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Supplementary Information for

# Bone resorption and body reorganization during maturation induce maternal transfer of toxic metals in anguillid eels

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Supplementary Text

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## Supplementary Text

### Sampling of animals:

A total of 22 live European eels (*Anguilla anguilla*) were originally obtained from German commercial fisheries and eventually divided into six groups according to their sex, life history and maturation stages: Yellow, silver, (hormone treated) maturing female, (hormone treated) fully matured female, silver males and (hormone treated) fully mature male developmental stages. Due to low availability, no male yellow eels were included in the analyses (*See Table S1 for detailed biological data of all used individuals*). In accordance with German animal welfare law (1), eels used for this study were killed by an overdose of 2-phenoxyethanol (ROTH, Karlsruhe, Germany) until final stop of the heart and subsequently stored at -20°C before further data collection.

### Hormone treatment:

All hormone-treated groups consisted of silver eels that were artificially matured by regular hormone injections prior to analyses. While the maturing female group consisted of fish that were incompletely stimulated due to shorter hormonal treatment, individuals in the mature female and mature male groups consisted of ready-to-spawn individuals with fully matured gonads. During the time of hormone treatment, these eels were held under a gentle but constant water flow in a round recirculation system equipped with aeration and a trickle filter for mechanical filtration and denitrification.

To induce maturation in female eels, individuals were treated with weekly intramuscular injections of aqueous salmon pituitary extract (SPE; Argent Aquaculture, Redmond, USA; 20 mg/kg). Injections were administered close to the dorsal fin into the dorsal muscle. Full maturation was indicated by a steep increase in weight caused by the hydration of oocytes. To reach this stage, between 26 to 32 weekly SPE injections per individual were required. Individuals in the group of maturing females did not reach the full maturation status and received between 13 and 27 SPE-injections before they died during the maturation process. They were immediately stored at 20°C when death was confirmed. Male eels were treated with weekly intraperitoneal injections of 150 IU human chorionic gonadotropin (hCG; Sigma-Aldrich, Schnelldorf, Germany). These fish were regarded mature when they produced active spermatozoa, which was regularly monitored and visually confirmed using a stereo microscope. It took ≥8 weeks of hCG-treatment to reach this stage.

### Sampling of skeletal elements:

The first spinal, postcranial vertebrae of each individual per group were excised for µCT scans. The same vertebrae of each, one representative individual per group was then also used for SEM scans and the third to fifth postcranial vertebrae taken from the same individuals were excised for bone histology. For body burden calculations and for inductively coupled plasma mass spectrometry (ICP-MS) measurements, all fish were carefully further dissected and body compartments of interest (muscle, liver, gonads, selected vertebrae and remaining spinal cord) were separated, weighed and subsequently freeze-dried and weighed again to record the water contents.

**Whole-body computed tomography (CT) imaging:**

Non-invasive whole-body scans from eels were performed at a clinical CT system (Somatom Definition Flash, Siemens Medical Solutions, Forchheim, Germany). All animals were scanned within one single CT scan (voltage 120 kV, current 54 mA, exposure time 285 ms, focal spot 1.2, filter type wedge 3) and CT slices were reconstructed using the Syngo software (Syngo CT 2012B, Siemens Medical Solutions) at voxel size 0.77 mm with a medium kernel (B35f). (*For further instrumental settings, see Table S3*) Individual whole-body volumes and skeletal structures were semi-automatically rendered based on contrast thresholding using Imalytics Preclinical Software, as previously described (2,3). Each individual animal was assigned a distinct class and color to facilitate visualization. BMD was estimated by comparing bone density with a hydroxyapatite calibration phantom (Osteo Phantom, Siemens Medical Solutions, Erlangen, Germany) of known BMD (200 mg / $cm^3$) scanned simultaneously with the eels.

**Vertebrae imaging (µCT):**

Making use of the same individuals from the whole-body CT scans, detailed imaging of the *second* postcranial spinal vertebrae was performed in a preclinical µCT system (TomoScope DUO 30s, CT-Imaging, Erlangen, Germany). Following the standard scanning protocol HQD-6565-360-90 as described by Gremse et al. (4), the vertebrae from individuals within the same sampling groups were scanned. Volumetric images were reconstructed at voxel size 70µm using a Feldkamp-type reconstruction with ring artifact correction. Vertebrae were segmented using a region-growing algorithm based on their increased image intensity, to determine the vertebrae volume per animal.

**Calcium maps:**

Calcium maps were derived from the clinical-CT (whole-body) and micro-CT (vertebrae) data, making use of proportionality between calcium-content and x-ray density (5). Those were employed to assess the mean and total calcium concentration (values normalized to g/$10^6$ $mm^3$) per animal, and for providing a color-coded volumetric depiction of the calcium distribution within the scanned specimen or body compartment.

**Scanning electron microscopy (SEM) of selected vertebrae:**

*Ex vivo* validation of bone demineralization was performed through high-resolution scanning electron microscopy (ESEM XL 30 FEG, FEI, Eindhoven, sputter coater EM SCD500, Leica, Wetzlar, Germany) of harvested vertebrae. Of each group, one representative, of the first three *postcranial* vertebra per developmental stage was scanned for overview, 50x, 500x and 2500x magnifications (BSE scan, 10 kV, VD: 10, Spot 3.0, Blender 3.0). Energy dispersive X-ray spectroscopy (EDXS) analyses were performed at 50x magnifications (Preset 50, Amp 35 µs), providing the semi-quantitative assessment of average Ca and P content within each sample Results were compared to the ICP-MS analytical evaluation.

**Bone histology:**

For histological analyses, frozen abdominal (type II) vertebrae of one representative individual (with median bone density) per group were thawed in 10% neutral-buffered formalin at room temperature and subsequently fixated in formalin for additional three days. After formalin fixation, these samples were rinsed in running tap water overnight and then transferred stepwise into 70% ethanol (storage solution). Then, prior to decalcification, the vertebrae were again transferred stepwise to 100% ethanol to remove tissue fat overnight on a shaker and after this, retransferred stepwise to tap water again. Decalcification was carried out with Decal™ Decalcifier (Statlab Medical, McKinney, USA) twice for 24 h; with each step performed on a shaker at room temperature. After decalcification, samples were rinsed overnight in tap water followed by dehydration in a series of ethanol solutions with increasing concentration (30-100% ethanol in water). In the paraffin embedding process, the bones were treated with HistoChoice (Sigma-Aldrich, St. Louis, USA), two times overnight on a shaker, followed by graded HistoChoice - paraffin solutions (30-100% paraffin at 60°C). Serial sections of 5 µm thickness were prepared in the sagittal plane of the vertebral column, starting at the lateral periphery of vertebral bodies and ending in the mediosagittal plane. Sections were mounted on treated microscope slides (Superfrost Plus Micro, VWR International, Radnor, USA) and stained with Heidenhain's Azan following the protocol of Presnell & Schreibman (6). DPX (Sigma-Aldrich, St. Louis, USA) was used to mount the sections on slides. All sections were analyzed and photographed at magnifications between 40x and 400x with a Leitz Dialux 22EB microscope (Leica, Wetzlar, Germany) equipped with a 5MP color CCD camera.

**Analytical validation by ICP-MS & TDA AAS**

Before further utilization, muscle, liver, bone and gonad samples were weighed, lyophilized (Lyovac GT 2; GEA Pharma Systems, Wommelgem, Belgium) and then weighed again before being homogenised using a laboratory mill (IKA A11; IKA, Staufen, Germany).

Concentrations of most minerals and metals were determined by means of ICP-MS. Briefly, samples were digested in Polytetrafluoroethylene (PTFE) high-pressure vessels (HPR-1000/10S, MLS GmBH, Leutkirch, Germany) using an MLS Ethos plus microwave oven with temperature and pressure control (MLS GmbH, Leutkirch, Germany). To this end, approx. 50-100 mg was weighed into a microwave vessel containing 5 mL nitric acid (65%, SupraPure, Sigma Aldrich), 7 mL hydrogen peroxide (SupraPure, Sigma Aldrich), and 1 mL internal rhodium standard (1µg $mL^{-1}$). The digestion temperature was ramped from room temperature to 210°C for 45 min, and held for 15 min. After digestion, samples were rehydrated 2- and 10-fold with ultrapure water. Instrumental ICP-MS analyses were performed on an ELAN DRC II system (PerkinElmer SCIEX™, PerkinElmer, Waltham, MA, USA). The technical parameters and operating conditions are summarized in supplementary material (S2). Calibration was performed using a commercial multi-element standard (CertiPUR®, Merck, Darmstadt, Germany) with element concentrations ranging from 0.1 – 1000 mg $L^{-1}$. Limits of detection (LODs) were calculated by applying the dilution factor to LODs based on the signal obtained from the analysis of 10 replicates of a solution containing a digested unexposed filter using the three standard deviation criteria.

Mercury concentrations in the body compartments of interest were determined using thermal decomposition, amalgamation, and atomic absorption spectrometry (TDA AAS) (Direct Mercury Analyzer, Milestone Inc, Shelton, USA) with integrated autosampler, control system lab-terminal 1024 and laboratory weighing scale (Precisa XT220, Milestone, Leutkirch, Germany). Dry samples were weighed to approximately 1,5 - 60 mg and quadruplicate measurements were conducted for each sample. If values varied more than 20 %, another 4-fold measurement was performed.

Analytically derived dry weight-based concentrations of metals and minerals in homogenized samples from individual organs were multiplied with the total dry-weight of the respective organ, which was estimated based on life-history stage-dependent percentages of the body wet-weight of each fish. For this, a reference database consisting of whole-body dry-weight distribution data from 120 European eels of different stages was used (*SI Table S4*). Results were expressed as relative percentages in the individual compartments, and as soft tissue to bone mass ratios.

**Statistical Analyses**

Statistical testing was carried out using GraphPad Prism 6.0h (GraphPad software Inc, California, USA). Analysis of variances (ANOVA) followed by Tukey's or Dunnett’s post hoc test was used to test for differences in volume and calcium map- derived calcium content of whole-body and single vertebrae sampling groups of female eels. For the two groups of male eels, student's t-test was used to test for differences in volume and calcium content of whole bodies and single vertebrae sampling groups. A significance level of $P < 0.05$ was used for all tests. (*Details on test results available in Table S2*)

**Table S1.**

**Detailed biological parameters of eels used in this study**

| ID | System / Origin | Sex | Stage (Durif ) | Length (cm) | Mass (g) | Liver mass (g) | Gonad mass (g) | Muscle sample (g) | Stomach & Gut (g) | Total muscle lipid *(fatmeter device)* | Swim-bladder (g) | Skeletal bone volume ($mm^3$) | Bone mineral density in whole skeleton (apatite / mm3) | Vertebral bone volume ($mm^3$) | Bone mineral density in Vertebrae (apatite / mm3) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| yf1 | Warnow / Peene | f | 2 | 51 | 177 | 1.25 | 0.28 | 17.39 | 6.12 | 14.5 | 1.13 | 11444.30 | 37.62 | 37.16 | 457.29 |
| yf2 | Warnow / Peene | f | 1 | 45 | 118 | 1.41 | 0.1 | 10.25 | 3.73 | 12.9 | 0.18 | 8675.78 | 40.09 | 17.99 | 491.39 |
| yf4 | Warnow / Peene | f | 3 | 55 | 302 | 4.0 | 3.04 | 21.53 | 7.44 | 33.1 | 0.77 | 11556.70 | 19.43 | 23.66 | 469.92 |
| sf1 | Warnow / Peene | f | 5 | 66 | 632 | 7.8 | 11.34 | 38.4 | 12.48 | 26.3 | 2.98 | 23699.70 | 24.36 | 85.03 | 411.02 |
| sf2 | Warnow / Peene | f | 5 | 73 | 818 | 8.4 | 11.45 | 41.45 | 19.05 | 30.2 | 2.96 | 27983.50 | 28.68 | 114.36 | 374.80 |
| sf3 | Warnow / Peene | f | 5 | 73 | 782 | 0.94 | 10.58 | 36.92 | 18.38 | 29.5 | 2.24 | 24316.50 | 23.76 | 74.28 | 450.60 |
| sf4 | Warnow / Peene | f | 5 | 76 | 877 | 10.53 | 12.59 | 34.52 | 21.48 | 38.2 | 4.89 | 34374.70 | 29.77 | 91.07 | 503.94 |
| gf1 | Ems | f | 5 | 71 | 528 | 4.8 | 74.07 | 18.95 | 2.84 | 20.9 | 1.35 | 19986.00 | 17.66 | 82.82 | 305.01 |
| gf2 | Arresö (Dk) | f | 5 | 73 | 709 | 10.64 | 10.9 | 33.58 | 6.95 | 31.5 | 3.72 | 30512.10 | 23.92 | 113.07 | 380.54 |
| gf4 | Ems | f | 5 | 73 | 675 | 8.52 | 124.1 | 29 | 4.78 | 20.7 | 1.47 | 20626.70 | 20.25 | 58.34 | 320.64 |
| mf1 | Weser | f | 5 | 69 | 634 | 4.66 | 155 | 13.39 | 1.66 | NA | 3.8 | 11152.30 | 13.04 | 38.06 | 244.46 |
| mf2 | Weser | f | 5 | 66 | 532 | 5.14 | 152 | 15.5 | 4.1 | NA | 0.5 | 9757.93 | 15.49 | 38.89 | 258.49 |
| mf3 | Weser | f | 5 | 77 | 826 | 14.21 | 295.6 | 12.46 | 1.92 | NA | 1.05 | 17118.30 | 1534 | 49.47 | 258.69 |
| mf4 | Weser | f | 4 | 81 | 1219 | 17.08 | 471.87 | 25.6 | 9.1 | NA | 2.81 | 21026.90 | 16.31 | 66.08 | 238.66 |
| sm1 | Warnow / Peene | m | 6 | 44 | 125 | 1.25 | 0.1 | 14.44 | 2.24 | 29.6 | 0.58 | 4071.39 | 10.50 | 14.49 | 348.71 |
| sm2 | Warnow / Peene | m | 6 | 39 | 107 | 1.38 | <LOQ | 10.82 | 2.6 | 27.3 | 1.09 | 3390.63 | 10.19 | 10.92 | 334.14 |
| sm3 | Warnow / Peene | m | 6 | 42 | 122 | 1.39 | <LOQ | 14.69 | 1.87 | 37.4 | 0.69 | 4799.40 | 9.95 | 12.00 | 388.27 |
| sm4 | Warnow / Peene | m | 6 | 43 | 141 | 1.25 | <LOQ | 10.39 | 1.53 | 33.4 | 0.58 | 4750.95 | 14.62 | 10.20 | 432.65 |
| mm1 | Raised in farm | m | 6 | 43 | 136 | 1.63 | 8.77 | 8.82 | 1.33 | 32.8 | 0.21 | 3926.61 | 7.41 | 8.70 | 330.84 |
| mm2 | Raised in farm | m | 6 | 41 | 155 | 1.96 | 14.94 | 9.68 | 0.94 | 34.2 | 0.11 | 3357.13 | 7.81 | 5.19 | 300.31 |
| mm3 | Raised in farm | m | 6 | 39 | 106 | 1.1 | 10.65 | 9.7 | 1.69 | 36.3 | 0.05 | 2929.22 | 8.30 | 6.75 | 323.04 |
| mm4 | Raised in farm | m | 6 | 43 | 88 | 0.85 | 2.32 | 8.65 | 0.78 | 37.6 | 0.05 | 4398.01 | 6.75 | 7.43 | 316.86 |

**Table S2.**

**Details of statistical tests between tested groups. Bold letters indicate significant results**

**Skeletal Bone Volume**

**FEMALES (one-way ANOVA and Tukey's multiple comparisons test)**

| | Mean 1 | Mean 2 | Mean Diff. | Summary | 95.00% CI of diff. | Adjusted P value |
|---|---|---|---|---|---|---|
| **Yellow vs. Silver** | **10559** | **27594** | **-17035** | ** | **-28238 to -5832** | **0.0042** |
| **Yellow vs. Maturing** | **10559** | **23708** | **-13149** | * | **-25126 to -1173** | **0.0310** |
| Yellow vs. Mature | 10559 | 14764 | -4205 | ns | -15408 to 6998 | 0.6701 |
| Silver vs. Maturing | 27594 | 23708 | 3885 | ns | -7318 to 15088 | 0.7194 |
| **Silver vs. Mature** | **27594** | **14764** | **12830** | * | **-2458 to 23202** | **0.0158** |
| Maturing vs. Mature | 23708 | 14764 | 8944 | ns | -2259 to 20147 | 0.1312 |

**Males (unpaired t-test)**

| | Mean 1 | Mean 2 | Mean Diff | Summary | 95.00% CI of diff. | P value | $R^2$ |
|---|---|---|---|---|---|---|---|
| Silver vs. Mature | 4253 | 3653 | -600 | ns | -1732 to 532 | 0.2423 | 0.2189 |

**Skeletal Bone Mineral Density**

**FEMALES (one-way ANOVA and Tukey's multiple comparisons test)**

| | Mean 1 | Mean 2 | Mean Diff. | Summary | 95.00% CI of diff. | Adjusted P value |
|---|---|---|---|---|---|---|
| Yellow vs. Silver | 32.4 | 26.64 | 5.757 | ns | -7.215 to 18.73 | 0.5504 |
| Yellow vs. Maturing | **32.4** | 20.61 | 11.79 | ns | -2.082 to 25.66 | 0.1030 |
| **Yellow vs. Mature** | **32.4** | **15.04** | **17.35** | ** | **4.382 to 30.33** | **0.0098** |
| Silver vs. Maturing | 26.64 | 20.61 | 6.029 | ns | -6.943 to 19 | 0.5147 |
| Silver vs. Mature | 26.64 | 15.04 | 11.6 | ns | -0.4131 to 23.61 | 0.0591 |
| Maturing vs. Mature | 20.61 | 15.04 | 5.568 | ns | -7.405 to 18.54 | 0.575 |

**Males (unpaired t-test)**

| | Mean 1 | Mean 2 | Mean Diff | Summary | 95.00% CI of diff. | P value | $R^2$ |
|---|---|---|---|---|---|---|---|
| Silver vs. Mature | 11.31 | 7.564 | -3.75 | ns | -6.577 to -0.9225 | 0.0176 | 0.6371 |

**Vertebral Volume**

**FEMALES (one-way ANOVA and Tukey's multiple comparisons test)**

| | Mean 1 | Mean 2 | Mean Diff. | Summary | 95.00% CI of diff. | Adjusted P value |
|---|---|---|---|---|---|---|
| Yellow vs. Silver | **26.27** | **91.19** | **-64.92** | ** | **-105.9 to -23.98** | **0.0031** |
| Yellow vs. Maturing | **26.27** | **84.74** | **-58.47** | ** | **-102.2 to -14.71** | **0.0099** |
| Yellow vs. Mature | 26.27 | 48.2 | -21.93 | ns | -62.87 to 19.01 | 0.4017 |
| Silver vs. Maturing | 91.19 | 84.74 | 6.442 | ns | -34.50 to 47.38 | 0.9615 |
| Silver vs. Mature | **91.19** | **48.2** | **42.99** | * | **5.083 to 80.89** | **0.0260** |
| Maturing vs. Mature | 84.74 | 48.2 | 36.54 | ns | -4.395 to 77.48 | 0.0840 |

**Males (unpaired t-test)**

| | Mean 1 | Mean 2 | Mean Diff | Summary | 95.00% CI of diff. | P value | $R^2$ |
|---|---|---|---|---|---|---|---|
| **Silver vs. Mature** | **11.9** | **7.018** | **-4.89** | ** | **-7.796 to -1.974** | **0.0063** | **0.7376** |

**Vertebral Bone Mineral Density**

**FEMALES (one-way ANOVA and Tukey's multiple comparisons test)**

| | Mean 1 | Mean 2 | Mean Diff. | Summary | 95.00% CI of diff. | Adjusted P value |
|---|---|---|---|---|---|---|
| Yellow vs. Silver | 0.04442 | 0.04351 | 0.0009096 | ns | -0.008852 to 0.01067 | 0.9919 |
| **Yellow vs. Maturing** | **0.04442** | **0.03354** | **0.01088** | * | **0.0003348 to 0.02142** | **0.0426** |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Yellow vs. Mature** | **0.04442** | **0.02501** | **0.01941** | ******* | **0.009649 to 0.02917** | **0.0005** |
| Silver vs. Maturing | 0.04351 | 0.03354 | 0.009969 | ns | -0.0005748 to 0.02826 | 0.0656 |
| **Silver vs. Mature** | **0.04351** | **0.02501** | **0.0185** | ******* | **0.008739 to 0.02826** | **0.0007** |
| Maturing vs. Mature | 0.03354 | 0.02501 | 0.008532 | ns | 0.0002012 to 0.02908 | 0.1273 |

**Males (unpaired t-test)**

| | Mean 1 | Mean 2 | Mean Diff | Summary | 95.00% CI of diff. | P value | $R^2$ |
|---|---|---|---|---|---|---|---|
| **Silver vs. Mature** | **0.03759** | **0.03178** | **-0.005818** | ***** | **-0.01145 to -0.0001844** | **0.0449** | **0.5156** |

## Concentration in Bones From ADULT FEMALES

## (one-way ANOVA with Dunnett's multiple comparisons test)

| | **Mean 1** | **Mean 2** | **Mean Diff.** | **Summary** | **95.00% CI of diff.** | **Adjusted P value** |
|---|---|---|---|---|---|---|
| **Cadmium** | | | | | | |
| Silver vs. Maturing | 43.75 | 40 | 3.75 | ns | -4.116 to 11.62 | 0.3810 |
| Silver vs. Mature | 43.75 | 40 | 3.75 | ns | -3.532 to 11.03 | 0.3337 |
| **Copper** | | | | | | |
| Silver vs. Maturing | 397.5 | 543 | -145.5 | ns | -451.1 to 160.1 | 0.3819 |
| Silver vs. Mature | 397.5 | 534 | -136.5 | ns | -419.4 to 146.4 | 0.3737 |
| **Manganese** | | | | | | |
| Silver vs. Maturing | 29625 | 26033 | 3592 | ns | -13428 to 20611 | 0.8013 |
| Silver vs. Mature | 29625 | 21025 | 8600 | ns | -7157 to 24357 | 0.2989 |
| **Mercury** | | | | | | |
| Silver vs. Maturing | 56 | 43.33 | 12.67 | ns | -85.56 to 110.9 | 0.9181 |
| **Silver vs. Mature** | **56** | **157.8** | **-101.8** | ***** | **-192 to -10.81** | **0.0310** |

## Concentration In Gonads From Adult Females

## (one-way ANOVA with Dunnett's multiple comparisons test)

| | **Mean 1** | **Mean 2** | **Mean Diff.** | **Summary** | **95.00% CI of diff.** | **Adjusted P value** |
|---|---|---|---|---|---|---|
| **Cadmium** | | | | | | |
| Silver vs. Maturing | **45** | **96** | **-51** | **ns** | -160.2 to 58.25 | 0.3941 |
| **Silver vs. Mature** | **45** | **168.4** | **-123.3** | ***** | **-224.4 to -22.11** | **0.0208** |
| **Copper** | | | | | | |
| **Silver vs. Maturing** | **3481** | **1261** | **2220** | ****** | **789.1 to 3651** | **0.0059** |
| **Silver vs. Mature** | **3481** | **1690** | **1791** | ***** | **465.9 to 3115** | **0.0124** |
| **Manganese** | | | | | | |
| **Silver vs. Maturing** | **612.8** | **2845** | **-2233** | ***** | **-4324 to -141.4** | **0.0381** |
| Silver vs. Mature | 612.8 | 1765 | -1153 | ns | -3089 to 783.6 | 0.2492 |
| **Mercury** | | | | | | |
| Silver vs. Maturing | 139 | 186 | -47 | ns | -408 to 314 | 0.9166 |
| **Silver vs. Mature** | **139** | **520.3** | **-381.3** | ***** | **-715.5 to -47.01** | **0.0284** |

## Concentration In Livers From Adult Females

## (one-way ANOVA with Dunnett's multiple comparisons test)

| | **Mean 1** | **Mean 2** | **Mean Diff.** | **Summary** | **95.00% CI of diff.** | **Adjusted P value** |
|---|---|---|---|---|---|---|
| **Cadmium** | | | | | | |
| Silver vs. Maturing | 58.75 | | -400.3 | ns | -1077 to 276.8 | 0.2530 |
| **Silver vs. Mature** | **58.75** | | **-1024** | ****** | **-1651 to -397.2** | **0.0044** |
| **Copper** | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Silver vs. Maturing | 32700 | 5510 | 27190 | ns | -1457 to 55838 | 0.0619 |
| Silver vs. Mature | 32700 | 22617 | 10083 | ns | -18565 to 38730 | 0.5760 |
| **Manganese** | | | | | | |
| Silver vs. Maturing | 4711 | 7795 | -3083 | ns | -8471 to 2304 | 0.2713 |
| **Silver vs. Mature** | **4711** | **9956** | **-5245** | ***** | **-10233 to -257.1** | **0.0406** |
| **Mercury** | | | | | | |
| Silver vs. Maturing | 629.3 | 300.7 | 328.6 | ns | -1098 to 1755 | 0.7700 |
| **Silver vs. Mature** | **629.3** | **2123** | **-1494** | ***** | **-2815 to -172.8** | **0.0295** |

**Concentration In Muscle From Adult Females**

**(one-way ANOVA with Dunnett's multiple comparisons test)**

| | **Mean 1** | **Mean 2** | **Mean Diff.** | **Summary** | **95.00% CI of diff.** | **Adjusted P value** |
|---|---|---|---|---|---|---|
| **Cadmium** | | | | | | |
| Silver vs. Maturing | 1532 | 584.5 | 947.5 | ns | -751.2 to 2646 | 0.2764 |
| Silver vs. Mature | 1532 | 1352 | 180 | ns | -1207 to 1567 | 0.9126 |
| **Copper** | | | | | | |
| Silver vs. Maturing | 58.75 | 45 | 13.75 | ns | -20.63 to 48.13 | 0.4897 |
| Silver vs. Mature | 58.75 | 45 | 13.75 | ns | -18.08 to 45.58 | 0.4423 |
| **Manganese** | | | | | | |
| Silver vs. Maturing | 414.3 | 450.3 | -36.08 | ns | -890.4 to 818.3 | 0.9907 |
| Silver vs. Mature | 414.3 | 1049 | -635 | ns | -1426 to 156 | 0.1110 |
| **Mercury** | | | | | | |
| Silver vs. Maturing | 767 | 383.3 | 383.7 | ns | -479.8 to 1247 | 0.4248 |
| **Silver vs. Mature** | **767** | **1922** | **-1155** | ****** | **-1954 to 355.3** | **0.0088** |

**Table S3.**

**Instrumental parameters for the analysis with ICP-MS**

| ICP-MS Operating Conditions and Parameters. | |
|---|---|
| ICP-MS | Elan-DRCII (Perkin-Elmer) |
| Nebulizer | Meinhard Type A quartz<br>Part No.: WE02-4372 |
| Spray Chamber | Quartz Cyclonic<br>Part No.: WE02-5222 |
| RF Power | 1100 Watt |
| Plasma Ar Flow | 15 L/min |
| Nebulizer Ar Flow | 0.93 L/min |
| Aux. Ar Flow | 1.1 L/min |
| Injector | 2.0 mm i.d. Quartz<br>Part No.: WE02-3916 |
| $CeO^+/Ce^+$ | <3% |

**Table S4.**

**Reference dataset with biological values for mass balance calculations**

| Origin | Yellow /Silver | Sex | Stage (Durif) | Length (cm) | Weight (g) | Fatmeter % in Filet | Fat (%ww) (analyt.) | Kidney (g) | Kidney (%) | Liver (g) | Liver (%) | Gonads (g) | Gonads (%) | Gut (g) | Gut (%) | Total muscle (g) | Muscle (%) | Skin (g) | Skin (%) | Carcass (g) | Carcass (%) | SB (g) | SB (%) | Gills (g) | Gills (%) | Spleen (g) | Spleen (%) | Brain (g) | Brain (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Aquaculture | y | f | 1 | 34 | 57.75 | NA | 7.35 | 0.72 | 1.24 | 0.80 | 1.38 | NA | NA | 2.25 | 3.90 | 26.31 | 45.56 | 6.36 | 11.01 | 12.87 | 22.28 | NA | NA | 0.97 | 1.68 | 0.09 | 0.16 | 0.05 | 0.08 |
| Aquaculture | y | f | 1 | 35 | 52.81 | NA | 7.35 | 0.86 | 1.62 | 0.80 | 1.51 | NA | NA | 2.15 | 4.06 | 23.25 | 44.02 | 5.79 | 10.96 | 13.93 | 26.37 | NA | NA | 0.52 | 0.98 | 0.12 | 0.23 | 0.03 | 0.06 |
| Aquaculture | y | f | 1 | 40 | 94.05 | NA | 8.72 | 1.26 | 1.34 | 1.29 | 1.37 | 0.33 | 0.35 | 3.78 | 4.02 | 40.24 | 42.79 | 9.77 | 10.38 | 28.75 | 30.57 | NA | NA | 1.73 | 1.84 | 0.20 | 0.21 | 0.05 | 0.05 |
| Aquaculture | y | f | 1 | 39 | 108.65 | NA | 20.99 | 0.90 | 0.83 | 1.37 | 1.26 | 0.37 | 0.34 | 5.67 | 5.22 | 56.53 | 52.03 | 9.65 | 8.89 | 23.58 | 21.70 | NA | NA | 1.29 | 1.19 | 0.14 | 0.13 | 0.05 | 0.05 |
| Aquaculture | y | m | 1 | 35 | 83.7 | NA | 23.24 | 0.61 | 0.73 | 1.28 | 1.53 | NA | NA | 4.85 | 5.79 | 39.90 | 47.67 | 9.07 | 10.84 | 19.19 | 22.93 | NA | NA | 1.18 | 1.41 | 0.11 | 0.13 | 0.04 | 0.05 |
| Aquaculture | y | m | 1 | 34 | 76.12 | NA | 23.50 | 0.46 | 0.60 | 1.04 | 1.37 | NA | NA | 3.61 | 4.75 | 38.77 | 50.93 | 8.38 | 11.01 | 18.58 | 24.41 | NA | NA | 0.80 | 1.06 | 0.05 | 0.06 | 0.03 | 0.04 |
| Aquaculture | y | f | 1 | 36 | 78.2 | NA | 11.32 | 0.82 | 1.04 | 0.93 | 1.18 | 0.10 | 0.13 | 2.11 | 2.69 | 44.91 | 57.43 | 8.55 | 10.94 | 13.69 | 17.51 | NA | NA | 0.93 | 1.19 | 0.07 | 0.10 | 0.05 | 0.06 |
| Aquaculture | y | f | 1 | 35 | 61.39 | NA | 9.91 | 0.45 | 0.73 | 0.86 | 1.41 | 0.16 | 0.27 | 3.40 | 5.53 | 27.34 | 44.54 | 5.49 | 8.95 | 17.71 | 28.85 | NA | NA | 0.65 | 1.06 | 0.07 | 0.12 | 0.04 | 0.06 |
| Aquaculture | y | f | 1 | 35 | 73.47 | NA | 21.35 | 0.84 | 1.14 | 0.98 | 1.34 | 0.31 | 0.42 | 4.14 | 5.64 | 41.36 | 56.30 | 6.35 | 8.65 | 12.86 | 17.50 | NA | NA | 1.27 | 1.73 | 0.10 | 0.14 | 0.07 | 0.09 |
| Aquaculture | y | f | 1 | 35 | 65.9 | NA | 15.10 | 0.45 | 0.68 | 0.90 | 1.36 | 0.42 | 0.64 | 2.31 | 3.51 | 38.40 | 58.27 | 4.57 | 6.93 | 9.97 | 15.13 | NA | NA | 1.47 | 2.23 | 0.05 | 0.08 | 0.06 | 0.09 |
| Aquaculture | y | f | 1 | 34 | 66.84 | NA | 13.75 | 0.73 | 1.09 | 0.91 | 1.36 | 0.10 | 0.14 | 2.63 | 3.93 | 32.93 | 49.27 | 7.73 | 11.56 | 15.61 | 23.36 | NA | NA | 0.89 | 1.33 | 0.14 | 0.21 | 0.05 | 0.07 |
| Aquaculture | y | f | 1 | 35 | 63.7 | NA | 17.02 | 0.72 | 1.13 | 0.84 | 1.32 | NA | NA | 1.63 | 2.56 | 34.81 | 54.65 | 6.48 | 10.18 | 11.91 | 18.70 | NA | NA | 0.91 | 1.42 | 0.08 | 0.13 | 0.04 | 0.06 |
| Aquaculture | y | f | 1 | 31 | 57.33 | NA | 26.49 | 0.48 | 0.84 | 0.61 | 1.06 | NA | NA | 2.48 | 4.32 | 29.68 | 51.78 | 5.85 | 10.20 | 12.77 | 22.28 | NA | NA | 0.42 | 0.73 | 0.06 | 0.10 | 0.03 | 0.05 |
| Aquaculture | y | m | 1 | 31 | 56.38 | NA | 25.35 | 0.54 | 0.96 | 0.92 | 1.63 | NA | NA | 1.54 | 2.74 | 35.77 | 63.44 | 4.65 | 8.25 | 6.52 | 11.56 | NA | NA | 0.54 | 0.95 | 0.07 | 0.13 | 0.04 | 0.07 |
| Aquaculture | y | m | 1 | 34 | 85.45 | NA | 27.37 | 0.61 | 0.71 | 1.01 | 1.18 | NA | NA | 3.29 | 3.85 | 47.30 | 55.35 | 9.49 | 11.11 | 17.29 | 20.23 | NA | NA | 0.81 | 0.94 | 0.04 | 0.05 | 0.04 | 0.04 |
| Aquaculture | y | f | 1 | 35 | 66.65 | NA | 20.91 | 0.60 | 0.89 | 0.82 | 1.23 | 0.04 | 0.06 | 2.73 | 4.09 | 39.37 | 59.07 | 5.89 | 8.83 | 10.27 | 15.41 | NA | NA | 0.79 | 1.18 | 0.04 | 0.06 | 0.05 | 0.08 |
| Eider | y | f | 1 | 41 | 115 | 11.1 | NA | NA | NA | 1.78 | 1.55 | 0.20 | 0.17 | 7.18 | 6.24 | 60.89 | 52.95 | 17.17 | 14.93 | 16.86 | 14.66 | 1.05 | 0.91 | 2.45 | 2.13 | NA | NA | NA | NA |
| Elbe | y | f | 2 | 52 | 265 | 34.9 | NA | NA | NA | 2.88 | 1.09 | 2.52 | 0.95 | 11.19 | 4.22 | 160.30 | 60.49 | 32.50 | 12.26 | 36.47 | 13.76 | 1.03 | 0.39 | 1.71 | 0.65 | NA | NA | NA | NA |
| Elbe | y | f | 2 | 56 | 295 | 35.2 | NA | NA | NA | 3.53 | 1.20 | 1.48 | 0.50 | 16.10 | 5.46 | 174.31 | 59.09 | 32.62 | 11.06 | 51.47 | 17.45 | 0.88 | 0.30 | 2.83 | 0.96 | NA | NA | NA | NA |
| Weser | y | f | 2 | 55 | 320 | 32.7 | NA | NA | NA | 4.28 | 1.34 | 1.60 | 0.50 | 23.19 | 7.25 | 191.16 | 59.74 | 34.41 | 10.75 | 46.48 | 14.53 | 2.60 | 0.81 | 3.59 | 1.12 | NA | NA | NA | NA |
| Weser | y | f | 2 | 61 | 406 | 32.8 | NA | NA | NA | 5.10 | 1.26 | 1.86 | 0.46 | 21.88 | 5.39 | 247.64 | 61.00 | 38.11 | 9.39 | 60.03 | 14.79 | 0.18 | 0.04 | 5.75 | 1.42 | NA | NA | NA | NA |
| Weser | y | f | 2 | 60 | 395 | 29.9 | NA | NA | NA | 9.08 | 2.30 | 2.44 | 0.62 | 18.51 | 4.69 | 247.90 | 62.76 | 36.94 | 9.35 | 56.37 | 14.27 | 1.66 | 0.42 | 5.71 | 1.45 | NA | NA | NA | NA |
| Weser | y | f | 2 | 52 | 285 | 31.4 | NA | NA | NA | 4.82 | 1.69 | 1.67 | 0.59 | 16.40 | 5.75 | 151.91 | 53.30 | 29.76 | 10.44 | 52.00 | 18.25 | 1.03 | 0.36 | 4.15 | 1.46 | NA | NA | NA | NA |
| Weser | y | f | 2 | 57 | 306 | 20.4 | NA | NA | NA | 4.79 | 1.57 | 2.18 | 0.71 | 17.45 | 5.70 | 183.99 | 60.13 | 32.56 | 10.64 | 43.61 | 14.25 | 2.04 | 0.67 | 3.95 | 1.29 | NA | NA | NA | NA |
| Weser | y | f | 2 | 55 | 288 | 30.7 | NA | NA | NA | 3.90 | 1.35 | 1.25 | 0.43 | 14.28 | 4.96 | 182.94 | 63.52 | 25.57 | 8.88 | 43.71 | 15.18 | 0.79 | 0.27 | 3.69 | 1.28 | NA | NA | NA | NA |

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Weser | y | f | 2 | 58 | 508 | 33.3 | NA | NA | NA | 8.80 | 1.73 | 4.03 | 0.79 | 29.10 | 5.73 | 323.47 | 63.68 | 48.60 | 9.57 | 62.87 | 12.38 | 0.95 | 0.19 | 6.31 | 1.24 | NA | NA | NA | NA |
| Weser | y | f | 2 | 53 | 314 | 28 | NA | NA | NA | 5.85 | 1.86 | 1.30 | 0.41 | 12.23 | 3.89 | 203.29 | 64.74 | 27.85 | 8.87 | 38.90 | 12.39 | 0.61 | 0.19 | 2.99 | 0.95 | NA | NA | NA | NA |
| Weser | y | f | 2 | 51 | 218 | 15.3 | NA | NA | NA | 2.48 | 1.14 | 0.91 | 0.42 | 12.84 | 5.89 | 127.34 | 58.41 | 19.88 | 9.12 | 34.73 | 15.93 | 1.00 | 0.46 | 3.51 | 1.61 | NA | NA | NA | NA |
| Weser | y | f | 2 | 53 | 290 | 21.6 | NA | NA | NA | 5.66 | 1.95 | 1.02 | 0.35 | 19.83 | 6.84 | 164.31 | 56.66 | 28.79 | 9.93 | 46.29 | 15.96 | 1.00 | 0.34 | 4.96 | 1.71 | NA | NA | NA | NA |
| Eider | y | f | 2 | 52 | 201 | 10.8 | NA | NA | NA | 2.95 | 1.47 | 0.51 | 0.25 | 9.58 | 4.77 | 85.87 | 42.72 | 28.89 | 14.37 | 60.77 | 30.23 | 0.81 | 0.40 | 4.65 | 2.31 | NA | NA | NA | NA |
| Eider | y | f | 2 | 55 | 355 | 7.9 | NA | NA | NA | 4.53 | 1.28 | 0.82 | 0.23 | 11.73 | 3.30 | 122.46 | 34.50 | 37.20 | 10.48 | 58.21 | 16.40 | 1.36 | 0.38 | 5.52 | 1.55 | NA | NA | NA | NA |
| Schlei | y | f | 2 | 55 | 323 | 11.2 | NA | NA | NA | 6.23 | 1.93 | 1.35 | 0.42 | 15.16 | 4.69 | 172.46 | 53.39 | 36.64 | 11.34 | 70.25 | 21.75 | 2.68 | 0.83 | 3.85 | 1.19 | NA | NA | NA | NA |
| Rhein | y | f | 2 | 56 | 281 | 19.6 | NA | NA | NA | 5.91 | 2.10 | 1.22 | 0.43 | 12.92 | 4.60 | 174.00 | 61.92 | 23.21 | 8.26 | 43.48 | 15.47 | 0.93 | 0.33 | 2.73 | 0.97 | NA | NA | NA | NA |
| Rhein | y | f | 2 | 51 | 206 | 21.2 | NA | NA | NA | 4.75 | 2.31 | 0.67 | 0.33 | 9.70 | 4.71 | 123.00 | 59.71 | 22.09 | 10.72 | 34.71 | 16.85 | 0.46 | 0.22 | 2.12 | 1.03 | NA | NA | NA | NA |
| Rhein | y | f | 2 | 52 | 206 | 18.3 | NA | NA | NA | 2.97 | 1.44 | 0.54 | 0.26 | 10.80 | 5.24 | 116.95 | 56.77 | 24.08 | 11.69 | 37.48 | 18.19 | 0.74 | 0.36 | 2.67 | 1.30 | NA | NA | NA | NA |
| Rhein | y | f | 2 | 55 | 276 | 29.8 | NA | NA | NA | 3.77 | 1.37 | 0.90 | 0.33 | 14.84 | 5.38 | 162.80 | 58.99 | 32.22 | 11.67 | 44.79 | 16.23 | 1.53 | 0.55 | 3.09 | 1.12 | NA | NA | NA | NA |
| Rhein | y | f | 2 | 53 | 272 | 30.2 | NA | NA | NA | 4.57 | 1.68 | 2.33 | 0.86 | 12.48 | 4.59 | 164.82 | 60.60 | 38.54 | 14.17 | 36.12 | 13.28 | 1.58 | 0.58 | 2.68 | 0.99 | NA | NA | NA | NA |
| Schlei/ Trave | y | f | 3 | 79 | 995 | 35.4 | NA | NA | NA | 11.93 | 1.20 | 13.61 | 1.37 | 30.95 | 3.11 | 668.23 | 67.16 | 110.57 | 11.11 | 121.43 | 12.20 | 2.58 | 0.26 | 8.42 | 0.85 | NA | NA | NA | NA |
| Elbe | y | f | 3 | 52 | 238 | 13.3 | NA | NA | NA | 2.68 | 1.13 | 0.89 | 0.37 | 15.99 | 6.72 | 127.18 | 53.44 | 26.86 | 11.29 | 47.09 | 19.79 | 2.53 | 1.06 | 2.86 | 1.20 | NA | NA | NA | NA |
| Weser | y | f | 3 | 59 | 354 | 21.3 | NA | NA | NA | 5.12 | 1.45 | 1.65 | 0.47 | 19.96 | 5.64 | 223.36 | 63.10 | 32.99 | 9.32 | 52.78 | 14.91 | 1.04 | 0.29 | 4.63 | 1.31 | NA | NA | NA | NA |
| Eider | y | f | 3 | 71 | 660 | 21.8 | NA | NA | NA | 8.15 | 1.23 | 3.10 | 0.47 | 25.05 | 3.80 | 403.63 | 61.16 | 76.32 | 11.56 | 106.66 | 16.16 | 3.04 | 0.46 | 9.35 | 1.42 | NA | NA | NA | NA |
| Eider | y | f | 3 | 63 | 442 | 15.3 | NA | NA | NA | 7.62 | 1.72 | 1.99 | 0.45 | 24.64 | 5.57 | 248.82 | 56.29 | 44.71 | 10.12 | 77.15 | 17.45 | 2.99 | 0.68 | 7.76 | 1.76 | NA | NA | NA | NA |
| Eider | y | f | 3 | 54 | 302 | 7 | NA | NA | NA | 3.97 | 1.31 | 0.72 | 0.24 | 18.73 | 6.20 | 167.84 | 55.58 | 37.04 | 12.26 | 47.98 | 15.89 | 1.57 | 0.52 | 5.16 | 1.71 | NA | NA | NA | NA |
| Eider | y | f | 3 | 54 | 276 | 10.1 | NA | NA | NA | 4.59 | 1.66 | 0.96 | 0.35 | 12.88 | 4.67 | 151.70 | 54.96 | 40.49 | 14.67 | 45.44 | 16.46 | 2.12 | 0.77 | 4.39 | 1.59 | NA | NA | NA | NA |
| Eider | y | f | 3 | 54 | 290 | 17.4 | NA | NA | NA | 4.34 | 1.50 | 0.80 | 0.28 | 13.97 | 4.82 | 163.55 | 56.40 | 36.80 | 12.69 | 47.79 | 16.48 | 1.72 | 0.59 | 2.72 | 0.94 | NA | NA | NA | NA |
| Eider | y | f | 3 | 56 | 303 | 9.3 | NA | NA | NA | 3.65 | 1.20 | 0.94 | 0.31 | 11.65 | 3.84 | 179.44 | 59.22 | 40.78 | 13.46 | 48.88 | 16.13 | 1.15 | 0.38 | 4.85 | 1.60 | NA | NA | NA | NA |
| Eider | y | f | 3 | 61 | 357 | 9.6 | NA | NA | NA | 5.37 | 1.50 | 1.00 | 0.28 | 16.64 | 4.66 | 183.65 | 51.44 | 46.63 | 13.06 | 82.70 | 23.17 | 3.30 | 0.92 | 7.22 | 2.02 | NA | NA | NA | NA |
| Eider | y | f | 3 | 60 | 374 | 18.2 | NA | NA | NA | 5.46 | 1.46 | 1.59 | 0.43 | 16.86 | 4.51 | 194.18 | 51.92 | 52.62 | 14.07 | 80.52 | 21.53 | 2.44 | 0.65 | 5.63 | 1.51 | NA | NA | NA | NA |
| Schlei/ Trave | y | f | 3 | 71 | 564 | 30 | NA | NA | NA | 7.63 | 1.35 | 6.28 | 1.11 | 22.28 | 3.95 | 310.54 | 55.06 | 73.09 | 12.96 | 113.52 | 20.13 | 1.39 | 0.25 | 6.18 | 1.10 | NA | NA | NA | NA |
| Schlei/ Trave | y | f | 3 | 84 | 1047 | 23.9 | NA | NA | NA | 12.80 | 1.22 | 14.51 | 1.39 | 22.33 | 2.13 | 590.01 | 56.35 | 141.73 | 13.54 | 217.14 | 20.74 | 3.41 | 0.33 | 10.95 | 1.05 | NA | NA | NA | NA |
| Rhein | y | f | 3 | 67 | 544 | 40 | NA | NA | NA | 6.76 | 1.24 | 3.65 | 0.67 | 19.98 | 3.67 | 333.08 | 61.23 | 47.84 | 8.79 | 87.15 | 16.02 | 1.51 | 0.28 | 5.85 | 1.08 | NA | NA | NA | NA |
| Rhein | y | f | 3 | 53 | 280 | 20.2 | NA | NA | NA | 4.42 | 1.58 | 1.16 | 0.41 | 11.01 | 3.93 | 184.20 | 65.79 | 21.87 | 7.81 | 44.84 | 16.01 | 1.29 | 0.46 | 2.82 | 1.01 | NA | NA | NA | NA |
| Eider | y | f | 3 | 63 | 544 | 22.5 | NA | NA | NA | 5.08 | 0.93 | 3.55 | 0.65 | 19.11 | 3.51 | 323.07 | 59.39 | 78.10 | 14.36 | 79.52 | 14.62 | 1.68 | 0.31 | 6.75 | 1.24 | NA | NA | NA | NA |
| Eider | y | f | 3 | 67 | 484 | 18 | NA | NA | NA | 5.90 | 1.22 | 3.12 | 0.64 | 21.10 | 4.36 | 293.55 | 60.65 | 49.87 | 10.30 | 86.78 | 17.93 | 1.66 | 0.34 | 8.39 | 1.73 | NA | NA | NA | NA |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Eider | y | f | 3 | 62 | 429 | 28.8 | NA | NA | NA | 4.43 | 1.03 | 3.01 | 0.70 | 16.96 | 3.95 | 266.63 | 62.15 | 54.53 | 12.71 | 62.32 | 14.53 | 1.76 | 0.41 | 5.42 | 1.26 | NA | NA | NA | NA |
| Eider | y | f | 3 | 61 | 450 | 24.1 | NA | NA | NA | 5.64 | 1.25 | 2.39 | 0.53 | 21.94 | 4.88 | 274.19 | 60.93 | 52.00 | 11.56 | 68.45 | 15.21 | 1.50 | 0.33 | 6.31 | 1.40 | NA | NA | NA | NA |
| Eider | y | f | 3 | 64 | 424 | 26.1 | NA | NA | NA | 6.14 | 1.45 | 2.16 | 0.51 | 20.17 | 4.76 | 256.11 | 60.40 | 51.95 | 12.25 | 64.84 | 15.29 | 1.57 | 0.37 | 4.80 | 1.13 | NA | NA | NA | NA |
| Eider | y | f | 3 | 62 | 376 | 19.2 | NA | NA | NA | 4.90 | 1.30 | 1.33 | 0.35 | 20.24 | 5.38 | 202.96 | 53.98 | 49.05 | 13.05 | 77.45 | 20.60 | 1.58 | 0.42 | 6.95 | 1.85 | NA | NA | NA | NA |
| Eider | y | f | 3 | 60 | 330 | 24.9 | NA | NA | NA | 4.98 | 1.51 | 1.23 | 0.37 | 15.64 | 4.74 | 180.08 | 54.57 | 44.78 | 13.57 | 67.26 | 20.38 | 1.68 | 0.51 | 6.10 | 1.85 | NA | NA | NA | NA |
| Eider | y | f | 3 | 57 | 351 | 22.3 | NA | NA | NA | 4.70 | 1.34 | 1.62 | 0.46 | 16.96 | 4.83 | 196.91 | 56.10 | 42.72 | 12.17 | 69.23 | 19.72 | 2.28 | 0.65 | 4.38 | 1.25 | NA | NA | NA | NA |
| Schlei/Trave | s | f | 4 | 91 | 1829 | 20.1 | NA | NA | NA | 20.68 | 1.13 | 25.81 | 1.41 | 48.78 | 2.67 | 1027.89 | 56.20 | 239.36 | 13.09 | 347.43 | 19.00 | 5.45 | 0.30 | 16.50 | 0.90 | NA | NA | NA | NA |
| Schlei/Trave | s | f | 4 | 97 | 2110 | 25.2 | NA | NA | NA | 19.86 | 0.94 | 27.80 | 1.32 | 61.18 | 2.90 | 1265.15 | 59.96 | 249.03 | 11.80 | 397.06 | 18.82 | 5.31 | 0.25 | 22.06 | 1.05 | NA | NA | NA | NA |
| Schlei/Trave | s | f | 4 | 91 | 1412 | 24.6 | NA | NA | NA | 14.49 | 1.03 | 16.68 | 1.18 | 45.96 | 3.25 | 797.11 | 56.45 | 178.38 | 12.63 | 282.62 | 20.02 | 3.84 | 0.27 | 14.25 | 1.01 | NA | NA | NA | NA |
| Schlei/Trave | s | f | 4 | 80 | 1283 | 18.5 | NA | NA | NA | 18.23 | 1.42 | 12.13 | 0.95 | 39.94 | 3.11 | 614.18 | 47.87 | 179.49 | 13.99 | 316.20 | 24.65 | 20.06 | 1.56 | 16.23 | 1.27 | NA | NA | NA | NA |
| Schlei/Trave | s | f | 4 | 90 | 1318 | 25.3 | NA | NA | NA | 15.02 | 1.14 | 16.56 | 1.26 | 43.37 | 3.29 | 738.89 | 56.06 | 177.96 | 13.50 | 258.80 | 19.64 | 4.11 | 0.31 | 11.46 | 0.87 | NA | NA | NA | NA |
| Schlei/Trave | s | f | 4 | 80 | 1153 | 24.5 | NA | NA | NA | 15.77 | 1.37 | 15.81 | 1.37 | 35.98 | 3.12 | 646.87 | 56.10 | 146.62 | 12.72 | 237.13 | 20.57 | 3.39 | 0.29 | 13.67 | 1.19 | NA | NA | NA | NA |
| Schlei/Trave | s | f | 4 | 91 | 1625 | 26.9 | NA | NA | NA | 19.82 | 1.22 | 15.13 | 0.93 | 52.94 | 3.26 | 706.67 | 43.49 | 208.03 | 12.80 | 253.61 | 15.61 | 5.65 | 0.35 | 14.69 | 0.90 | NA | NA | NA | NA |
| Schlei/Trave | s | f | 4 | 86 | 1283 | 20.9 | NA | NA | NA | 12.91 | 1.01 | 16.54 | 1.29 | 35.43 | 2.76 | 715.71 | 55.78 | 189.25 | 14.75 | 245.41 | 19.13 | 3.86 | 0.30 | 12.61 | 0.98 | NA | NA | NA | NA |
| Schlei/Trave | s | f | 5 | 66 | 620 | 24.7 | NA | NA | NA | 6.97 | 1.12 | 8.72 | 1.41 | 26.69 | 4.30 | 367.78 | 59.32 | 80.91 | 13.05 | 98.65 | 15.91 | 2.92 | 0.47 | 7.09 | 1.14 | NA | NA | NA | NA |
| Eider | s | f | 5 | 69 | 611 | 22.5 | NA | NA | NA | 7.96 | 1.30 | 8.07 | 1.32 | 21.29 | 3.48 | 350.80 | 57.41 | 87.56 | 14.33 | 95.78 | 15.68 | 3.07 | 0.50 | 8.28 | 1.36 | NA | NA | NA | NA |
| Eider | s | f | 5 | 70 | 681 | 24.7 | NA | NA | NA | 9.04 | 1.33 | 8.52 | 1.25 | 20.47 | 3.01 | 411.36 | 60.41 | 102.97 | 15.12 | 92.79 | 13.63 | 3.89 | 0.57 | 7.47 | 1.10 | NA | NA | NA | NA |
| Eider | s | f | 5 | 62 | 528 | 27.9 | NA | NA | NA | 7.19 | 1.36 | 4.07 | 0.77 | 26.02 | 4.93 | 300.07 | 56.83 | 62.78 | 11.89 | 94.47 | 17.89 | 4.47 | 0.85 | 5.91 | 1.12 | NA | NA | NA | NA |
| Eider | s | f | 5 | 62 | 602 | 21.9 | NA | NA | NA | 6.42 | 1.07 | 7.57 | 1.26 | 17.57 | 2.92 | 375.28 | 62.34 | 88.35 | 14.68 | 71.52 | 11.88 | 3.11 | 0.52 | 5.62 | 0.93 | NA | NA | NA | NA |
| Eider | s | f | 5 | 67 | 579 | 28.7 | NA | NA | NA | 5.13 | 0.89 | 6.52 | 1.13 | 19.89 | 3.44 | 360.54 | 62.27 | 75.03 | 12.96 | 78.58 | 13.57 | 3.21 | 0.55 | 7.88 | 1.36 | NA | NA | NA | NA |
| Eider | s | f | 5 | 62 | 405 | 6 | NA | NA | NA | 8.20 | 2.02 | 0.94 | 0.23 | 21.03 | 5.19 | 190.43 | 47.02 | 47.57 | 11.75 | 107.10 | 26.44 | 1.63 | 0.40 | 9.56 | 2.36 | NA | NA | NA | NA |
| Weser | s | f | 5 | 70 | 683 | 19.8 | NA | NA | NA | 9.73 | 1.42 | 10.17 | 1.49 | 31.42 | 4.60 | 326.87 | 47.86 | 117.24 | 17.17 | 8.09 | 1.18 | 2.67 | 0.39 | 152.49 | 22.33 | NA | NA | NA | NA |
| Weser | s | f | 5 | 63 | 519 | 18.7 | NA | NA | NA | 8.12 | 1.56 | 9.56 | 1.84 | 22.35 | 4.31 | 252.25 | 48.60 | 102.92 | 19.83 | 96.47 | 18.59 | 1.90 | 0.37 | 4.77 | 0.92 | NA | NA | NA | NA |
| Schlei/Trave | s | f | 5 | 72 | 736 | 23.7 | NA | NA | NA | 11.83 | 1.61 | 8.03 | 1.09 | 23.80 | 3.23 | 366.53 | 49.80 | 97.07 | 13.19 | 194.97 | 26.49 | 3.69 | 0.50 | 6.11 | 0.83 | NA | NA | NA | NA |
| Schlei/Trave | s | f | 5 | 84 | 988 | 22.1 | NA | NA | NA | 10.84 | 1.10 | 13.99 | 1.42 | 32.05 | 3.24 | 540.07 | 54.66 | 147.61 | 14.94 | 195.55 | 19.79 | 5.07 | 0.51 | 12.47 | 1.26 | NA | NA | NA | NA |
| Schlei/Trave | s | f | 5 | 74 | 709 | 25 | NA | NA | NA | 7.44 | 1.05 | 6.87 | 0.97 | 27.09 | 3.82 | 401.27 | 56.60 | 100.49 | 14.17 | 128.73 | 18.16 | 2.71 | 0.38 | 8.44 | 1.19 | NA | NA | NA | NA |
| Schlei/Trave | s | f | 5 | 78 | 807 | 27.1 | NA | NA | NA | 8.96 | 1.11 | 8.75 | 1.08 | 22.10 | 2.74 | 464.95 | 57.61 | 116.61 | 14.45 | 147.42 | 18.27 | 2.80 | 0.35 | 10.10 | 1.25 | NA | NA | NA | NA |
| Schlei/Trave | s | f | 5 | 64 | 459 | 25.6 | NA | NA | NA | 6.55 | 1.43 | 7.07 | 1.54 | 16.53 | 3.60 | 234.38 | 51.06 | 80.69 | 17.58 | 82.45 | 17.96 | 4.00 | 0.87 | 10.38 | 2.26 | NA | NA | NA | NA |
| Eider | s | f | 5 | 63 | 484 | 11.1 | NA | NA | NA | 7.05 | 1.46 | 2.19 | 0.45 | 20.34 | 4.20 | 263.19 | 54.38 | 57.18 | 11.81 | 103.64 | 21.41 | 2.14 | 0.44 | 8.93 | 1.85 | NA | NA | NA | NA |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Eider | s | f | 5 | 64 | 427 | 17.4 | NA | NA | NA | 4.90 | 1.15 | 2.06 | 0.48 | 20.32 | 4.76 | 245.93 | 57.59 | 47.48 | 11.12 | 78.23 | 18.32 | 1.73 | 0.41 | 7.91 | 1.85 | NA | NA | NA | NA |
| Eider | s | f | 5 | 60 | 521 | 24.5 | NA | NA | NA | 7.32 | 1.40 | 5.92 | 1.14 | 22.86 | 4.39 | 315.51 | 60.56 | 71.24 | 13.67 | 81.64 | 15.67 | 1.60 | 0.31 | 6.74 | 1.29 | NA | NA | NA | NA |
| Weser | s | m | 6 | 43 | 143 | 23.2 | NA | 1.01 | NA | 1.99 | 1.39 | 0.01 | 0.01 | 4.06 | 2.84 | 75.78 | 52.99 | 27.27 | 19.07 | 19.36 | 13.54 | 0.66 | 0.46 | 1.67 | 1.17 | NA | NA | NA | NA |
| Weser | s | m | 6 | 39 | 120 | 23.8 | NA | 1.09 | NA | 1.72 | 1.43 | 0.07 | 0.06 | 3.25 | 2.71 | 66.51 | 55.43 | 19.55 | 16.29 | 14.92 | 12.43 | 0.53 | 0.44 | 1.37 | 1.14 | NA | NA | NA | NA |
| Weser | s | m | 6 | 40 | 117 | 20.9 | NA | 1.39 | NA | 1.60 | 1.37 | 0.09 | 0.08 | 4.54 | 3.88 | 61.82 | 52.84 | 15.66 | 13.38 | 16.66 | 14.24 | 0.22 | 0.19 | 1.23 | 1.05 | NA | NA | NA | NA |
| Weser | s | m | 6 | 38 | 106 | 20.4 | NA | 1.18 | NA | 2.06 | 1.94 | 0.08 | 0.08 | 2.66 | 2.51 | 45.98 | 43.38 | 11.99 | 11.31 | 13.44 | 12.68 | 1.23 | 1.16 | 1.21 | 1.14 | NA | NA | NA | NA |
| Weser | s | m | 6 | 40 | 130 | 25.3 | NA | 1.83 | NA | 3.20 | 2.46 | 0.21 | 0.16 | 4.18 | 3.22 | 74.87 | 57.59 | 16.89 | 12.99 | 15.29 | 11.76 | 1.07 | 0.82 | 1.30 | 1.00 | NA | NA | NA | NA |
| Weser | s | m | 6 | 39 | 92 | 24.6 | NA | 0.55 | NA | 1.41 | 1.53 | 0.03 | 0.03 | 2.88 | 3.13 | 49.89 | 54.23 | 11.05 | 12.01 | 14.74 | 16.02 | 0.29 | 0.32 | 0.86 | 0.93 | NA | NA | NA | NA |
| Weser | s | m | 6 | 43 | 120 | 25.5 | NA | 0.94 | NA | 1.83 | 1.53 | 0.09 | 0.08 | 3.03 | 2.53 | 67.66 | 56.38 | 16.55 | 13.79 | 15.43 | 12.86 | 0.58 | 0.48 | 1.09 | 0.91 | NA | NA | NA | NA |
| Weser | s | m | 6 | 36 | 81 | 25.4 | NA | 0.81 | NA | 1.33 | 1.64 | 0.01 | 0.01 | 3.22 | 3.98 | 46.66 | 57.60 | 9.63 | 11.89 | 10.56 | 13.04 | 0.31 | 0.38 | 0.62 | 0.77 | NA | NA | NA | NA |
| Weser | s | m | 6 | 43 | 129 | 21.5 | NA | 0.81 | NA | 1.95 | 1.51 | 0.11 | 0.09 | 2.97 | 2.30 | 72.04 | 55.84 | 18.08 | 14.02 | 16.49 | 12.78 | 0.42 | 0.33 | 1.26 | 0.98 | NA | NA | NA | NA |
| Weser | s | m | 6 | 36 | 90 | 27.9 | NA | 0.57 | NA | 1.72 | 1.91 | 0.01 | 0.01 | 3.92 | 4.36 | 45.99 | 51.10 | 11.64 | 12.93 | 14.63 | 16.26 | 0.31 | 0.34 | 0.69 | 0.77 | NA | NA | NA | NA |
| Ems | **mature** | f | M | 81 | 1385 | NA | 34.39 | NA | NA | 18.38 | 1.33 | 743 | 53.65 | 13.20 | 0.95 | 249.66 | 18.03 | 131.20 | 9.47 | 82.50 | 5.96 | 4.20 | 0.30 | 11.24 | 0.81 | NA | NA | NA | NA |
| Ems | **mature** | f | M | 65 | 584 | NA | 22.32 | NA | NA | 6.39 | 1.09 | 282 | 48.34 | 7.60 | 1.30 | 123.67 | 21.18 | 69.34 | 11.87 | 35.34 | 6.05 | 2.63 | 0.45 | 5.43 | 0.93 | NA | NA | NA | NA |
| Ems | **mature** | f | M | 79 | 1073 | NA | 21.85 | NA | NA | 16.61 | 1.55 | 457 | 42.61 | 11.91 | 1.11 | 263.56 | 24.56 | 107.35 | 10.00 | 55.63 | 5.18 | 3.40 | 0.32 | 9.15 | 0.85 | NA | NA | NA | NA |
| Schlei | **mature** | f | M | 80 | 1177 | NA | 35.03 | NA | NA | 14.28 | 1.21 | 659 | 55.99 | 10.38 | 0.88 | 176.53 | 15.00 | 112.22 | 9.53 | 69.24 | 5.88 | 4.20 | 0.36 | 9.43 | 0.80 | NA | NA | NA | NA |
| Schlei | **mature** | f | M | 63 | 567 | NA | 25.00 | NA | NA | 6.38 | 1.13 | 325 | 57.32 | 5.40 | 0.95 | 93.88 | 16.56 | 48.25 | 8.51 | 23.63 | 4.17 | 2.03 | 0.36 | 7.82 | 1.38 | NA | NA | NA | NA |
| Arresö | **mature** | f | M | 76 | 808 | NA | NA | NA | NA | 7.82 | 0.97 | 397.3 | 49.17 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Elbe | **mature** | f | M | 70 | 609 | NA | NA | NA | NA | 6.65 | 1.09 | 302.1 | 49.61 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Arresö | **mature** | f | M | 81 | 992 | NA | NA | NA | NA | NA | NA | 342.0 | 34.48 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Arresö | **mature** | f | M | 81 | 934 | NA | NA | NA | NA | 11.23 | 1.20 | 473.9 | 50.74 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Arresö | **mature** | f | M | 78 | 912 | NA | NA | NA | NA | 14.22 | 1.56 | 432.3 | 47.40 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Elbe | **mature** | f | M | 64 | 601 | NA | NA | NA | NA | 7.86 | 1.31 | 238.0 | 39.60 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Elbe | **mature** | f | M | 79 | 1049 | NA | NA | NA | NA | 13.77 | 1.31 | 569.2 | 54.26 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Arresö | **mature** | f | M | 73 | 730 | NA | NA | NA | NA | 13.30 | 1.82 | 366.0 | 50.14 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Arresö | **mature** | f | M | 75 | 1013 | NA | NA | NA | NA | 18.51 | 1.83 | 403.0 | 39.78 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Weser | **mature** | f | M | 82 | 966 | NA | NA | NA | NA | 13.50 | 1.40 | 428.2 | 44.33 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Weser | **mature** | f | M | 69 | 628 | NA | NA | NA | NA | 9.60 | 1.53 | 230.1 | 36.64 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Ploen lake | **mature** | f | M | 91 | 1484 | NA | NA | NA | NA | NA | NA | 393 | 26.48 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Weser | **mature** | f | M | 71 | 654 | NA | NA | NA | NA | 11.30 | 1.73 | 309.8 | 47.37 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Weser | **mature** | f | M | 76 | 791 | NA | NA | NA | NA | NA | NA | 305.3 | 38.60 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Ploen lake | **mature** | f | M | 94 | 1941 | NA | NA | NA | NA | 23.60 | 1.22 | 871.2 | 44.89 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Ploen lake | **mature** | f | M | 84 | 1153 | NA | NA | NA | NA | 20.70 | 1.80 | 322.5 | 27.97 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Weser | **mature** | f | M | 72 | 777 | NA | NA | NA | NA | 12.70 | 1.63 | 368.2 | 47.39 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Ploen lake | **mature** | f | M | 85 | 1104 | NA | NA | NA | NA | 13.29 | 1.20 | 307.8 | 27.88 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Weser | **mature** | f | M | 77 | 967 | NA | NA | NA | NA | 14.21 | 1.47 | 471.4 | 48.75 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Weser | **mature** | f | M | 67 | 633 | NA | NA | NA | NA | 10.00 | 1.58 | 269.7 | 42.61 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Weser | **mature** | f | M | 75 | 891 | NA | NA | NA | NA | 13.20 | 1.48 | 298.5 | 33.50 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Weser | **mature** | f | M | 66 | 786 | NA | NA | NA | NA | 9.01 | 1.15 | 321.4 | 40.89 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| Ploen lake | **mature** | f | M | 78 | 1159 | NA | NA | NA | NA | 16.10 | 1.39 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |

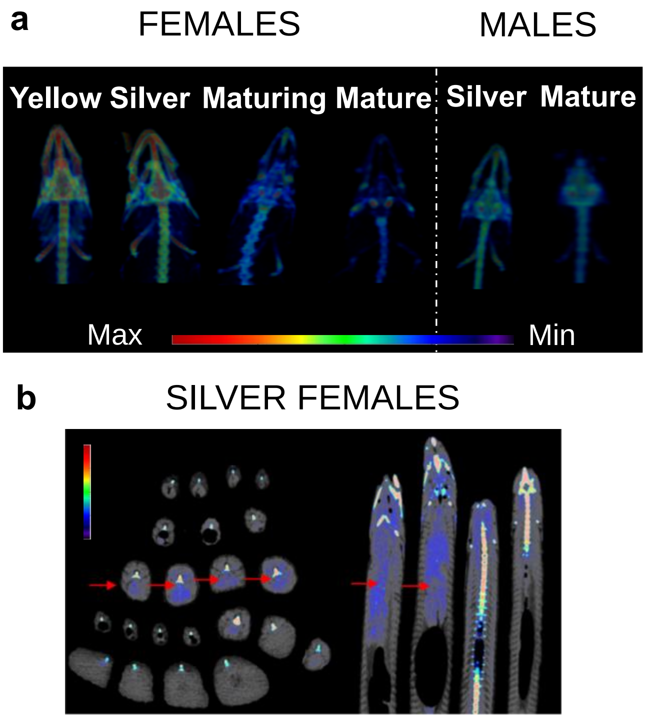


**Figure S1: Bone loss in skeletal elements from eels in different maturation stages and mineral accumulation in female silver eels.**
(a) Clinical tomography calcium maps pf skull and skeletal elements of eels showed declining bone density along progressing maturation. (b) Note accumulated mineral signals around gonadal tissue in silver females at onset of maturation. Images obtained by clinical computed tomography.

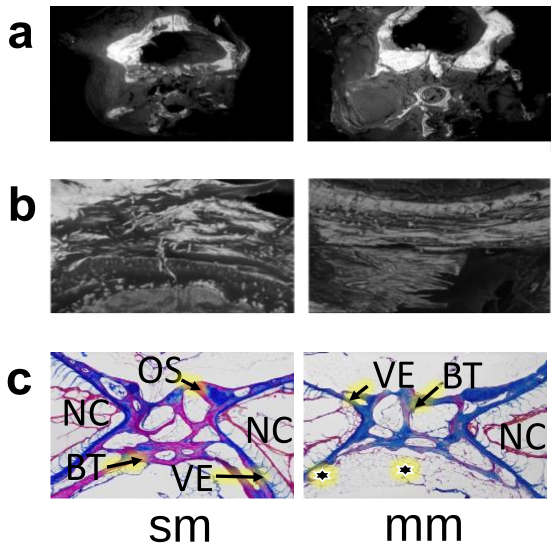


**Figure S2: Structural aspects of bone loss during sexual maturation in male eels.**
Superior view (a) and (500x) magnified (b) SEM images of entire vertebral body endplates of male eels in different maturation stages depict the successive bone loss on a supracellular level. (c) Bone histology is based on azan-dyed, para-sagittal sections of vertebral bodies and illustrates changes of bone structures along the maturation process on a cellular level. Defined structures are marked and labeled by abbreviations: (NC = Notochord, BT = Bone Trabeculae, VE = Vertebral Body Endplate, OS = Osteon, * = indication of bone resorption). (sm= silver male; mm= mature male).

**Supplementary References**


1. Anonymous (2013) Tierschutz-Versuchstierverordnung vom 1. August 2013 (BGBI. I S. 3125, 3126)

2. Brinkmann M, Rizzo L, Lammers T, Gremse F, Schiwy S, Kiessling F, Hollert H (2016) Micro-computed tomography (µCT) as a novel method in ecotoxicology - determination of morphometric and somatic data in rainbow trout (*Oncorhynchus mykiss*) Science of The Total Environment 543(Pt A):135-139. DOI:10.1016/j.scitotenv.2015.11.020

3. Gremse F, Stark M, Ehling J, Menzel JR, Lammers T, Kiessling F. (2016) Imalytics Preclinical: Interactive Analysis of Biomedical Volume Data. Theranostics; 6(3):328–341. pmid:26909109

4. Gremse F, Doleschel D, Zafarnia S, Babler A, Jahnen-Dechent W, Lammers T, et al. (2015) Hybrid microCT-FMT imaging and image analysis. J Vis Exp;(100).

5. Yang CC, Nagarajan MB, Huber MB, Carballido-Gamio J, Bauer JS, Baum T et al (2014) Improving bone strength prediction in human proximal femur specimens through geometrical characterization of trabecular bone microarchitecture and support vector regression. J. Electron. Imaging. 2014;23: 013013.

6. Presnell JK, Schreibman MP (1998) Humason's Animal Tissue Techniques. Fifth edition, the John Hopkins University Press, Baltimore. 572 pages