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Otolith chemistry indicates recent worsened Baltic cod condition is linked to hypoxia exposure

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Abstract

Deoxygenation worldwide is increasing in aquatic systems with implications for organisms' biology, communities and ecosystems. Eastern Baltic cod has experienced a strong decline in mean body condition (i.e. weight at a specific length) over the past 20 years with effects on the fishery relying on this resource. The decrease in cod condition has been tentatively linked in literature to increased hypoxic areas potentially affecting habitat range, but also to benthic prey and/or cod physiology directly. To date, no studies have been performed to test these mechanisms. Using otolith trace element microchemistry and hypoxia-responding metrics based on Manganese (Mn) and Magnesium (Mg), we investigated the relation between fish body condition at capture and exposure to hypoxia. Cod individuals collected after 2000 with low body condition had a higher level of Mn/Mg in the last year of life, indicating higher exposure to hypoxic waters than cod with high body condition. Moreover, lifetime exposure to hypoxia was even more strongly correlated to body condition, suggesting that condition may reflect long-term hypoxia status. These results were irrespective of fish age or sex. This implies that as Baltic cod visit poor-oxygen waters, perhaps searching for benthic food, they compromise their own performance. This study specifically sheds light on the mechanisms leading to low condition of cod and generally points to the impact of deoxygenation on ecosystems and fisheries.

Keywords: hypoxia, body condition, *Gadus morhua*, otoliths, trace element analyses

Introduction

Cod (*Gadus morhua*) is a key demersal fish species in the North Atlantic, both ecologically and economically. In the Baltic Sea, since the mid-1980s, the frequency of very slender specimens of Eastern Baltic cod has been increasing progressively, and the mean body condition of individuals has decreased by around 30% [1-2]. The average weight of a 40-cm long cod has dropped from 900g to 600g from the early 1990s to 2018. This is of biological concern in terms of affecting population reproductive potential [3] and mortality [4], but also changing trophic interactions [5-6]. Additionally, the increase of slender cod has been detrimental for the fisheries industry that complained about increased catches of scrawny individuals with little or no commercial value.

A number of hypotheses have been proposed to explain the decline in cod condition, including increased extent of hypoxic waters, decreased abundance of pelagic prey, increased parasite infection, or a combination of these factors [1-2]. A recent study [2] found a strong statistical correlation between the temporal changes in the extent of hypoxic areas and changes in cod mean condition in the central Baltic Sea. Hypoxic areas could affect cod condition directly via physiological stress induced by exposure to hypoxia, indirectly by reducing the availability of benthic prey, or by contraction of suitable habitat [2]. However, no direct evidence has been provided to date to support or refute any of these hypotheses.

Otolith chemistry may offer a direct test of whether low condition of individual fish relates to past hypoxia exposure. Otoliths, the small aragonitic concretions within the hearing/balance system in teleost fishes, readily take up the trace element manganese (Mn) when present in the environment, and Mn^{2+} and Mn^{3+} become available (dissolved) under suboxic/hypoxic conditions [7-8]. Otolith Mn/Ca ratios distinguish fish from hypoxic vs normoxic environments, but manganese uptake is also affected by growth rate [8]. Therefore, a new otolith chemical proxy for hypoxia has been recently developed [9], that is the ratio of Mn to the trace element magnesium (Mg), which is also taken up in otoliths but is regulated by growth processes (eg [10-12]). Thus, in this paper we investigated whether direct exposure to hypoxia, as proxied by the Mn/Mg ratio accumulated in the otoliths, could explain the difference in condition between cod individuals collected in the open Baltic Sea in the period of worsening hypoxia (i.e., after 2000) [2].

Methods

Otoliths from 134 cod individuals sampled in February-March during the Baltic International Trawl Survey (BITS) in ICES subdivisions 25 and 27 (Fig. 1) were extracted from archives; this is within the range of the Eastern Baltic cod population where the occurrence of Western Baltic cod is considered minor [13]. Fish were collected in 1990-1995, 2000 (N = 57 up to 2000), 2005, 2010-2015, and 2017 (N = 79 for 2000 onward). Otoliths from fish in good body condition (Fulton's condition factor K [$K = (\text{total Weight (g)} / (\text{total Length}^3 \text{ (mm)})) \times 10^5$] ≥ 0.9) and poor condition ($K < 0.9$) at capture were randomly selected for each time period. Transverse thin sections exposed each otolith's entire depositional sequence from core formation (birth) to the outer edges (death). Microchemical analyses were made with laser ablation inductively coupled plasma mass spectrometry; lasered transects ran from core to outer edge, along the major dorsal growth axis (for details see [8]). Post-processing included parsing the data contained within a year's otolith growth by superimposing chemical transects on an otolith image and assigning annulus marks (Fig. 2A, B).

The data analyzed were mean and cumulative Mn/Mg within annual otolith growth zones. Duration of hypoxia exposure within a year was defined as the distance (in micrometers), from one annulus to the next, on the otolith transect where Mn/Mg exceeded the age-based median values for all the samples [9]. These durations were then expressed as percentages of years by dividing the "hypoxic" distances within a given annulus by its total distance. Percent durations were subsequently grouped into quartiles (< 25%, 25-49.9%, 50-74.9%, and ≥ 75%) to define "hypoxia exposure groups" (HEGs), where HEG-1 were the least exposed and HEG-4 the most exposed [9].

Analysis of variance (ANOVA) tested whether cod in good versus poor condition at time of capture were exposed to different levels of hypoxia during their lifetime, examining the period prior to 2000 (characterized by relatively good oxygen levels) separately from 2000 onward (period of chronic Baltic hypoxia). We tested the average and cumulative lifetime exposure, as well as the average and cumulative exposure during the most recent year of life. We tested both levels of Mn/Mg (degree of exposure) and duration of exposure (as defined above). Additionally, we tested the proxy of metabolic activity (Mg/Ca, see [12]), i.e. the lifetime accumulated Mg/Ca ratio, against age and HEG to test for long-term metabolic effects. Analyses were checked for normality and homogeneity of variances, and transformed or variance-weighted as needed.

Results

A total of 134 cod with equal sex ratios were analyzed. Fish lengths ranged between 340-969 mm and the estimated ages ranged between 3 to 9 years. Cod in poor condition (mean $K = 0.721 \pm 0.088$ s.d., range 0.482–0.889, N = 64) were distinct in the data set from high-condition fish (mean

K = 1.105 ± 0.084 s.d., range 0.90–1.380, N = 70). Example otolith transects showing Mn/Ca (hypoxia proxy uncorrected for growth) and corresponding Mg/Ca (proposed proxy of metabolic activity and growth) for a poor condition cod (Fig. 2A, left) vs. a high condition cod (Fig. 2B, left) demonstrate how fish of either condition status may experience summertime hypoxia (peaks in Mn/Ca), but the magnitudes of exposure are higher in the low condition fish. Additionally, Mg/Ca tracks the seasonal pattern of Mn/Ca in the healthy fish (Fig. 2B, left), but decouples from the Mn/Ca pattern in the fish with low K (Fig. 2A. left). Dividing the Mn by Mg results in the proxy of hypoxia exposure (Fig. 2A and B, right).

Proxies of hypoxia exposure differed considerably between time periods (Table 1). Overall, Mn/Mg proxies were elevated during the 2000s, the period of chronic hypoxia intensity. Mean Mn/Mg during the last year of life differed by condition class significantly in the 2000s (Table 1, Part A), irrespective of fish sex and age. Mean Mn/Mg values were much more similar and not significantly different in the pre-2000 (Table 1, Part A). Duration of hypoxia in the final year of life was nearly significant for the 2000s ($p = 0.06$) but not so for the period pre-2000 ($p = 0.86$), irrespective of fish sex and age. Over entire lifetimes, mean and cumulative Mn/Mg ratio and lifetime duration of hypoxia exposure (one-way ANOVA, Table 1, part B) were also strongly separated by condition class in the 2000s but not in the pre-2000s. In the 2000s low and high condition classes differed significantly ($p = 0.012$) from Age 2 onwards, with increasing divergence observed during fish life (variance-weighted ANOVA, Fig. 2C), irrespective of sex.

Lifetime cumulative Mg/Ca, our proxy of lifetime metabolism [12], when tested against age and HEG groups, showed highly significant divergences (Fig. 2D): the least hypoxia exposed (HEG-1) and most exposed (HEG-4) separated the most, whereas the intermediate groupings HEG-2 and HEG-3 largely overlapped each other (Fig. 2D). The (Age x HEG) interactions were significant for the period 2000s onward ($p = 0.021$) and both periods combined ($p = 0.008$), but not for the period pre-2000 ($p = 0.916$).

Discussion

During the past two decades (2000 onwards), a period of rapidly increasing, chronic hypoxia, cod in poor condition at capture had experienced a higher degree of hypoxia exposure, as suggested in our analyses by the higher Mn/Mg ratio, both in the last year of life and over entire lifetimes. Additionally, cumulative indices of duration of exposure were significantly parsed by condition classes (Table 1), becoming more so with increasing age (Fig. 2C). This suggests an accumulative effect of recurring hypoxia exposures on condition. In strong contrast, both low and high condition fish collected before 2000 experienced relatively little hypoxia as indexed by our proxies. This suggests that other factors affected cod condition prior to 2000, such as pelagic prey availability and density-dependent processes [2]; and that perhaps a change in system functioning occurred after 2000 due to deoxygenation.

Beginning in the mid-1990s, the mean body condition of Eastern Baltic cod decreased by around 30% [2] and the proportion of fish with condition close to lethal levels (Fulton's $K < 0.8$) has increased, reaching up to 35% in recent years [13]. These changes in cod body condition co-occurred with expansion of hypoxic and anoxic areas, mirroring a general deoxygenation of the central Baltic Sea [14]. Our analyses independently support the conclusions of Casini et al. [2]

linking declines in body condition to increasing hypoxia, as evidenced directly by otolith chemistry.

Our results shed light on some of the processes leading to low condition in Baltic Sea cod. The findings indicate that cod do not entirely avoid hypoxic waters but instead at least partially persist there, likely in search of benthic organisms [2] which constitute a key food resource for adults [15]. Moreover, the exposure to hypoxia appears to increase during the second year of life, when cod switch from a diet of semi-pelagic invertebrates to a predominance of benthic prey. Cod otolith chemistry (Sr/Ca ratios) indicates directed offshore movements into deeper, saltier water at about that age [7]. Tagging experiments have shown that cod undertake short, frequent visits to hypoxic deep waters [16], presumably to forage. Our study suggests that these sojourns in oxygen-poor waters (indexed by Mn/Mg) produce physiological stress in cod (indexed by lower Mg/Ca), mirrored by a decrease in body condition as shown in our analyses and also demonstrated in controlled experiments in fish including cod [17-18]. Lifetime cumulative Mg/Ca, an index of lifetime metabolic activity, split out by hypoxia exposure group (Fig. 2D), with highest cumulative metabolic activity in the least exposed group, and vice-versa. We suggest this is further evidence of the long-term impact of living in environments with recurring seasonal hypoxia.

As deoxygenation spreads due to climate warming and continued eutrophication [19], more organisms and ecological communities will be confronted with low oxygen as a metabolic constraint (eg [17, 20-23]. Eastern Baltic Sea cod present a dramatic case of a population being driven into decline by a combination of environmental pressures and overfishing [24]. Hypoxia and weakened condition appear to have made this population susceptible to a cascade of ecological changes, including increased predation by seals and parasitic infections [1] as well as heightened competition from flounder [25]. More study of the complex responses of ecological communities to hypoxia will be urgently needed as hypoxia continues to spread. This also points out the immediate societal need to address the drivers of hypoxia.

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Supplementary material.

Table S-1. Annulus data used in the study.

Table S-2. Lifetime average data used in the study.

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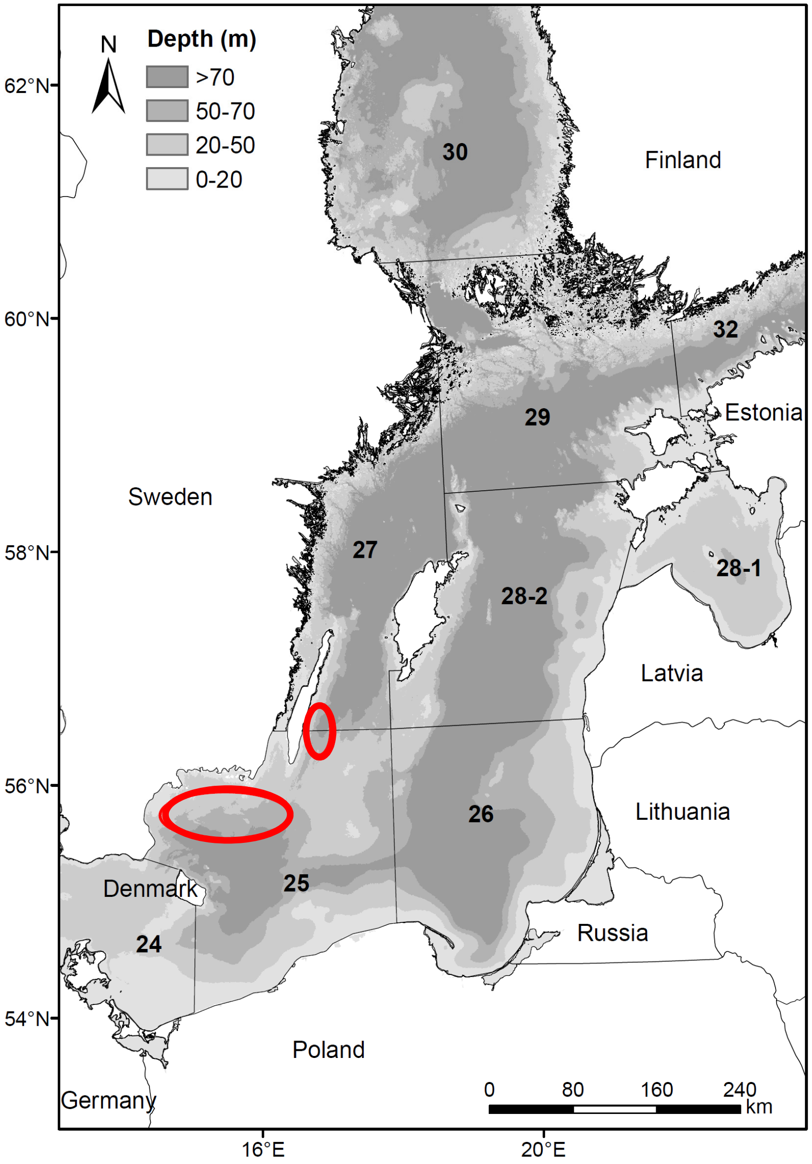
List of Figures.

1. Left: examples of cod taken from different areas of the Baltic sea indicative of wild cod in very good and very poor condition. Right: Map of the Baltic Sea showing the sampling areas (red ellipses). Waters with oxygen concentration < 2 ml/l (defined as hypoxia limit in the Baltic Sea) are frequently found below 70 m depth in the sampling areas. Numbers indicate ICES subdivisions, see Tables S-1 and S-2. Photos: Y. Heimbrand and J. Pönni.

2. Differences in otolith chemistry as related to hypoxia and fish condition (measured by Fulton's K). **A, B**. Otolith cross sections with Mn/Ca (blue), a hypoxia proxy partly affected by growth, and Mg/Ca (orange), a proxy for metabolic activity and growth; arrows point to transects (righ-hand panels) made by dividing Mn by Mg, to correct for growth effects on Mn. Yellow dots indicate the locations of winter annuli (left panels). X-axis denotes the distance (in microns) from the otolith core. **A**, fish 420 mm long and Age-5, was caught in February 2014 and had a low Fulton's K value; note persistently high seasonal hypoxia events and decoupling of Mg/Ca in third year. **B**, fish 450 mm long and Age-3 was caught in March 2005 and had high Fulton's K, lower Mn/Ca and higher Mg/Ca. **C**, Lifetime accumulated metric of hypoxia exposure duration measured by the otolith proxy as the lifetime Mn/Mg exceeding year-specific thresholds vs. age and categorized condition factor (high condition is 0.9 or greater) for pre-2000 and 2000s; p-values shown are calculated for (Fulton's K x Age) separately for each period (joint p-value of Fulton's K x Age x Period = 0.15). **D**, Cube root-transformed lifetime cumulative Mg/Ca, a metabolic proxy, as a function of age and hypoxia exposure group (HEG, quartiles of hypoxia duration) for pre-2000 and 2000s. Error bars for **C** and **D** are 95% confidence intervals.


N
Depth (m)
>70
50-70
20-50
0-20
62°N
60°N
58°N
56°N
54°N
16°E
20°E
30
Finland
32
29
Estonia
Sweden
27
28-1
28-2
Latvia
26
Lithuania
Denmark
25
24
Russia
Poland
Germany
0
80
160
240
km

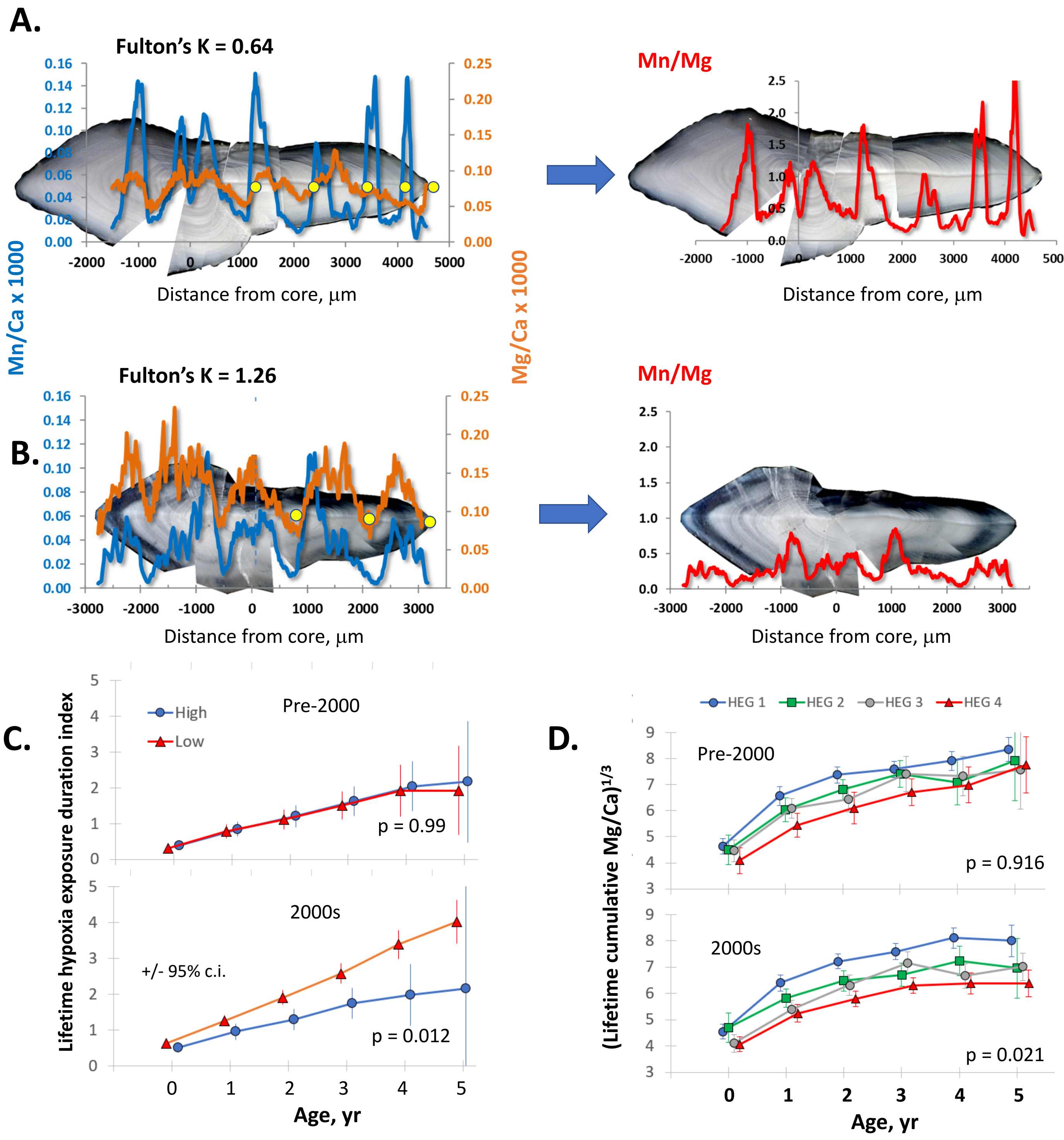

A.
Fulton's K = 0.64
Mn/Mg
B.
Fulton's K = 1.26
Mn/Mg
Mn/Ca x 1000
Mg/Ca x 1000
Distance from core, µm
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0.25
0.20
0.15
0.10
0.05
0.00
2.5
2.0
1.5
1.0
0.5
0.0
-3000
-2000
-1000
0
1000
2000
3000
4000
5000
C.
Lifetime hypoxia exposure duration index
High
Low
Pre-2000
p = 0.99
2000s
+/- 95% c.i.
p = 0.012
Age, yr
D.
HEG 1
HEG 2
HEG 3
HEG 4
(Lifetime cumulative Mg/Ca)^1/3
Pre-2000
p = 0.916
2000s
p = 0.021
Age, yr
0
1
2
3
4
5

Table 1. Analysis of variance results for hypoxia exposure proxies and fish condition (Fulton's K)[1] ; s.e. = standard error

A. During last year of life (* = 2 high extreme outliers deleted based on Q-Q plots)

Proxy	**Period**	**High K**	**s.e.**	**Low K**	**s.e.**	**df**	**p**
Mean Mn/Mg	pre-2000	0.094	0.01	0.08	0.01	44 (*)	0.377
	2000s	0.163	0.03	0.294	0.03	63 (*)	0.006
Cumulative Mn/Mg	pre-2000	54.3	9.1	62.9	11.9	47	0.565
	2000s	103.5	17.3	127.9	16	63 (*)	0.304
Duration of hypoxia proxy	pre-2000	0.339	0.06	0.321	0.008	47	0.855
(as fraction of last year)	2000s	0.479	0.06	0.639	0.06	66	0.058

B. Over entire lifetime

Proxy	**Period**	**High K**	**s.e.**	**Low K**	**s.e.**	**df**	**p**
Lifetime mean Mn/Mg	pre-2000	0.304	0.02	0.298	0.02	53	0.849
	2000s	0.406	0.06	0.539	0.05	77	0.069
LN(Lifetime cumulative Mn/Mg)	pre-2000	7.00	0.079	7.09	0.084	54	0.434
	2000s	7.07	0.073	7.34	0.063	75	0.008
LN(1 + Mn/Mg duration over lifetime)	pre-2000	0.894	0.081	0.908	0.096	52	0.918
	2000s	0.968	0.071	1.444	0.055	76	$< 10^{-6}$

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[1] Fulton's K mean values (± s.e.) are pre-2000 high K: 1.12 (0.015); pre-2000 low K: 0.72 (0.019); 2000s high K: 1.07 (0.015); 2000s low K: 0.72 (0.013).