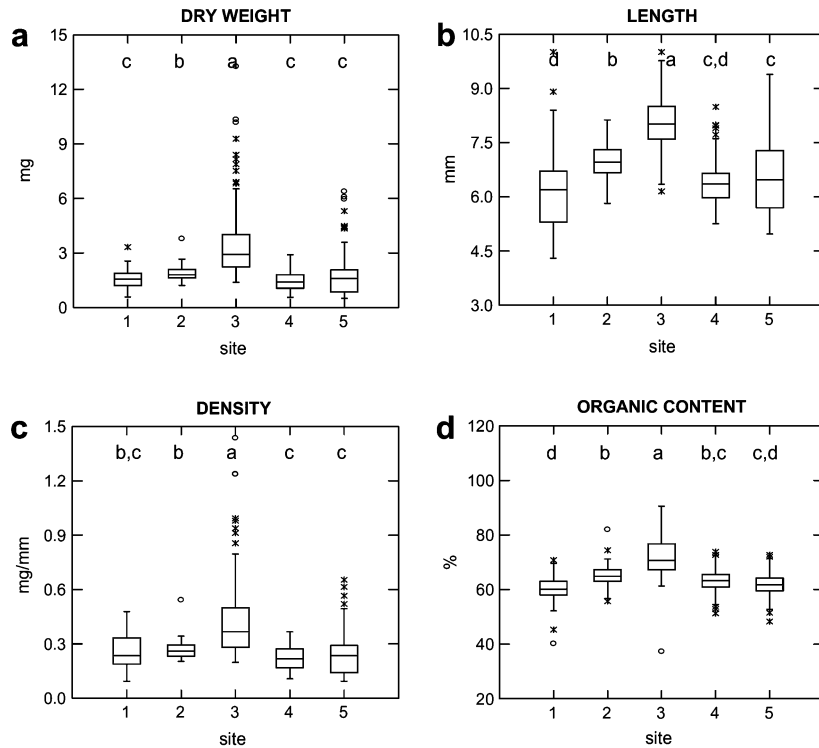


Figure 3. Box plots displaying integrated descriptive parameters for populations collected at site 1: hospital outlet, 2: marina, 3: harbor, 4: outer bay and 5: Frederik VII's Island. (a) Dry weight (mg), (b) length (mm), (c) density (mg dry weight/mm), and (d) organic content (% dw). Sites with a letter in common are not significantly different ($p > 0.05$ Kruskal-Wallis).

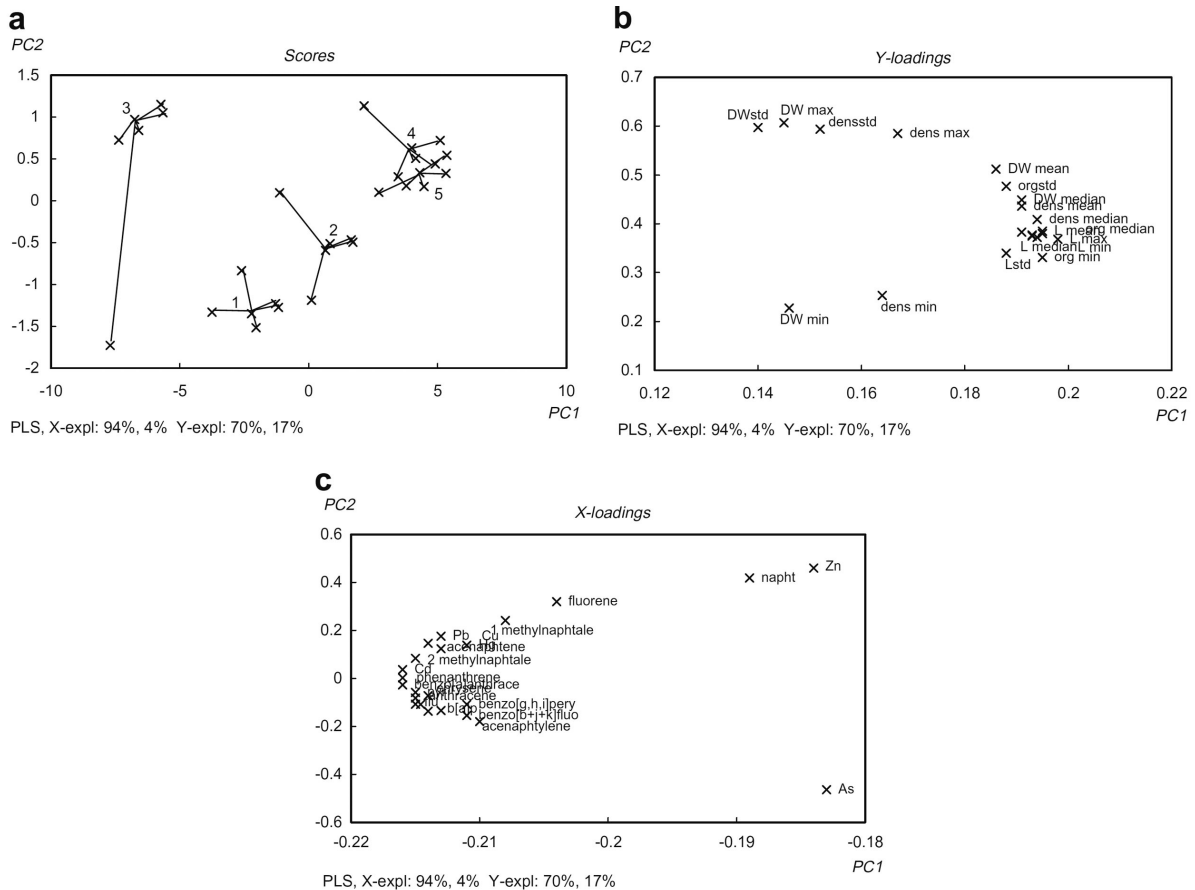


Figure 4. (a) Partial Least Square analysis of the relation between sites, based on (b) normalized population characteristics as explained by (c) the contamination level. Variation within sites in (a) is given by the Martens' uncertainty test.

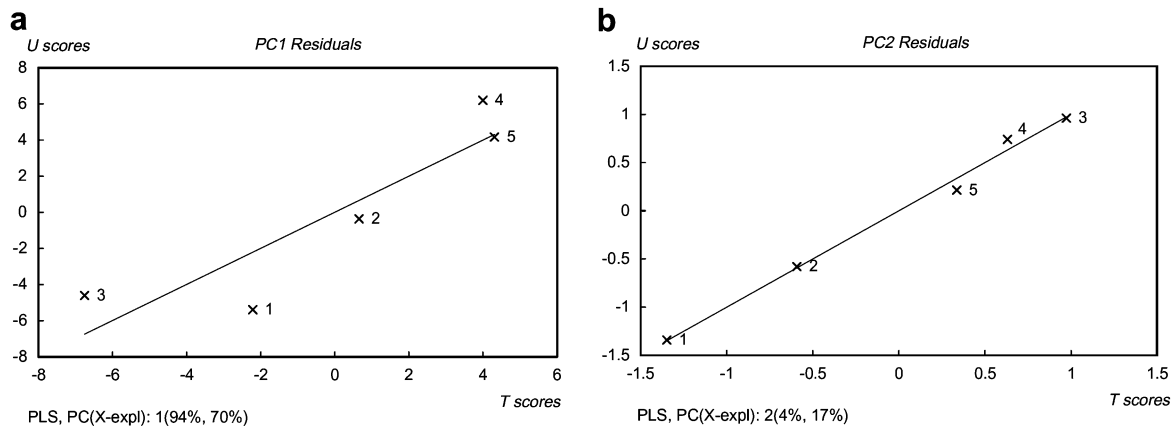


Figure 5. Regression between measured and predicted residuals for (a) PC1 ($r^2 = 0.81$, $p = 0.039$) and (b) PC2 ($r^2 = 0.99$, $p = 0.0003$).

contaminated site for all contaminants apart from As, but the proportion of lighter PAHs and Zn was larger at the other sites. However, analyses of the residuals showed that the model for PC1 (Figure 5a), which explained the separation due to the total contamination level, could not adequately predict the population characteristics for the most contaminated sites (the harbor, site 3), whereas the model for PC2 (Figure 5b), which explained the distribution between contaminants, was much stronger.

4. Discussion

The aim of this study was to determine if *O. pinguis* has the potential to be a bioindicator for contamination in the Arctic by investigation of its occurrence and population characteristics at different contaminated and reference sites.

O. pinguis in this study was shown to be present also at sites with high contamination, such as the harbor where concentrations of metals and PAHs were within one order of magnitude lower than the measured concentrations in the highly contaminated Boston Harbor (Durell et al., 2008). The Sisimiut harbor sediment concentrations also fall under the categories of "further evaluation for risk" or "risk" when comparing to the EAC values given by the OSPAR Commission (OSPAR, 1997).

The population characteristics of *O. pinguis* could however, not be directly linked to contamination level, as the most contaminated site (the harbor) consisted of a population with the largest body length and dry weight whereas the second most contaminated site (the hospital outlet) consisted of a population with the smallest individuals, more similar in size to the populations found at the reference sites and at the marina.

Besides the differences in contaminant levels, sediment organic matter also varied, with the two most contaminated sites containing a much higher fraction of organic matter than found at the other sites. For amphipods the amount and quality of the organic matter may help in overcoming other stressors (e.g., Costa et al., 2005; Spadaro et al., 2008). The differences in population characteristics may therefore reflect a balance between a positive factor (food availability) and a negative factor (contamination level).

The normalization of the population characteristics to sediment carbon content was an attempt to reduce the confounding influence of food availability on the effect of contamination. The multivariate model then showed a relationship between population characteristics and contamination, albeit with large uncertainties in the prediction. It must also be taken into consideration that contamination levels per se do not necessarily reflect either bioavailability, which can both be reduced and enhanced due to organic enrichment (Gunnarsson et al., 1995), or differ-

ences in toxicity between contaminants, and thus the model can not be expected to be conclusive. However, the question of how the actual population characteristics (un-normalized) can vary so much between the two most contaminated sites, still remains. One other reason for the smaller size at the hospital site can be that it is exposed to other contaminants (e.g., pharmaceuticals), that are discharged with the waste water effluent. But, part of the explanation can also be that different population traits and strategies have been selected at these two sites, resulting in different population characteristics, hereunder skewed sex ratios. Selection toward tolerant populations has previously been demonstrated in laboratory exposure systems (e.g., Luoma et al., 1983; Klerks and Levinton, 1989; Galletly et al., 2007; Janssens et al., 2009), where for example Klerks and Levinton (1989) found population-level resistance acquired after only one to four generations in an oligochaete cultured in the presence of cadmium. Anthropogenically impacted sediments are however rarely contaminated with single chemicals, and resistance to contaminant mixtures appears to be more difficult to achieve than resistance to single substances Klerks (1999).

Our results indicate that measuring population characteristics, such as body size and weight, is not sufficient if *O. pinguis* is to be used as a bioindicator. There is a need for consistency in the association between stress and effects as previously suggested (Adams, 2003; Theodorakis, 2003). Our study does not show such consistency and as discussed by Collier (2003) and Adams (2005), the establishment of the causal relationship between stressors and effects on marine biota is not straightforward in field studies due to the physicochemical and biological complexity of the systems, the range of biotic and abiotic factors that can act on the responses of biota to stressors, and the many pathways by which stressors may affect the ecosystem. However, this study enables us to suggest other relevant endpoints such as reproductive success (Sundelin and Eriksson, 1998; Strand et al., 2004; Camus and Olsen, 2008) and tolerance. Tolerance can be used as an endpoint, either through comparing the phenotypic response to contaminants of populations from contaminated sites with reference sites, or by comparing genotypic effects on functional genes and total genetic variation (Street et al., 1998; Bickham et al., 2000; Belfiore and Anderson, 2001; Ross et al., 2002; Gardeström et al., 2006; Hoffmann and Daborn, 2007).

5. Conclusion

O. pinguis fulfils the criteria of being a suitable bioindicator for the Arctic in that it has been shown to occur in high numbers in both clean and contaminated areas. The differences in popula-

tion traits between different types of contaminated areas suggest that the endpoints measured in the present study (body length, amphipod organic content and weight) will need to be supplemented with other endpoints, e.g., reproductive effects, tolerance and genetic diversity, when using this species to assess contamination impacts in complex field settings.

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