Mercury in gray wolves (Canis lupus) in Alaska: Increased exposure through consumption of marine prey

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Abstract

Mercury (Hg) bioaccumulates in the tissues of organisms and biomagnifies within food-webs. Gray wolves (Canis lupus) in Alaska primarily acquire Hg through diet; therefore, comparing the extent of Hg exposure in wolves, in conjunction with stable isotopes, from interior and coastal regions of Alaska offers important insight into their feeding ecology. Liver, kidney, and skeletal muscle samples from 162 gray wolves were analyzed for total mercury (THg) concentrations and stable isotopic signatures (δ13C, δ15N, and δ34S). Median hepatic THg concentrations were significantly higher in wolves with coastal access compared to wolves from interior Alaska. Stable isotope ratios, in conjunction with THg concentrations, provide strong evidence that coastal wolves are utilizing marine prey representing several trophic levels. The utilization of cross-ecosystem food resources by coastal wolves is clearly contributing to increased THg exposure, and may ultimately have negative health implications for these animals.

Keywords

bioaccumulation; biomagnification; mercury; Canis lupus; stable isotopes; feeding ecology

1. Introduction

A variety of stressors exist in the environment that may ultimately affect the health and functioning of organisms (Lafferty and Holt, 2003). Monomethylmercury (MeHg+)
bioaccumulates in the tissues of organisms, and elevated exposure to this form of mercury (Hg) can be particularly harmful to the fetus or neonate, especially in consumers that occupy high trophic positions (Chumchal et al., 2011). In some areas of the Arctic, Hg concentrations in marine food-webs have dramatically increased (Braune et al., 2005) to concentrations at which adverse biological effects might be expected (Dietz et al., 2013). While gray wolves (Canis lupus) have commonly been recognized as obligate carnivores that prey primarily on ungulates, recent studies have shown that the diets of some wolf populations are subsidized with marine organisms (Adams et al., 2010; Christensen et al., 2005; Watts et al., 2010). Therefore, gray wolves in Alaska serve as an optimal species for studying Hg exposure in apex-predator wildlife populations. While utilization of cross-ecosystem food sources (which has been demonstrated in a variety of higher-level consumers and predators) can be beneficial to species comprising recipient food-webs, the potential negative implications (low nutrients, infectious and toxic agents) of dietary components are not commonly explored (Christensen et al., 2005; Walters, Fritz, and Otter, 2008).

Stable isotope analysis is an integrated approach used to improve the overall understanding of food-webs (Hansen, Burmeister, and Sommer, 2009). Stable carbon (C)-, nitrogen (N)-, and sulfur (S)-isotopic compositions, in particular, have been widely recognized as established proxies of the trophic architecture. Enrichment of the heavier isotope of N (15N) compared to the lighter form (14N), occurs with each trophic level (Minagawa and Wada, 1984; Newsome et al., 2010), and marine organisms tend to have higher N isotope signatures relative to terrestrial biota (Benson and Parker, 1961; Miyake and Wada, 1967). Thus, N stable isotopes have been used extensively to quantify marine-based dietary sources for a variety of mammalian consumers, including wolves (Adams et al., 2010), grizzly bears (Ursus arctos horribilis) (Christensen et al., 2011), polar bears (Ursus maritimus) (Dehnh et al., 2006), and mink (Mustela vison) (Lake et al., 2007). Because isotopic enrichment in marine versus terrestrial biomes also exists for C and S, feeding ecology studies have additionally incorporated these isotopes, to determine the contribution of marine versus terrestrial prey sources in consumer diets (Inger et al., 2006). The purpose of this study was, therefore, to evaluate the feeding ecology of gray wolves in Alaska, as determined by stable isotopes signature, and total Hg (THg) tissue concentrations.

2. Materials and Methods

2.1 Sample collection

Tissues subsamples from a total of 162 gray wolves were collected by the Alaska Department of Fish and Game (ADF&G) from 2006 through 2009 as part of ongoing projects. Samples of liver (n=145), kidney (n=143), skeletal muscle (n=60), and cardiac muscle (n=16) were placed into individual Whirlpac™ bags and frozen immediately at −20°C. Samples were transferred to the University of Alaska Fairbanks (UAF) and stored at −80°C until laboratory analyses. Tissues were not systematically collected from all organs of each animal due to logistical constraints. The collection site for each wolf was assigned based on Game Management Unit (GMU) as defined by ADF&G (http://www.adfg.alaska.gov/index.cfm?adfg=huntingmaps.gmuinfo). Sex and age class (van Belle et al., 2004) were determined for each animal. Animals were classified as <12 months or ≥12 months.

2.2 Stable isotope analyses

In preparation for stable isotope analysis (C, N, and S), liver and skeletal muscle samples were freeze-dried and ground to a fine powder using a mortar and pestle, followed by further homogenization using a ball mill (mini-bead beater, BioSpec). Approximately 1.5–2.0 mg of
tissue was loaded into 5 × 9 mm tin capsules for C and N isotope analyses; 5.5 mg of sample amended with 2.0 mg of Vanadium oxide V₂O₅ was loaded into tin capsules for S isotope analysis.

Stable isotope ratios were analyzed by continuous-flow isotope-ratio mass spectrometry using an elemental analyzer (Carlo Erba NC1500 or Thermo Flash 2000) interfaced to a mass spectrometer (Micromass Optima or Thermo-Finnigan Delta Plus XP), as described elsewhere (Butala, Scanlan, and Chandhuri, 2006). Isotope values are reported in delta (δ) notation:

\[ \delta X = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \]

where X represents ¹³C, ¹⁵N, or ³⁴S in parts per thousand (‰) deviation relative to a standard (monitoring) gas and R_{sample} and R_{standard} represent the ratio of ¹³C/¹²C, ¹⁵N/¹⁴N, or ³⁴S/³²S for sample and standard, respectively. Isotopic data were normalized to V-PDB, Air, and V-CDT using the primary standards USGS 40 (−26.24‰ and −4.52‰ for δ¹³C and δ¹⁵N, respectively), USGS 41 (37.76‰ and 47.57‰ for δ¹³C and δ¹⁵N, respectively), NBS127 (21.1‰ for δ³⁴S), and IAEA-SO6 (−34.05‰ for δ³⁴S). Analytical error was assessed by replicate measures of primary standards (<0.2‰ for all three isotopes across all analytical sequences) and quality control was assessed using secondary standards analyzed within individual analytical sequences (<0.3 ‰). Accuracy was assessed using primary standards as unknowns, and was within 0.2‰ for all three isotopes. Sample reproducibility, determined via duplicate measurements, was better than 0.2‰ for all three isotopes.

### 2.3 Total mercury (THg) analysis

All samples were thawed to room temperature and sub-sampled (70–150 mg) in duplicate, using stainless steel forceps and scissors. Instruments were washed with ultrapure water and dried between each sample. THg concentrations are reported on a wet weight (ww) basis. Samples were analyzed using a Milestone DMA-80 instrument (Butala, Scanlan, and Chandhuri, 2006; EPA 600-R-04-012, 2004). The method detection limit for THg determination was 0.005 ng/g, ww. Quality assurance and quality control were based on method blanks, standard reference materials (SRMs), check standards, and sample duplicates. All samples were run in duplicate and re-analyzed if the percent difference between samples was >10%. The SRM utilized was DORM-3 (National Resource Council Canada; 0.382 +/- 0.060 ng THg/g). Percent recovery for check standards (5, 20, and 100 ng aqueous Hg) was >90%. Analysis of the standard reference material was within 10% of the certified value for THg.

### 2.4 Statistical analyses

Collection sites were grouped into two location categories based on whether or not a coastline was present in the assigned GMU. Thus, wolves in GMUs that contained coastline were defined as “coastal” and those in GMUS that had no coastline were defined as “interior” animals. Specific pack assignment was not possible based on sample collection design.

Data distributions were assessed for normality using graphical techniques including histograms and box-and-whisker plots, supplemented by quantile-quantile plots (Henderson, 2006). Log-transformation did not satisfy the assumptions of normality; therefore, non-parametric statistical analyses were employed and median values and ranges are reported. Chi-square analyses were first used to evaluate the dichotomous variables of age class, sex, and location. The Mann-Whitney U test was used to evaluate differences in liver, kidney,
and muscle THg concentrations based on location, sex, and age class. Isotope values were also compared based on location, sex, and age class. Alpha was set ≥ 0.05. Statistical analyses were performed using StatCrunch 5.0 statistical software (Integrated Analytics LLC, Pearson Education, 2007–2009).

3. Results

Of the 162 wolves, 30 occupied regions with coastal access and 132 wolves were from interior locales. Sex information was available for 161/162 wolves and age for 159/162 wolves. Among interior wolves, 20 males and 25 females were < 12 months, whereas 40 males and 44 females were ≥ 12 months. Among coastal wolves, 4 males and 2 females were < 12 months, whereas 11 males and 12 females were ≥ 12 months. Chi-Square analyses demonstrated there was no significant association between age class and location, location and sex, or age class and sex.

Among all wolves, THg concentrations varied widely among the four tissues examined, particularly for liver and kidney (Table 1). No significant differences in THg concentrations were observed based on sex (Table 2); however, wolves that were ≥ 12 months had significantly higher hepatic and renal THg concentrations, and coastal wolves had significantly higher THg concentrations in all tissues (Tables 3–4).

C and N isotope analysis was conducted on 132 liver samples and 59 skeletal muscle samples (Figs. 1A & 1B, 2A & 2B). S isotope analysis was carried out on 131 liver samples and 56 skeletal muscle samples. Median liver $\delta^{13}$C, $\delta^{15}$N, and $\delta^{34}$S signatures (and ranges) were $-24.4 \%e (-20.0$ to $-26.8$), $7.2 \%e (5.7$ to $15.9$), and $5.2 \%e (-1.64$ to $17.1$), respectively. Median values for all stable isotopic compositions were found to be significantly higher in coastal animals for all tissues ($p<0.01$). Interior wolves had significantly lower S values and liver THg concentrations (Fig. 3A). A similar trend was observed for skeletal muscle (Fig. 3B).

4. Discussion

Stable isotope analysis has been shown to be an excellent technique for assessing questions relating to food webs and trophic structure, and in understanding the basic foraging habits of mammals (Crawford, McDonald, and Bearhop, 2008). Few studies have used mammalian stable isotope signatures and Hg tissue concentrations together to evaluate foraging ecology questions in apex-predator systems (Cardona-Marek et al., 2009; Horton et al., 2009; Young et al., 2010); and typically, only C and N isotope ratios are assessed. This work is unique in that THg concentrations and stable isotope ($\delta^{13}$C, $\delta^{15}$N, and $\delta^{34}$S) signatures were used in conjunction to better understand the feeding ecology of Alaskan gray wolves and how it relates to dietary-based toxicant exposure. Wolves have been described as opportunistic, generalist carnivores (Watts et al., 2010), with considerable dietary plasticity among and within populations. This feeding strategy has been proposed to play a role in the success of wolves in parts of Alaska (Szepanski, 1999). However, the importance of marine resources in coastal wolves, and the effect of prey choices on individuals and populations, remains unknown. A recent study by Bocharova et al. (2013) showed that THg concentrations in Arctic foxes were reflective of foraging strategies, rather than variation in overall THg concentrations in the environment, and that high THg concentrations acquired predominantly through diet may represent a prominent risk for top predators. The present study, which is the first to evaluate Hg in Alaskan gray wolves, also suggests that cross-ecosystem utilization of food resources may contribute to increased Hg exposure, particularly in coastal populations.
The stable isotope values (C, N, and S) in liver and skeletal muscle tissue from coastal wolves were significantly higher than values in interior wolves, suggesting a marine-based diet in coastal wolves. Carbon and nitrogen isotopes are two measures that have been used to demonstrate marine resource utilization in populations (Adams et al., 2010), as δ¹³C and δ¹⁵N increase with the marine content of diet (Hilderbrand et al., 1996). The additional measurement of δ³⁴S was implemented in this study, since it can further aid in differentiating between the dietary contributions of marine and terrestrial resources (Crawford, McDonald, and Bearhop, 2008; Hansen, Burmeister, and Sommer, 2009). Our isotope analyses strengthen the contention that marine resources are contributing to the diets of coastal wolves in Alaska.

Among all wolves, THg concentrations varied based on tissue type. The deposition of Hg in tissues may be dependent on a number of biological factors including diet, age, sex, body condition, and health. It has been suggested that the affinity for Hg in kidney and liver tissue may be due to the preferential bonds of the organic Hg compounds to SH-groups of the plasma proteins in these organs (Kacmár et al., 1992). The highest THg concentrations in this study were observed in liver and kidney, which has also been reported elsewhere (Agusa et al., 2011; Misztal-Szkudlińska et al., 2010; Sures, 2004). Isotopic differences among tissues within an individual are largely due to differential routing of macronutrients to the various organs and differences in tissue turnover rates (Martínez del Rio et al., 2009). Liver turns over faster than skeletal muscle, and therefore has a shorter dietary integration window. For this reason, it tends to be more reflective of recent feeding, and could explain some of the variance noted in both stable isotopes and THg.

Coastal wolves in this study had significantly higher THg concentrations in all tissues examined, relative to interior wolves. In wildlife populations, Hg has been reported to cause reproductive impairment, alterations in growth and behavior, and even death (Facemire et al., 1995; Osowski et al., 1995; Wren, 1986). As obligate predators and opportunists, gray wolves have been previously described to eat a variety of small mammals, fish, and birds, when available (Darimont et al., 2004). Alaskan wolves with coastal access have been reported to utilize a wide variety of marine sources of prey, including harbor seals (Phoca vitulina) (Klein, 1995, Szepanski, 1999), various marine mammal carrion (Darimont et al., 2002; Mech, 1970), anadromous eulachon smelt (Thaleichthys pacificus), seabirds, marine invertebrates (Mech et al., 1998), and salmon (Adams et al., 2010; Darimont et al., 2002; Szepanski, 1999). Our results are in agreement with the work by Szepanski (1999), in which the diets of coastal wolves in Alaska were more varied, relative to interior animals, and trophic diversity was broader; likewise, these results are in agreement with findings for Arctic foxes, where coastal animals had higher variance in C isotopes, compared to those from inland regions (Angerbjorn, 1994).

In the present study, interior wolves tended to subsist on terrestrial resources, consequently resulting in low THg tissue concentrations. In contrast, coastal wolves tended to utilize marine resources resulting in higher THg concentrations. However, individual variation did exist. A few interior wolves with high THg had stable isotopic signatures more similar to the coastal animals; conversely, there were 6 coastal wolves with relatively low S values (<5 ‰), more reflective of the interior wolves. While wolves belong to packs that tend to hunt cooperatively, not all pack members have access to the same resources. Unstable packs and lone individuals often have larger home ranges, are more mobile than established, stable packs (Mech and Botani, 2003), and may have a more varied or specialized diet (Urton and Hobson, 2005). These particular coastal animals may have occupied more inland regions of the coastal GMU without access, or limited access, to marine resources. Furthermore, landscape modifications and anthropogenic influences may alter prey distribution and
abundance, and behavioral interactions within and across species may affect accessibility to prey.

Our integrated approach provides insight into how toxicant exposure relates to foraging ecology in Alaskan gray wolves. However, evaluation of the physiological, environmental, and social factors affecting diet variation of wolves is needed in order to more clearly elucidate possible exposure pathways in individuals.

5. Conclusion

THg concentrations and C, N and S isotope values in various tissues provide four separate measures supporting the contention that, when accessible, Alaskan gray wolves exploit marine resources. If apex predators such as Alaskan gray wolves increase their utilization of marine subsidies, individuals and/or populations may be at risk for harmful effects associated with Hg exposure (e.g., in utero exposure of fetus) and other marine-based food-web toxicants. This work, and other reports, clearly demonstrates the need to better understand foraging behaviors of gray wolves in Alaska, and how THg exposure varies temporally and spatially based on foraging ecology. Stable isotope mixing model studies (Debridge et al., 2012; Fortin et al., 2007; Semmens et al., 2009; Szepanski, 1999; Urton and Hobson, 2005) have been used to quantify diet composition in wildlife populations, and may be one useful approach in addressing this need in the future.

Acknowledgments

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References


Mech, LD.; Adams, LG.; Meier, TJ.; Burch, JW.; Dale, BW. The wolves of Denali. University of Minnesota Press; Minneapolis, Minnesota, USA: 1998.


Highlights

- Stable isotopes (C, N, & S) indicate the use of marine resources in Alaskan wolves
- Coastal wolves had significantly higher THg concentrations versus interior wolves
- Cross-ecosystem feeding behavior partly explains high THg concentrations in wolves
Figure 1.
Stable isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$) in A) liver, and B) skeletal muscle, based on the designation of coastal versus interior gray wolves; $\triangle$ = coastal wolves, $\bigcirc$ = interior wolves.
Figure 2.
Stable isotopic compositions ($\delta^{15}\text{N}$ and $\delta^{34}\text{S}$) in A) liver, and B) skeletal muscle, based on the designation of coastal versus interior gray wolves; $\triangle$ = coastal wolves, $\circ$ = interior wolves.
Figure 3.
THg and $\delta^{34}S$ in wolf liver, based on the designation of coastal versus interior gray wolves; $\triangle$ = coastal wolves, $\bigcirc$ = interior wolves.
Table 1

Median and range of THg concentrations for all gray wolf (*Canis lupus*) tissues expressed in ug/kg wet weight (ww).

<table>
<thead>
<tr>
<th>Organ/Tissue</th>
<th>n</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>145</td>
<td>34.7</td>
<td>5.5–7260.7</td>
</tr>
<tr>
<td>Kidney</td>
<td>143</td>
<td>95.2</td>
<td>8.3–11,173.3</td>
</tr>
<tr>
<td>Skeletal Muscle</td>
<td>60</td>
<td>12.5</td>
<td>4.4–546.0</td>
</tr>
<tr>
<td>Cardiac Muscle</td>
<td>16</td>
<td>206.7</td>
<td>3.9–640.0</td>
</tr>
</tbody>
</table>
**Table 2**

Median and range of THg concentrations in male and female gray wolves (*Canis lupus*), expressed in ug/kg wet weight (ww).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>n</th>
<th>Median</th>
<th>Range</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td>0.39</td>
</tr>
<tr>
<td>Male</td>
<td>68</td>
<td>36.9</td>
<td>5.7–7260.7</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>76</td>
<td>30.9</td>
<td>5.5–7206.0</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td></td>
<td>0.40</td>
</tr>
<tr>
<td>Male</td>
<td>68</td>
<td>106.4</td>
<td>13.5–11,173.3</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>74</td>
<td>92.9</td>
<td>8.3–4638.2</td>
<td></td>
</tr>
<tr>
<td>Skeletal Muscle</td>
<td></td>
<td></td>
<td></td>
<td>0.39</td>
</tr>
<tr>
<td>Male</td>
<td>27</td>
<td>13.0</td>
<td>4.4–388.6</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>33</td>
<td>12.2</td>
<td>4.8–545.9</td>
<td></td>
</tr>
<tr>
<td>Cardiac Muscle</td>
<td></td>
<td></td>
<td></td>
<td>0.84</td>
</tr>
<tr>
<td>Male</td>
<td>7</td>
<td>226.8</td>
<td>4.2–306.5</td>
<td></td>
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<tr>
<td>Female</td>
<td>9</td>
<td>186.6</td>
<td>3.9–639.5</td>
<td></td>
</tr>
</tbody>
</table>
Table 3

Median and range of THg concentrations in gray wolves (*Canis lupus*), expressed in ug/kg wet weight (ww) based on age class (<12 months or ≥12 months).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>n</th>
<th>Median</th>
<th>Range</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liver</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 12 months</td>
<td>45</td>
<td>15.3</td>
<td>5.5–6086.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>≥12 months</td>
<td>97</td>
<td>48.4</td>
<td>13.3–7260.7</td>
<td></td>
</tr>
<tr>
<td><strong>Kidney</strong></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&lt; 12 months</td>
<td>46</td>
<td>33.9</td>
<td>8.3–3373.0</td>
<td></td>
</tr>
<tr>
<td>≥12 months</td>
<td>96</td>
<td>154.3</td>
<td>37.6–11,173.0</td>
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</tr>
<tr>
<td><strong>Skeletal Muscle</strong></td>
<td></td>
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<td></td>
<td>0.07</td>
</tr>
<tr>
<td>&lt; 12 months</td>
<td>9</td>
<td>8.1</td>
<td>4.4–545.9</td>
<td></td>
</tr>
<tr>
<td>≥12 months</td>
<td>49</td>
<td>13.0</td>
<td>5.0–465.6</td>
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</tr>
<tr>
<td><strong>Cardiac Muscle</strong></td>
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<td></td>
<td>0.125</td>
</tr>
<tr>
<td>&lt; 12 months</td>
<td>1</td>
<td>3.9</td>
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<tr>
<td>≥12 months</td>
<td>15</td>
<td>226.8</td>
<td>116.2–335.8</td>
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</table>
### Table 4

Median and range of THg concentrations in coastal and interior gray wolves (*Canis lupus*), expressed in ug/kg wet weight (ww).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>n</th>
<th>Median</th>
<th>Range</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td></td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interior</td>
<td>117</td>
<td>28.4</td>
<td>5.5–2226.9</td>
<td></td>
</tr>
<tr>
<td>Coastal</td>
<td>28</td>
<td>1159.0</td>
<td>33.8–7260.7</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interior</td>
<td>116</td>
<td>80.5</td>
<td>8.3–4567.3</td>
<td></td>
</tr>
<tr>
<td>Coastal</td>
<td>27</td>
<td>1939.0</td>
<td>78.3–11,173.0</td>
<td></td>
</tr>
<tr>
<td>Skeletal Muscle</td>
<td>&lt;0.001</td>
<td>10.9</td>
<td>4.4–258.4</td>
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</tr>
<tr>
<td>Cardiac Muscle</td>
<td>&lt;0.001</td>
<td>317.2</td>
<td>90.3–545.9</td>
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<tr>
<td>Interior</td>
<td>3</td>
<td>4.2</td>
<td>3.9–179.0</td>
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<tr>
<td>Coastal</td>
<td>13</td>
<td>232.8</td>
<td>70.5–639.5</td>
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