Tracing the first steps of American sturgeon pioneers in Europe

Arne Ludwig*1, Ursula Arndt1,2,3, Sebastian Lippold1, Norbert Benecke4, Lutz Debus5, Timothy L King6 and Shuichi Matsumura7,8

Background:
A Baltic population of Atlantic sturgeon was founded ~1,200 years ago by migrants from North America, but after centuries of persistence, the population was extirpated in the 1960s, mainly as a result of over-harvest and habitat alterations. As there are four genetically distinct groups of Atlantic sturgeon inhabiting North American rivers today, we investigated the genetic provenance of the historic Baltic population by ancient DNA analyses using mitochondrial and nuclear markers.

Results:
The phylogeographic signal obtained from multilocus microsatellite DNA genotypes and mitochondrial DNA control region haplotypes, when compared to existing baseline datasets from extant populations, allowed for the identification of the region-of-origin of the North American Atlantic sturgeon founders. Moreover, statistical and simulation analyses of the multilocus genotypes allowed for the calculation of the effective number of individuals that originally founded the European population of Atlantic sturgeon. Our findings suggest that the Baltic population of *A. oxyrinchus* descended from a relatively small number of founders originating from the northern extent of the species’ range in North America.

Conclusion:
These results demonstrate that the most northerly distributed North American *A. oxyrinchus* colonized the Baltic Sea ~1,200 years ago, suggesting that Canadian specimens should be the primary source of broodstock used for restoration in Baltic rivers. This study illustrates the great potential of patterns obtained from ancient DNA to identify population-of-origin to investigate historic genotype structure of extinct populations.

Background
Sturgeons (Acipenseriformes: Acipenseridae), the producers of caviar, are remnant survivors of the once flourishing chondrosteans, dominant fishes of the Permian period. The continued persistence of these 'living fossils' is threatened throughout North America, Europe, and Asia. Today there are two species of Atlantic sea sturgeons; the European sturgeon *Acipenser sturio*, found in France (Gironde basin), and the Atlantic sturgeon *A. oxyrinchus* inhabiting the rivers and coastal waters from the Gulf of Mexico to...
the Canadian Maritime Provinces. Although classified as sister species and showing some phenotypic similarities, approximately 60 million years of isolation [1] has resulted in physiological differences between these two species. For example, European sturgeons prefer spawning temperatures ≥ 20°C, while Atlantic sturgeons exhibit latitudinal variation in spawning temperatures ranging from as low as 13°C in Canada to 26°C in the southeastern U.S. [2].

According to archaeological and molecular dating, a population of Atlantic sturgeon was founded in the Baltic Sea during the Middle Ages (8th and 10th century) by migrants from North America [3]. These founders created a self-sustaining population, which became disjunct from the western Atlantic populations. This Baltic population has been over-exploited by commercial fisheries and was extirpated in the 20th century. A group of international fishery managers are now seeking to re-establish the extirpated population using fish from the original source population(s), on the grounds that North American A. oxyrinchus exhibit sufficient ecological and genetic potential for a successful restoration. To increase the probability of success of such a restoration in the long-term, the ideal scenario would be to identify and use a founder group that is genetically closely related to the extinct population. Although the utility of ancient DNA studies to elucidate evolutionary relationships and guide restoration projects has been recognized [4-7], the full extent of management applications from these studies have not yet been realized.

In this study, we investigated the evolutionary and demographic characteristics of the historic founders, by performing an extensive genetic characterization of the extinct Baltic population derived from medieval tissue samples representing their first generations starting at the 8th century. We focused on identifying the region-of-origin of the North American founders, and on calculating the effective number of individuals that originally founded the Baltic population ~1,200 years ago.

Results

Mitochondrial DNA (mtDNA)

Two hundred and twenty seven DNA samples from 586 ancient bony scutes (8th – 13th c.) were successfully screened for their mtDNA control region haplotypes. The species A. sturio and A. oxyrinchus were differentiated by 22 diagnostic substitutions (> 10% sequence divergence) [see Additional file 1]. Two hundred and twenty scutes had A. oxyrinchus control region haplotypes (218 haplotype A, and one haplotype BS1 [EU684143] and BS2 [EU684144] each, respectively). Seven scutes shared haplotype AS17 from A. sturio.

Morphological classification

The morphology of 210 bony scutes was preserved sufficiently to identify species. Of this number, 176 were classified as A. oxyrinchus; whereas 34 showed typical A. sturio surfaces. Morphological classifications were subject to error depending on the state of scute preservation. However, 183 (87%) samples were classified as the same species based on morphology and mitochondrial DNA. Four scutes yielding A. sturio haplotypes showed A. oxyrinchus morphology; in contrast 23 scutes had A. oxyrinchus mtDNA and A. sturio morphology.

Amplification of nuclear DNA

Allelic profiles of 29 (out of 50) randomly selected scutes from Ralswiek, Island of Rugia Germany were successfully amplified. The 29 randomly selected scutes yielded unique multilocus genotypes. Locus Afu-39 was monomorphic in two populations (Table 1). Profiles of seven polymorphic microsatellite loci were used for the assignment analysis: Afu-19 (trinucleotide), Afu-39 (trinucleotide), Afu-68 (tetranucleotide), Afu-54 (tetranucleotide), Aox-45 (trinucleotide), Aox-23 (trinucleotide) and Aox-12 (imperfect nucleotide). All loci used in this study showed allelic patterns of disomic inheritance. The detected structure (four clusters) of A. oxyrinchus populations was related to their geographic distribution. Baltic and Canadian sturgeons grouped together (Figure 1A). STRUCTURE results showed a high allele-frequency similarity of Baltic samples with Canadian samples (28 samples were assigned to the Canadian population). A single sample was assigned to the Mid-Atlantic population. Probability values for region-of-origin assignment are given in Table 2. FST estimates (10100 permutations) (Table 3) and AMOVA values (Table 4) were calculated using Arlequin v. 3.0 [8] based on haplotype frequencies of mtDNA control region sequences.

Identification of hybrids

Flanking sequences of locus Aox-23 were successfully amplified for 47 (of 50) scutes as previously described [3]. Three hybrids (fish with nuclear sequences from both species) and four introgressed specimens (mtDNA = A. sturio and nDNA = A. oxyrinchus) were identified. Additional assignment tests calculated in STRUCTURE including 100 artificial hybrids generated between fishes from source populations (Canadian, Mid-Atlantic) and European sturgeons (A. sturio) designed in HYBRIDLAB 1.0 clustered Baltic sturgeon together with Atlantic sturgeon, and produced no evidence for a historic hybrid population (Figure 2).

Inference of the founder population size

Using ancient and contemporary DNA data for eight genetic loci (7 autosomal microsatellites and mtDNA), the size of the founding population to the Baltic Sea was
Table 1: Heterozygosity.

| Locus     | n   | H_o | H_e | p    | s.d. | Steps done |
|-----------|-----|-----|-----|------|------|------------|
| **Canadian population**                          |
| Afu19     | 39  | 0.31| 0.33| 1.00 | 0    | 10100      |
| Afu39     | 39  | 0.26| 0.28| 1.00 | 0    | 10100      |
| Afu54     | 39  | 0.49| 0.49| 0.34 | > 0  | 10100      |
| Aox23     | 39  | 0.36| 0.58| 0.28 | > 0  | 10100      |
| Aox45     | 39  | 0.72| 0.77| 0.83 | > 0  | 10100      |
| Aox12     | 39  | 0.77| 0.83| 0.01 | > 0  | 10100      |
| **Mid-Atlantic population**                      |
| Afu19     | 54  | 0.74| 0.65| 0.01 | > 0  | 10100      |
| Afu39     | 54  | 0.04| 0.05| 1    | 0    | 10100      |
| Afu54     | 54  | 0.35| 0.31| 0.68 | > 0  | 10100      |
| Aox23     | 54  | 0.72| 0.75| 0.63 | > 0  | 10100      |
| Aox45     | 54  | 0.76| 0.59| 0.61 | > 0  | 10100      |
| Aox12     | 53  | 0.85| 0.84| 0    | > 0  | 10100      |
| **Southeast population**                         |
| Afu19     | 37  | 0.67| 0.65| 0.59 | > 0  | 10100      |
| Afu39     | 37  | 0.38| 0.41| 0.31 | > 0  | 10100      |
| Afu54     | 37  | 0.27| 0.24| 1    | 0    | 10100      |
| Aox23     | 37  | 0.87| 0.82| 0.02 | > 0  | 10100      |
| Aox45     | 37  | 0.84| 0.84| 0.07 | > 0  | 10100      |
| **Gulf population**                              |
| Afu19     | 48  | 0.58| 0.59| 0.59 | > 0  | 10100      |
| Afu39     | 48  | 0.35| 0.31| 0.32 | > 0  | 10100      |
| Afu54     | 48  | 0.54| 0.51| 0.56 | > 0  | 10100      |
| Aox23     | 48  | 0.23| 0.23| 0.35 | > 0  | 10100      |
| Aox45     | 48  | 0.73| 0.67| 0.68 | > 0  | 10100      |
| Aox12     | 48  | 0.73| 0.72| 0    | > 0  | 10100      |
| **Baltic population (aDNA)**                     |
| Afu19     | 24  | 0.56| 0.67| 0.29 | > 0  | 10100      |
| Afu39     | 18  | 0.29| 0.42| 0.13 | > 0  | 10100      |
| Afu54     | 29  | 0.54| 0.73| 0.25 | > 0  | 10100      |
| Aox23     | 26  | 0.52| 0.67| 0    | > 0  | 10100      |
| Aox45     | 29  | 0.61| 0.76| 0.07 | > 0  | 10100      |
| Aox12     | 30  | 0.53| 0.65| 0.07 | > 0  | 10100      |

Heterozygosity based on microsatellites calculated in Arlequin v. 3.0 [8,40].

Inferred using the Approximate Bayesian Computation (ABC) method. When the posterior densities obtained for the 8 genetic loci are combined, the effective founding population size is likely to be less than 10 (Table 5, the baseline case). To evaluate the sensitivity of the results to the assumptions and methods used, different population histories, parameter values and estimation methods were tested. These included a more limited source population (Canadian only), the larger/smaller sizes of the modern North American/Baltic populations, different time points for colonization, and different rejecting/weighing procedures. Although the 95% HPD (Highest Probability Density) intervals varied, the estimated total population sizes were less than 20 individuals in most cases. A strong bottleneck signal was exhibited by both mtDNA data and also a few microsatellite loci. The assumption about the source population had a strong impact on the results. The 95% HPD interval became bigger when the Canadian population was assumed to be the only source, because the resolving power of the statistical analysis declined due to their low genetic diversity.

**Discussion**

Restoration projects are often faced with the problem that little information is available when choosing a founder group for restorative breeding, especially when native populations became extinct many decades ago. One powerful way of obtaining more information is to analyze the genetic structure of historic populations and their relationships to extant populations [7]. Recent progress in ancient DNA analysis enables investigations of historic population structures [5,6]. This information can be used to select specimens for introduction from appropriate regional groups, taking under consideration that individuals from different environments may exhibit evolutionarily important differences in adaptively significant traits.

Congruent patterns of population structuring among collections of extant *A. oxyrinchus* have been identified in both mitochondrial [9,10] and microsatellite DNA [11] which consisted of four regional clusters in the western Atlantic: 1) Gulf (A. *o. desotoi* in tributaries of the Gulf of Mexico), 2) southeastern (rivers in Georgia and South Carolina), 3) Mid-Atlantic (Hudson and Delaware rivers), and 4) Canadian (Kennebec, St. Lawrence and St. John) (Figure 1B). In the present analysis of the microsatellite profiles of the ancient Baltic population, 28 out of 29 (97%) individuals were assigned to the Canadian regional grouping and one fish was assigned to the Mid-Atlantic grouping (Figure 1A) as identified in previous studies. An overwhelming predominance of Canadian *A. oxyrinchus* genotypes within the ancient Baltic population was similarly observed in the mtDNA sequence data set (Figure 1C); 218 of 227 (96%) bony scutes shared haplotype A while the two remaining specimens had haplotypes BS1 and BS2, which are likely recent derivatives from haplotype A (Figure 3). However, it is difficult to decide when and where these "new" haplotypes evolved; prior to colonization in North America, or after the founding event in
Figure 1

Genetic variation and assignment test. A) Assignment test conducted in STRUCTURE based on seven polymorphic microsatellites showing Atlantic sturgeon genotype structuring and the assignment of Baltic individuals; B) Pie charts are the frequencies of the assignment to each sub-population calculated in STRUCTURE. Colors are identical with the population subdivision observed in the assignment test A; C) Histograms illustrates mitochondrial haplotype frequencies from each locality. Baltic sturgeon data were taken from this study (n = 227 ancient DNA samples) and 10 archived specimens previously published [3]. Atlantic sturgeon data from 3, 9 and Gulf sturgeon A. oxyrinchus desotoi were published by 10.
Table 2: Probability of assignment values.

| Sample         | Cluster 1 | Cluster 2 | Cluster 3 | Cluster 4 |
|----------------|-----------|-----------|-----------|-----------|
| Canadian_01    | 0.960     | 0.021     | 0.006     | 0.013     |
| Canadian_02    | 0.803     | 0.024     | 0.135     | 0.038     |
| Canadian_03    | 0.963     | 0.011     | 0.009     | 0.017     |
| Canadian_04    | 0.970     | 0.017     | 0.007     | 0.005     |
| Canadian_05    | 0.913     | 0.013     | 0.010     | 0.064     |
| Canadian_06    | 0.982     | 0.007     | 0.007     | 0.004     |
| Canadian_07    | 0.951     | 0.018     | 0.026     | 0.006     |
| Canadian_08    | 0.575     | 0.113     | 0.307     | 0.005     |
| Canadian_09    | 0.982     | 0.005     | 0.006     | 0.006     |
| Canadian_10    | 0.978     | 0.013     | 0.006     | 0.004     |
| Canadian_11    | 0.947     | 0.033     | 0.015     | 0.004     |
| Canadian_12    | 0.980     | 0.008     | 0.007     | 0.005     |
| Canadian_13    | 0.954     | 0.010     | 0.029     | 0.007     |
| Canadian_14    | 0.930     | 0.026     | 0.034     | 0.010     |
| Canadian_15    | 0.928     | 0.043     | 0.02      | 0.009     |
| Canadian_16    | 0.981     | 0.006     | 0.007     | 0.006     |
| Canadian_17    | 0.975     | 0.011     | 0.005     | 0.009     |
| Canadian_18    | 0.918     | 0.020     | 0.056     | 0.007     |
| Canadian_19    | 0.960     | 0.017     | 0.006     | 0.017     |
| Canadian_20    | 0.636     | 0.017     | 0.339     | 0.008     |
| Canadian_41    | 0.964     | 0.015     | 0.015     | 0.006     |
| Canadian_42    | 0.966     | 0.014     | 0.005     | 0.014     |
| Canadian_43    | 0.757     | 0.078     | 0.112     | 0.053     |
| Canadian_44    | 0.202     | 0.039     | 0.742     | 0.017     |
| Canadian_45    | 0.599     | 0.371     | 0.024     | 0.006     |
| Canadian_46    | 0.962     | 0.018     | 0.012     | 0.009     |
| Canadian_47    | 0.844     | 0.145     | 0.007     | 0.004     |
| Canadian_48    | 0.613     | 0.047     | 0.304     | 0.036     |
| Canadian_49    | 0.973     | 0.008     | 0.011     | 0.007     |
| Canadian_50    | 0.937     | 0.025     | 0.014     | 0.024     |
| Canadian_51    | 0.898     | 0.071     | 0.022     | 0.008     |
| Canadian_52    | 0.951     | 0.012     | 0.010     | 0.028     |
| Canadian_53    | 0.896     | 0.065     | 0.025     | 0.014     |
| Canadian_54    | 0.892     | 0.033     | 0.068     | 0.007     |
| Canadian_55    | 0.977     | 0.011     | 0.007     | 0.006     |
| Canadian_56    | 0.976     | 0.012     | 0.007     | 0.006     |
| Canadian_57    | 0.970     | 0.012     | 0.012     | 0.005     |
| Canadian_58    | 0.974     | 0.009     | 0.006     | 0.011     |
| Canadian_59    | 0.980     | 0.006     | 0.008     | 0.006     |
| Mid-American_01| 0.011     | 0.973     | 0.008     | 0.008     |
| Mid-American_02| 0.015     | 0.971     | 0.008     | 0.006     |
| Mid-American_03| 0.023     | 0.951     | 0.020     | 0.005     |
| Mid-American_04| 0.006     | 0.972     | 0.017     | 0.004     |
| Mid-American_05| 0.006     | 0.932     | 0.029     | 0.033     |
| Mid-American_06| 0.198     | 0.775     | 0.010     | 0.018     |
| Mid-American_07| 0.006     | 0.966     | 0.008     | 0.020     |
| Mid-American_08| 0.007     | 0.984     | 0.005     | 0.004     |
| Mid-American_09| 0.007     | 0.977     | 0.004     | 0.012     |
| Mid-American_10| 0.286     | 0.39      | 0.315     | 0.009     |
| Mid-American_11| 0.299     | 0.635     | 0.062     | 0.004     |
| Mid-American_12| 0.015     | 0.435     | 0.545     | 0.005     |
| Mid-American_13| 0.017     | 0.972     | 0.006     | 0.005     |
| Mid-American_14| 0.004     | 0.988     | 0.005     | 0.004     |
| Mid-American_15| 0.169     | 0.685     | 0.137     | 0.008     |
| Mid-American_16| 0.008     | 0.978     | 0.010     | 0.004     |
| Mid-American_17| 0.094     | 0.851     | 0.017     | 0.039     |
| Mid-American_18| 0.020     | 0.962     | 0.009     | 0.009     |
| Mid-American_19| 0.064     | 0.905     | 0.027     | 0.004     |
| Mid-American_20| 0.011     | 0.973     | 0.008     | 0.008     |
|                 | 0.019 | 0.952 | 0.019 | 0.01   |
|-----------------|-------|-------|-------|--------|
| Mid-American_22 | 0.006 | 0.984 | 0.007 | 0.003  |
| Mid-American_23 | 0.059 | 0.916 | 0.01  | 0.014  |
| Mid-American_24 | 0.007 | 0.975 | 0.015 | 0.003  |
| Mid-American_25 | 0.151 | 0.695 | 0.129 | 0.025  |
| Mid-American_26 | 0.008 | 0.983 | 0.005 | 0.003  |
| Mid-American_27 | 0.160 | 0.829 | 0.006 | 0.005  |
| Mid-American_28 | 0.405 | 0.403 | 0.158 | 0.035  |
| Mid-American_29 | 0.015 | 0.969 | 0.011 | 0.005  |
| Mid-American_31 | 0.017 | 0.903 | 0.045 | 0.035  |
| Mid-American_97 | 0.016 | 0.862 | 0.065 | 0.057  |
| Mid-American   | 0.049 | 0.505 | 0.013 | 0.023  |
| Mid-American_97 | 0.052 | 0.932 | 0.013 | 0.004  |
| Mid-American_97 | 0.043 | 0.926 | 0.013 | 0.018  |
| Mid-American_97 | 0.005 | 0.981 | 0.009 | 0.005  |
| Mid-American_97 | 0.159 | 0.814 | 0.024 | 0.003  |
| South-East_01   | 0.015 | 0.008 | 0.971 | 0.007  |
| South-East_02   | 0.023 | 0.008 | 0.898 | 0.071  |
| South-East_03   | 0.005 | 0.006 | 0.981 | 0.008  |
| South-East_04   | 0.006 | 0.005 | 0.98  | 0.008   |
| South-East_05   | 0.037 | 0.280 | 0.677 | 0.006  |
| South-East_06   | 0.007 | 0.041 | 0.947 | 0.005  |
| South-East_07   | 0.005 | 0.007 | 0.984 | 0.004  |
| South-East_08   | 0.036 | 0.018 | 0.939 | 0.006  |
| South-East_09   | 0.023 | 0.009 | 0.962 | 0.006  |
| South-East_10   | 0.048 | 0.013 | 0.928 | 0.011  |
| South-East_11   | 0.010 | 0.017 | 0.966 | 0.008  |
| South-East_12   | 0.006 | 0.007 | 0.982 | 0.005  |
| South-East_13   | 0.007 | 0.006 | 0.981 | 0.005  |
| South-East_14   | 0.005 | 0.007 | 0.984 | 0.004  |
| South-East_15   | 0.009 | 0.006 | 0.978 | 0.007  |
| South-East_16   | 0.008 | 0.006 | 0.98  | 0.005  |
| South-East_17   | 0.020 | 0.032 | 0.944 | 0.004  |
| South-East_18   | 0.013 | 0.012 | 0.984 | 0.007  |
| South-East_19   | 0.007 | 0.007 | 0.983 | 0.004  |
| South-East_20   | 0.007 | 0.013 | 0.975 | 0.005  |
| South-East_21   | 0.007 | 0.008 | 0.978 | 0.006  |
| South-East_22   | 0.011 | 0.008 | 0.973 | 0.008  |
| South-East_23   | 0.008 | 0.011 | 0.975 | 0.006  |
| South-East_24   | 0.007 | 0.008 | 0.975 | 0.01   |
| South-East_25   | 0.010 | 0.008 | 0.979 | 0.004  |
| South-East_26   | 0.048 | 0.024 | 0.920 | 0.008  |
| South-East_27   | 0.006 | 0.066 | 0.923 | 0.005  |
Table 2: Probability of assignment values. (Continued)

| Name                | Assignment Value | Assignment Value | Probability | Assignment Value |
|---------------------|------------------|------------------|-------------|------------------|
| South-East_28       | 0.005            | 0.005            | 0.985       | 0.005            |
| South-East_29       | 0.013            | 0.007            | 0.965       | 0.015            |
| South-East_30       | 0.008            | 0.009            | 0.977       | 0.005            |
| South-East_31       | 0.023            | 0.009            | 0.953       | 0.015            |
| South-East_32       | 0.021            | 0.014            | 0.956       | 0.009            |
| South-East_33       | 0.007            | 0.005            | 0.983       | 0.004            |
| South-East_34       | 0.004            | 0.004            | 0.985       | 0.006            |
| South-East_35       | 0.034            | 0.009            | 0.951       | 0.006            |
| South-East_36       | 0.005            | 0.013            | 0.975       | 0.006            |
| South-East_37       | 0.004            | 0.008            | 0.984       | 0.004            |
| Gulf sturgeon_073   | 0.018            | 0.008            | 0.005       | 0.969            |
| Gulf sturgeon_074   | 0.005            | 0.003            | 0.004       | 0.988            |
| Gulf sturgeon_075   | 0.005            | 0.004            | 0.005       | 0.986            |
| Gulf sturgeon_076   | 0.005            | 0.004            | 0.004       | 0.987            |
| Gulf sturgeon_077   | 0.004            | 0.013            | 0.026       | 0.953            |
| Gulf sturgeon_078   | 0.004            | 0.004            | 0.004       | 0.988            |
| Gulf sturgeon_079   | 0.006            | 0.006            | 0.005       | 0.983            |
| Gulf sturgeon_080   | 0.012            | 0.006            | 0.006       | 0.977            |
| Gulf sturgeon_081   | 0.008            | 0.016            | 0.024       | 0.953            |
| Gulf sturgeon_082   | 0.008            | 0.006            | 0.006       | 0.981            |
| Gulf sturgeon_083   | 0.004            | 0.009            | 0.007       | 0.980            |
| Gulf sturgeon_084   | 0.004            | 0.004            | 0.005       | 0.988            |
| Gulf sturgeon_085   | 0.005            | 0.005            | 0.004       | 0.986            |
| Gulf sturgeon_086   | 0.005            | 0.005            | 0.005       | 0.986            |
| Gulf sturgeon_087   | 0.004            | 0.007            | 0.007       | 0.982            |
| Gulf sturgeon_088   | 0.010            | 0.009            | 0.026       | 0.955            |
| Gulf sturgeon_089   | 0.005            | 0.005            | 0.006       | 0.985            |
| Gulf sturgeon_090   | 0.005            | 0.014            | 0.007       | 0.974            |
| Gulf sturgeon_091   | 0.008            | 0.007            | 0.007       | 0.978            |
| Gulf sturgeon_092   | 0.006            | 0.005            | 0.005       | 0.984            |
| Gulf sturgeon_093   | 0.007            | 0.007            | 0.005       | 0.981            |
| Gulf sturgeon_094   | 0.005            | 0.006            | 0.007       | 0.982            |
| Gulf sturgeon_095   | 0.013            | 0.016            | 0.006       | 0.965            |
| Gulf sturgeon_096   | 0.011            | 0.009            | 0.014       | 0.967            |
| Gulf sturgeon_137   | 0.006            | 0.004            | 0.005       | 0.984            |
| Gulf sturgeon_138   | 0.004            | 0.006            | 0.005       | 0.985            |
| Gulf sturgeon_139   | 0.007            | 0.006            | 0.006       | 0.980            |
| Gulf sturgeon_140   | 0.005            | 0.004            | 0.004       | 0.987            |
| Gulf sturgeon_141   | 0.017            | 0.012            | 0.005       | 0.965            |
| Gulf sturgeon_142   | 0.007            | 0.006            | 0.007       | 0.98            |
| Gulf sturgeon_143   | 0.006            | 0.004            | 0.005       | 0.985            |
| Gulf sturgeon_144   | 0.005            | 0.005            | 0.005       | 0.985            |
| Gulf sturgeon_145   | 0.006            | 0.005            | 0.005       | 0.984            |
| Gulf sturgeon_146   | 0.007            | 0.006            | 0.007       | 0.980            |
| Gulf sturgeon_147   | 0.006            | 0.007            | 0.023       | 0.962            |
| Gulf sturgeon_148   | 0.009            | 0.009            | 0.065       | 0.917            |
| Gulf sturgeon_149   | 0.004            | 0.005            | 0.004       | 0.987            |
| Gulf sturgeon_150   | 0.037            | 0.017            | 0.018       | 0.929            |
| Gulf sturgeon_151   | 0.009            | 0.008            | 0.006       | 0.977            |
| Gulf sturgeon_152   | 0.009            | 0.006            | 0.007       | 0.978            |
| Gulf sturgeon_153   | 0.009            | 0.008            | 0.006       | 0.977            |
| Gulf sturgeon_154   | 0.004            | 0.005            | 0.005       | 0.985            |
| Gulf sturgeon_155   | 0.004            | 0.004            | 0.005       | 0.988            |
| Gulf sturgeon_156   | 0.006            | 0.005            | 0.005       | 0.984            |
| Gulf sturgeon_157   | 0.008            | 0.008            | 0.008       | 0.975            |
| Gulf sturgeon_158   | 0.005            | 0.005            | 0.004       | 0.987            |
| Gulf sturgeon_159   | 0.005            | 0.004            | 0.004       | 0.987            |
| Gulf sturgeon_160   | 0.005            | 0.005            | 0.004       | 0.985            |

| Name                | Assignment Value | Assignment Value | Probability | Assignment Value |
|---------------------|------------------|------------------|-------------|------------------|
| Baltic_01           | 0.575            | 0.186            | 0.232       | 0.008            |
| Baltic_03           | 0.982            | 0.008            | 0.005       | 0.005            |
Table 2: Probability of assignment values. (Continued)

| Population | 0.943 | 0.046 | 0.007 | 0.003 |
|------------|-------|-------|-------|-------|
| Baltic_05  | 0.974 | 0.008 | 0.013 | 0.005 |
| Baltic_07  | 0.975 | 0.013 | 0.005 | 0.008 |
| Baltic_08  | 0.986 | 0.005 | 0.004 | 0.005 |
| Baltic_09  | 0.479 | 0.005 | 0.004 | 0.005 |
| Baltic_10  | 0.891 | 0.074 | 0.032 | 0.004 |
| Baltic_11  | 0.900 | 0.088 | 0.005 | 0.007 |
| Baltic_12  | 0.917 | 0.012 | 0.006 | 0.006 |
| Baltic_13  | 0.979 | 0.008 | 0.007 | 0.005 |
| Baltic_14  | 0.971 | 0.017 | 0.007 | 0.005 |
| Baltic_15  | 0.975 | 0.006 | 0.009 | 0.010 |
| Baltic_16  | 0.848 | 0.14  | 0.005 | 0.007 |
| Baltic_17  | 0.971 | 0.015 | 0.006 | 0.008 |
| Baltic_18  | 0.976 | 0.015 | 0.005 | 0.004 |
| Baltic_19  | 0.978 | 0.01  | 0.007 | 0.005 |
| Baltic_20  | 0.936 | 0.014 | 0.043 | 0.007 |
| Baltic_21  | 0.902 | 0.082 | 0.005 | 0.011 |
| Baltic_22  | 0.942 | 0.039 | 0.014 | 0.005 |
| Baltic_23  | 0.975 | 0.014 | 0.004 | 0.006 |
| Baltic_24  | 0.954 | 0.019 | 0.022 | 0.005 |
| Baltic_25  | 0.969 | 0.004 | 0.020 | 0.006 |
| Baltic_26  | 0.949 | 0.034 | 0.012 | 0.004 |
| Baltic_27  | 0.977 | 0.013 | 0.005 | 0.006 |
| Baltic_28  | 0.972 | 0.009 | 0.015 | 0.004 |
| Baltic_29  | 0.983 | 0.008 | 0.005 | 0.004 |
| Baltic_30  | 0.986 | 0.006 | 0.005 | 0.004 |
| Baltic_31  | 0.984 | 0.006 | 0.005 | 0.006 |

Probability of assignment values conducted in STRUCTURE based on microsatellites. Highest probabilities are listed in bold. Inferred clusters are given in Figure 1A.

The Baltic. If there were 3 or more female founders, it is possible that BS1 and BS2 may have evolved in North America. Prior this study, 45 North American haplotypes from this control region fragment have been described. Many of them were observed in only a few fish, sometimes single specimens [e.g. [9,10,12,13]]. Considering the fact that most North American populations are representing less than 10% of their population sizes 100 years ago [14], haplotypes BS1 and BS2 could have become regionally extinct in North America. By contrast, if there was only one female founder, the two haplotypes must have evolved in the Baltic Sea.

Table 3: Fst statistics.

| Population         | 1  | 2  | 3  | 4  | 5  |
|--------------------|----|----|----|----|----|
| Canadian           | 0  | ***| ***| ***| ns.|
| Mid-Atlantic       | 0.42| 0  | ***| ***| ***|
| Southeast          | 0.38| 0.15| 0  | ***| ***|
| Gulf of Mexico     | 0.65| 0.41| 0.27| 0  | ***|
| Baltic             | 0.02| 0.44| 0.43| 0.67| 0  |

Pairwise Fst estimates based on partial control region sequences calculated in Arlequin v. 3.0. by computing conventional F-Statistics from haplotype frequencies [8,41].

These results demonstrate that the most northerly distributed A. oxyrinchus successfully colonized the Baltic Sea, suggesting that Canadian specimens may have characteristics suitable for the environmental and ecological conditions that existed during the original founding. The IUCN reintroduction guidelines state that the organisms used for reintroduction should be as closely related as possible genetically to those originally inhabiting the habitat [15]. We suggest therefore that Canadian specimens should dominate the broodstock for reintroduction.

As recent physiological and biogeographic studies implicate temperature as a primary selection force for species survival and persistence of populations [16,17], a second factor for consideration might be including specimens from populations with broader thermal tolerances in order to minimize risk to the restored population through climate change. The inclusion of specimens from the Mid-Atlantic population could potentially extend the thermal amplitude in associated physiological responses.

In any case, from an ecological point of view, there are potentially many factors which might contradict each restoration plan [18,19] (e.g. climate change, concurrence with other species, introduction of parasites or diseases).
We observed a small number of hybrids and introgressed specimens indicating a historic Baltic population of *A. sturio*; a conclusion that is supported by the archaeological record [2]. Recently, the Baltic population was suggested to be a hybrid population between European sturgeons and Atlantic sturgeons [20]. However, this conclusion is not supported by the outcome of this study. Taking genotype distribution observed for the 8th–13th centuries [this study] and 18th–19th centuries [3] under consideration, most likely both species were sympatric during the founding event. Later, *A. sturio* was likely displaced by Atlantic sturgeons due to the cooling during the Little Ice Age. *A. sturio* may have evolved characteristics suitable for a warmer environment [A. sturio needs spawning temperatures ≥ 20°C whereas spawning temperatures of *A. oxyrinchus* range between 13–26°C; reviewed in [2]] rendering the species physically unable to persist permanently in the Baltic region. Baltic region was the most eastern historic distribution area of *A. sturio*. This region is characterized by a cold, continentally influenced climate. However, immigrations (colonization attempts) of *A. sturio* from the North Sea (e.g., Elbe River) into Baltic rivers can not be excluded during warmer decades until the North Sea population became extinct during the last century.

**Table 4: AMOVA.**

| Source of variation | d.f. | Sum of squares | Variance components | Percentage of variation |
|---------------------|-----|---------------|---------------------|------------------------|
| Among populations   | 4   | 78.63         | 0.16                | Va 47.98               |
| Within populations  | 625 | 110.19        | 0.18                | Vb 52.02               |
| Total               | 629 | 188.81        | 0.34                |                        |

Fixation Index Fst: 0.48

Analysis of molecular variance (AMOVA) based on mitochondrial haplotype distances calculated in Arlequin v. 3.0 [8].

Figure 2

**Hybrid assignments.** Assignment test using STRUCTURE clustering Baltic founders (ancient DNA), source populations (Mid-Atlantic and Canadian sturgeons), Gironde sturgeons (*A. sturio*) and artificially generated hybrids between Gironde sturgeons and specimens from the Mid-Atlantic and Canadian source populations (different groups separated by black lines, cluster associated with colors).
Assuming Canadian and Mid-Atlantic populations of *A. oxyrinchus* as the original founders, our simulations suggested that the Baltic Sea was colonized by fewer than 10 founders (females and males). The estimated number of founders changed as components of the simulation model were varied, but the estimated mean was 20 individuals at the largest. This finding was based on a discrete-generation model and relatively simple population dynamics. It must be noted that the assumption of constant population size is not likely to be valid, as intensive harvest caused drastic changes to population sizes. However, testing of several different scenarios indicated that this result was quite robust. There may have been several colonization events, but the outcome of this study indicates that only one of them is likely to have succeeded.

From a genetic point of view, our study suggests that it

---

**Table 5: Demographic modeling.**

| Mean | 95% HPD | $N_A$ | $N_B$ | $T_F$ | $T_{bot}$ | Source | Remark |
|------|---------|-------|-------|-------|-----------|--------|--------|
| 1    | 3.8     | 2–10  | 2000  | 2000  | 60        | 1  Ca+Mid | baseline |
| 2    | 3.0     | 2–6   | 10000 | 2000  | 60        | 1  Ca+Mid |          |
| 3    | 20.4    | 2–82  | 2000  | 6000  | 60        | 1  Ca    |          |
| 4    | 3.6     | 2–10  | 2000  | 2000  | 50        | 1  Ca+Mid |          |
| 5    | 18.6    | 2–38  | 2000  | 2000  | 60        | 10 Ca+Mid|          |
| 6    | 10.4    | 2–26  | 2000  | 2000  | 60        | --- Ca+Mid| an exponential growth from $N_A$ to $N_B$ |

Estimated size of the founding population ($N_f$) to the Baltic Sea at the Early Middle Ages. The ABC method was applied to 1,000,000 simulated genetic data sets (mtDNA control region and 7 microsatellite loci). The following population history was assumed as a baseline 1: a small part of the source (Canadian, Ca, and Mid-Atlantic, Mid) populations colonized the Baltic Sea at 1200 years or 60 generations before present ($T_F$), experienced single-generation bottleneck ($T_{bot}$), then the populations of both sides of the Atlantic ($N_A$, $N_B$) kept a constant size (effective population size = 2,000) until the Baltic population became extinct. Modified population assumptions were tested in the scenarios 2–6; 95% HPD (highest probability density) intervals are listed.

**Figure 3**

Phylogenetic relationships of ancient and recent Atlantic sturgeon haplotypes. Median-Joining network of American Atlantic and Baltic sturgeon haplotypes calculated in Network 4.2.0.1 based on control region sequences. Black circle white dots represent mutations and orange circle white dots represent inferred haplotypes introduced by the algorithm. Dot colors for haplotypes are congruent with colors used for mitochondrial haplotypes in figure 1.
may be possible for a small number of founders to result in a sustainable population.

**Conclusion**

Ancient DNA population genetic studies are a valuable tool for obtaining more information on historic population structure and information to select specimens for introduction from appropriate regional groups. Furthermore, our results indicate that only a small number of individuals may have been sufficient for the establishment and persistence of a self-sustaining population. This agrees with recent studies which suggest that successful colonization from a small number of individuals probably occurs more often than previously thought [21]. Our findings suggest that given a suitable environment, a long-term viable population may result from even a small founding population with limited genetic diversity, thus encouraging ongoing efforts to preserve and restore populations.

**Methods**

**Archaeological samples**

Bony scutes were excavated from two Medieval sites at the German Baltic coast, i.e. Ralswiek (Isle of Rugia, n = 538) and Wilhelmshof (Peninsula Usedom; n = 48). According to the historic record Ralswiek was a marine trading port in the late 8th and 9th centuries [22]. In the succeeding centuries (10th – 12th c.) the site lost its importance and became an agrarian settlement. Excavations (1972–1984) revealed a large faunal collection with numerous fish remains. The bony scutes of sturgeons studied here are from the early period in which sturgeons were very common and important in human diet during this time for consumption as indicated by the archaeological context. In the succeeding centuries (10th – 12th c.) sturgeons are rare among the fish remains from the cultural layers, indicating a decline in sturgeon occurrence. Similar temporal changes in the importance of sturgeon as a fish for consumption have been observed at other important Medieval sites of the Baltic coast, i.e. Gdansk (Poland) and Staraja Ladoga (Russia). Wilhelmshof is a non-agrarian settlement of the 12th–13th centuries with evidence for local handicraft and trade [24]. A small collection of fish remains (n = 178) is available from this site. Sturgeon is represented by 48 bony scutes, which were targets of the morphological and genetic analyses. Both species have different scute surfaces [25,26]. Scute surfaces of *A. oxyrinchus* are alveolar, while *A. sturio* have tubercular surfaces [drawings of scutes were published recently in [2]].

**Authenticity of DNA Sequences**

DNA extraction and PCR were performed at the ancient DNA Laboratory at the Paleogenetics Group at the Institute of Anthropology of the University of Mainz, a laboratory dedicated to ancient DNA analyses following strict guidelines. We applied the criteria for the authenticity of ancient DNA as previously described [27]. DNA was extracted from bony scutes after UV irritation from each side for 30 minutes. For each scute 0.25–0.5 g material was milled and incubated overnight in 2 ml EDTA buffer, 200 μl N-Laurylsarcosidase and 20 μl Proteinase-K followed by a phenol-chlorophorm extraction with a final concentration step using Centricron®-100 columns. Blank controls were included in every DNA extraction as well as in every PCR. Sturgeons had never been analyzed in the ancient DNA laboratory before. No evidence for contamination was detected during the entire study.

**Mitochondrial DNA analysis**

Cloning (Invitrogen) and sequencing (3100 ABI capillary sequencer; Applied Biosystems) were performed at the Leibniz Institute for Zoo and Wildlife Research, Berlin using standard procedures. PCR was performed using primers Hetero I and Hetero II or RevA, amplifying a short fragment of the control region (~200 bp) as previously described [28]. PCR products were purified by treatment with ExoSAP-IT™ (USB). A minimum of two independent PCRs were performed for each DNA extraction. Analysis of molecular variance (AMOVA) was calculated in Arlequin v. 3.0. Intraspecific relationships were calculated using NETWORK 4.2.0.1.

**Nuclear DNA analysis**

Microsatellite PCR’s were performed as previously described [11,29]. Length detection using 3100 ABI capillary sequencer (Applied Biosystems) were performed at the Leibniz Institute for Zoo and Wildlife Research, Berlin using standard procedures. Again, blank controls were included in every PCR setup. We used the procedure previously described [30] to minimize allelic dropout or artifacts: all loci were amplified from two independent DNA extractions. In case of differences between both runs (homozygous vs. heterozygous), this procedure was repeated until a sufficiently secure result was achieved otherwise the sample was discarded. Samples with ambiguous amplifications of multiple alleles were discarded for that locus. Allele length standardization between previously published data of *A. oxyrinchus* from rivers St. Lawrence and St. John (n = 39, Canadian population), Hudson and Delaware (n = 54, Mid-Atlantic), Albermarle Sound and Altamaha River (n = 37, South East), and Suwannee River (n = 48, Gulf) [11] and our ancient samples (taking into account different running conditions and devices between both labs) were performed on sample exchanges and validation of allele lengths after finishing ancient DNA analysis because shifts of +/- one allele can be found between genotyping platforms. A model-based assignment test was performed based on microsatellite data using STRUCTURE 2.0 [31]. Neither hybrids nor introgressed specimens (see below) were included in
assignment tests. All 29 ancient samples included in the assignment test were classified as *A. oxyrinchus* based on their morphology and shared mtDNA *A. oxyrinchus*-haplotype A. No signs of hybridization or introgression as indicated by their microsatellite locus *Aox*-23 flanking-region sequences were observed. Each scute produced a unique multilocus genotype. Population subdivision of *A. oxyrinchus* (Canadian, Mid-Atlantic, Southeast and Gulf populations – see [11]) was investigated using the admixture model and MCMC simulations (50,000 burn-in steps followed by 100,000 replications) for different numbers of clusters (K = 2–10). For each different K, the estimates of posterior probability Pr(X|K) (simulation summary Ln P(D)) were compared [32] choosing the ΔK showing a clear peak (K = 4–5). After this, Baltic samples (aDNA) were included using the admixture model (K = 4; 100,000 burn-in steps; 1,000,000 replicates). Ten replicated runs were calculated for comparison of Ln P(D)-values and the clustering.

**Hybrid detection**

*A. sturio* and *A. oxyrinchus* have several diagnostic substitutions in the flanking region of the microsatellite locus *Aox*-23 [3,29]. These substitutions were used as a hybrid marker. Hybrid detection was focused on scutes: i) showing a disagreement between morphology and mtDNA (n = 27), ii) all scutes having *A. sturio* haplotype AS17 (n = 7), and iii) to bring the sample size up to fifty we added 16 randomly selected scutes with *A. oxyrinchus* haplotype A. PCR products were cloned using the TOPO TA Cloning Kit® (Invitrogen). Approximately 20 clones of each sample (n = 901 clones) were sequenced. Additionally, HYBRID-LAB 1.0 [33] was used to simulate an artificial hybrid population between *A. sturio* (Gironde population, France – allelic data were published in [34]) and *A. oxyrinchus* (Canadian population). One hundred F1-hybrid genotypes were modeled. An additional assignment test using STRUCTURE included artificially generated hybrids, potential founders (Canadian and Mid-Atlantic sturgeons), Baltic sturgeons, and Gironde sturgeons (*A. sturio*).

**Inference of the founding population size**

The size of the founding population in the Baltic Sea in the Early Middle Ages was inferred from seven microsatellites and mtDNA control region sequences. The following population history was assumed in our simulations: a small part of the source (Canadian and Mid-Atlantic) populations colonized the Baltic Sea at 1,200 years before present (ybp), then the populations of both sides of the Atlantic kept a constant size (effective size = 1,000 with a 50:50 sex ratio) until the Baltic population became extinct. The Baltic founder population was assumed to experience a single-generation bottleneck, because the population size of species having a potential to produce a huge number of offspring is expected to show a dramatic increase after they settle themselves in a suitable environment. However, we also tested bottleneck periods of different lengths, as well as a gradual increase of the population size after the colonization, to check the sensitivity of the results to this assumption. Coalescent simulations were iterated 1,000,000 times, varying the effective population size of the founders as well as the source. Uniform prior distributions are assumed for both founder [1, 500] and source [100, 10,000] females as well as mutation rates (one mutation in [10,000, 100,000] years). In general, fishes are characterized by very low mutation rates and sturgeons have one of the lowest mutation rates within all vertebrates [35]. As we analyzed ancient Baltic samples (microsatellites: n = 18–30, mtDNA n = 218) and NA modern samples (microsatellites: n = 93, mtDNA n = 183) as real data, we took an equivalent number of ancient samples from the simulated Baltic population at 800 ybp as well as of modern samples from the simulated NA population. A stepwise mutation model was used for microsatellite evolution, while an infinite site model was used for mtDNA evolution. Generation time was assumed to be 20 years [36]. A discrete-generation coalescent method [37] was used to follow the change in the allele frequencies.

The approximate Bayesian computation (ABC) method [38] was applied to the simulated data set. The analyses were carried out using functions of the statistical package R provided by Mark Beaumont (University of Reading, UK). Out of the three elements (local regression, local weighing, and local density estimation) of the original ABC, the local regression procedure has a potential problem. The actual founder size used in each simulation iteration is increased or decreased by local regression on the basis of the deviation of simulated genetic data from the observed data. Because the range of founder sizes is rather small in the present study, the mathematical treatment can produce zero or negative founder sizes which never happen in the real world. Therefore, we carried out the full ABC analysis after log transformation of the variable. We also confirmed that our conclusions were unchanged if we used the untransformed data and applied the ABC without local regression to them. Posterior probability was calculated for each locus based on the following summary statistics: number of alleles, number of private alleles, and Nei’s gene diversity (for both microsatellites and mtDNA); and number of segregating sites (mtDNA only). Normalized Euclidian distances between the summary statistics values of the simulated data and those of the observed data [39] were calculated for each iteration. Each locus showed a different bottleneck signal, but our main discussion was based on the combined posterior probability. One thousand out of 1,000,000 simulated data (p0.001 = 0.001) with the smallest distances were selected and
used in the final analyses. Local weighing and calculations of the posterior density functions were carried out for each locus using the R functions.

**Abbreviations**

mt: mitochondrial; a: ancient; NA: North America.

**Authors’ contributions**

AL initiated the study, did statistical analysis and wrote the paper. UD did experimental work. SL did statistical analysis (hybrid detection). NB provided archaeological samples and background information. LD did morphological analyses. TLK did analysis of NA populations and revised the language. SM did demographic modelling. All authors read and approved the final manuscript.

**Additional material**

**Additional file 1**

Alignment of partial d-loop sequences of A. sturio (As) and A. oxyrinchus (Ao) (haplotypes AodF-AodK were taken from Ong et al. 1996,COPEIA 1996(2):464–9; no accession numbers are archived in Genbank).

Click here for file [http://www.biomedcentral.com/content/supplementary/1471-2148-8-221-S1.doc](http://www.biomedcentral.com/content/supplementary/1471-2148-8-221-S1.doc)

**Acknowledgements**

This work was funded by the German Research Foundation (DFG LU 852/5-1). We thank Joachim Burger and Michael Hofreiter for their support prior and during ancient DNA analysis, Dietmar Lieckfeldt for technical assistance; Jenny Giles, Jorns Fickel, Michael Hofreiter and three reviewer for their valuable and helpful comments.

**References**

1. Peng ZG, Ludwig A, Wang DQ, Wang D, Diogo R, Wei Q, He S: Age and biogeography of major clades in sturgeons and paddlefishes (Pisces: Acipenseriformes). Mol Phylogenet Evol 2007, 42:854-862.

2. Ludwig A, Gessner J: What makes the difference? – Sea sturgeon on both sides of the Atlantic Ocean. In Anadromous sturgeons: habitats, threats, and management Volume S6. Edited by: Munro J, Hatin D, Highower JE, McKown K, Sulak KJ, Kahlhe AW, Caron F. American Fisheries Society Symposium; 2007:285-300.

3. Ludwig A, Debus L, Lieckfeldt D, Wirgin I, Benecke N, Jenneckens I, Williot F, Waldman JR, Pitra C: When the American sea sturgeon swam east. Nature 2002, 419:447-448.

4. Cooper A, Rhymer J, James HF, Olson SL, McIntosh CE, Sorensen MD, Fleischer RC: Ancient DNA and island endemics. Nature 1996, 381:484.

5. Newsome SD, Etzioni MA, Gifford-Gonzalez D, Phillips DL, van Tuinen M, Hadly EA, Costa DP, Kennett DJ, Guilderson TP, Koch PL: The shifting baseline of northern fur seal ecology in the northeast Pacific Ocean. PNAS 2007, 104:9709-9714.

6. Borge T, Bachmann L, Bjørnsdø G, Wig O: Genetic variation in Holocene bowhead whales from Svalbard. Mol Ecol 2007, 16:2223-2235.

7. Nichols C, Herman J, Gaggiotti OE, Dobney KM, Parsons K, Hoelzel AR: Genetic isolation of a now extinct population of bottlenose dolphins (Tursiops truncatus). Proc R Soc B 2007, 274:1611-1616.

8. Schneider S, Roessli D, Excoffier L: Arlequin: A software for population genetics data analysis. In Ver 3.000 Genetics and Biome Lab, Dep. of Anthropology, University of Geneva: 2000.

9. Wirgin I, Waldman JR, Roisio J, Gross R, Collins MR, Rogers SG, Stabile J: Genetic structure of Atlantic sturgeon populations based on mitochondrial DNA control region sequences. Trans Am Fish Soc 2000, 129:476-486.

10. Stabile J, Waldman JR, Parauska F, Wirgin I: Stock structure and homing fidelity in Gulf of Mexico sturgeon (Acipenser oxyrinchus desotoi) based on restriction fragment length polymorphism and sequence analyses of mitochondrial DNA. Genetics 1996, 144:767-775.

11. King TA, Lubinski BA, Spidle AP: Microsatellite DNA variation in Atlantic sturgeon (Acipenser oxyrinchus oxyrinchus) and cross-species amplification in the Acipenseridae. Conserv Genetics 2001, 2:103-119.

12. Ong TL, Stabile J, Wirgin I, Waldman JR: Genetic divergence between A. transmontanus and A. o. desotoi as assessed by mitochondrial DNA sequencing analysis. COPEIA 1996, 1996(2):464-469.

13. Wirgin I, Grunwald C, Stabile J, Waldman JR: Genetic evidence for mid-Atlantic coast relict Atlantic sturgeon stocks. NA Fish 2007, 27:1214-1229.

14. Waldman JR: Restoring Acipenser sturio L., 1758 in Europe: Lessons from the Acipenser oxyrinchus Mitchell, 1815 experience in North America. Boletín Instituto Espanol de Oceanografía 2000, 16:237-244.

15. IUCN: Guidelines for re-introductions. Prepared by the IUCN/SSC Re-introduction Specialist Group. IUCN 1998 [http://www.iucnssc.org].

16. Parmesan C: Ecological and evolutionary responses to recent climate change. Annu Rev Ecol Evol Syst 2006, 37:637-669.

17. Hochachka PW, Somero GN: Biochemical adaptation – Mechanism and process in physiological evolution New York: Oxford University Press; 2002.

18. Armstrong DP, Seddon P: Directions in reintroduction biology. Trends Ecol Evol 2008, 23:20-25.

19. Ludwig A: A sturgeon view on conservation genetics. Euir J Wildl Res 2006, 52:3-8.

20. Tiedemann R, Moll K, Paulus KB, Scheer M, Williot P, Bartel R, Gessner J, Kirschbaum F. Atlantic sturgeons (Acipenser sturio, Acipenser oxyrinchus): American females successful in Europe. Naturwissenschaften 2007, 94:213-217.

21. Zayed A, Constantin SA, Packer L: Successful biological invasion despite a severe genetic load. PLoS ONE 2007, 2:e686.

22. Herrmann J: Ralswiek auf Rügen. Die slawisch-wikingsischen Siedlungen und deren Hinterland – Teil I. Beiträge zur Ur- und Frühgeschichte Mecklenburg-Vorpommerns 1997-1-32.

23. Benecke N: Some remarks on sturgeon fishing in the southern Baltic region in Medieval Time. In Fish and Archaeology Volume 294. Edited by: Brinkhuizen DC, Clason AT. British Archaeological Reports International Series; 1986:11-17.

24. Mangelsdorf G, Benecke N, Biermann F: Untersuchungen zum frühgeschichtlichen Wirtschafts- und Herrschaftszentrum Usedom II – Die spätslawische Siedlung am Priesterkamp. Bodendenkmäppfege in Mecklenburg-Vorpommern 2005, 52:397-545.

25. Artyukhin E, Vecsei P: On the status of Atlantic sturgeon: conspecificity of European Acipenser sturio and North American Acipenser oxyrinchus. J Appl Ichthyol 1999, 15:35-37.

26. Debus L: Meristic and morphological features of the Baltic sturgeon (Acipenser sturio L.). J Appl Ichthyol 1999, 15:36-45.

27. Mangelsdorf G, Benecke N, Biermann F: Untersuchungen zum frühgeschichtlichen Wirtschafts- und Herrschaftszentrum Usedom II – Die spätslawische Siedlung am Priesterkamp. Bodendenkmäppfege in Mecklenburg-Vorpommern 2005, 52:397-545.

28. Artyukhin E, Vecsei P: On the status of Atlantic sturgeon: conspecificity of European Acipenser sturio and North American Acipenser oxyrinchus. J Appl Ichthyol 1999, 15:35-37.

29. Mangelsdorf G, Benecke N, Biermann F: Untersuchungen zum frühgeschichtlichen Wirtschafts- und Herrschaftszentrum Usedom II – Die spätslawische Siedlung am Priesterkamp. Bodendenkmäppfege in Mecklenburg-Vorpommern 2005, 52:397-545.

30. Miller CR, Joyce P, Waits LP: Assessing allelic dropout and genotype reliability using maximum likelihood. Genetics 2002, 160:357-366.

31. Pritchard JK, Stephens M, Donnelly P: Inference of population structure using multilocus genotype data. Genetics 2000, 155:945-959.
32. Evanno GS, Regnaut S, Goudet J: Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 2005, 14:2611-2620.
33. Nielsen EE, Bach AL, Kotlicki P: Hybridlab (version 1.0): a program for generating simulated hybrids from population samples. Mol Ecol Notes 2006, 6:971-973.
34. Ludwig A, Williot F, Kirschbaum F, Lieckfeldt D: Genetic variability of the Gironde sturgeon population. BfN-Skripten 2004, 101:54-72.
35. Krieger J, Fuerst PA: Evidence for a slowed rate of molecular evolution in the order Acipenseriformes. Mol Biol Evol 2002, 19:891-897.
36. Caron F, Hatin D, Fortin R: Biological characteristics of adult Atlantic sturgeon (Acipenser oxyrinchus) in the St Lawrence River estuary and the effectiveness of management rules. J Appl Ichthyol 2002, 18:580-585.
37. Laval G, Excoffier L: SIMCOAL 2.0: a program to simulate genic diversity over large recombining regions in a subdivided population with a complex history. Bioinformatics 2004, 20:2485-87.
38. Beaumont MA, Zhang WY, Balding D: Approximate Bayesian computation in population genetics. Genetics 2002, 162:2025-2035.
39. Chan YL, Anderson CNK, Hadly EA: Bayesian estimation of the timing and severity of a population bottleneck from ancient DNA. PLoS Genetics 2006, 2:451-460.
40. Guo S, Thompson E: Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 1992, 48:361-372.
41. Tajima F: Measurement of DNA polymorphism. In Mechanisms of Molecular Evolution. Introduction to Molecular Paleopopulation Biology Edited by: Takahata N, Clark AG. Tokyo: Sunderland MA Japan Scientific Societies Press – Sinauer Associates Inc.; 1993:37-59.