Supplementary Table S1. Coordinates of Sampling sites and sampling date

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **River** | **Site** | **River\_km\_Danube** | **Sampling\_Date** | **sampling\_coordinates\_E** | **sampling\_coordinates\_N** |
| Danube | Kilia\_Arm\_Vylkove | 18 | 19.07.2019 | 29.55243 | 45.4058 |
| Danube | Reni | 130 | 19.07.2019 | 28.25887 | 45.45484 |
| Danube | Giurgeni | 235 | 18.07.2019 | 27.87538 | 44.74902 |
| Danube | Chiciu\_Silistra | 375 | 17.07.2019 | 27.22339 | 44.11828 |
| Danube | Downstream\_Ruse | 488 | 16.07.2019 | 26.06157 | 43.91233 |
| Danube | Nikopol | 586 | 15.07.2019 | 24.79351 | 43.70594 |
| Danube | Upstream\_Koslodouy | 700 | 14.07.2019 | 23.74877 | 43.80344 |
| Danube | Upstream\_Timok | 849 | 13.07.2019 | 22.68845 | 44.26321 |
| Danube | IGR\_Tekija\_Orsova | 954 | 12.07.2019 | 22.40348 | 44.6949 |
| Danube | Banatska\_Palanka | 1071 | 11.07.2019 | 21.36535 | 44.82346 |
| Danube | Downstream\_Pancevo | 1151 | 10.07.2019 | 20.72213 | 44.6829 |
| Danube | Upstream\_Tsiza | 1216 | 09.07.2019 | 20.26094 | 45.1427 |
| Danube | Ilok\_Backa\_Palanka | 1300 | 08.07.2019 | 19.37331 | 45.23304 |
| Danube | Hercegszanto | 1434 | 07.07.2019 | 18.77992 | 45.92931 |
| Danube | Dunafoldvar | 1560 | 06.07.2019 | 18.92613 | 46.81529 |
| Danube | US\_Budapest | 1660 | 06.07.2019 | 19.09385 | 47.58872 |
| Danube | Szob | 1707 | 05.07.2019 | 18.85776 | 47.81198 |
| Danube | Gonyu | 1790 | 05.07.2019 | 17.76693 | 47.74659 |
| Danube | Hainburg\_Upstream\_Morava | 1882 | 04.07.2019 | 16.90238 | 48.14247 |
| Danube | Wien | 1920 | 06.08.2019 | 16.4865556 | 48.1731944 |
| Danube | Oberloiben | 2007 | 02.07.2019 | 15.52546 | 48.38552 |
| Danube | Enghagen\_US\_dam | 2120 | 01.07.2019 | 14.35401 | 48.27762 |
| Danube | Jochenstein | 2205 | 01.07.2019 | 13.65559 | 48.54461 |
| Danube | Niederaltaich\_Muhlau | 2282 | 30.06.2019 | 12.94444 | 48.82803 |
| Danube | Kelheim | 2415 | 30.06.2019 | 11.86653 | 48.91581 |
| Danube | Bittenbrunn | 2497 | 29.06.2019 | 10.93058 | 48.73496 |
| Danube | Böfinger\_Halde | 2588 | 29.06.2019 | 9.93696 | 48.33919 |
| Danube | Gutenstein | 2686 | 04.07.2019 | 9.1206389 | 48.0782222 |
| Danube | Geisingen | 2796 | 04.07.2019 | 8.6666389 | 47.9190278 |
| Prut |  | 72 | 19.07.2019 | 28.20448 | 45.46981 |
| Siret |  | 155 | 18.07.2019 | 27.9344 | 45.40638 |
| Arges |  | 432 | 17.07.2019 | 26.59135 | 44.14795 |
| Russenski Lom | | 498 | 16.07.2019 | 25.93166 | 43.83467 |
| Olt |  | 604 | 15.07.2019 | 24.79032 | 43.72704 |
| Velika Morava | | 1103 | 10.07.2019 | 21.0698056 | 44.6207778 |
| Sava |  | 1170 | 09.07.2019 | 20.3507778 | 44.7681667 |
| Tsiza |  | 1215 | 09.07.2019 | 20.3130556 | 45.2100278 |
| Drava |  | 1383 | 08.07.2019 | 18.686 | 45.5628056 |
| Ipel |  | 1708 | 27.07.2019 | 18.7631111 | 47.8854722 |
| Hron |  | 1716 | 27.06.2019 | 18.6451389 | 47.9141111 |
| Raab |  | 1788 | 27.06.2019 | 17.5313889 | 47.6008333 |
| Morava |  | 1880 | 26.06.2019 | 16.9 | 48.2801389 |
| Traun |  | 2125 | 02.07.2019 | 14.0553611 | 48.1577222 |
| Enns |  | 2112 | 02.07.2019 | 14.4342778 | 48.0623056 |
| Inn |  | 2225 | 12.05.2020 | 12.82631488 | 48.26681454 |
| Isar |  | 2281 | 03.07.2019 | 12.8663889 | 48.7608056 |
| Lech |  | 2497 | 04.07.2019 | 10.8758611 | 48.5889167 |

Supplementary Table S2. Total number of reads, % positive PCR and number of read Acipenser ruthenus (Aci\_rut).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **River** | **Site** | **% positive PCR** | | **Sum of all reads (all PCRs)** | | **Sum reads Aci\_rut** | | **relative species abundance** | |
| Danube | Kilia\_Arm\_Vylkove | 62.5 | | 1 015 117 | | 26 009 | | 2.562 | |
| Danube | Reni | 12.5 | | 733 228 | | 4 567 | | 0.623 | |
| Danube | Giurgeni | 4.2 | | 1 113 824 | | 329 | | 0.030 | |
| Danube | Chiciu\_Silistra |  | | 863 624 | |  | |  | |
| Danube | Downstream\_Ruse |  | | 661 228 | |  | |  | |
| Danube | Nikopol |  | | 880 139 | |  | |  | |
| Danube | Upstream\_Koslodouy |  | | 882 552 | |  | |  | |
| Danube | Upstream\_Timok | 12.5 | | 787 919 | | 1 204 | | 0.153 | |
| Danube | IGR\_Tekija\_Orsova | 20.8 | | 1 078 410 | | 4 709 | | 0.437 | |
| Danube | Banatska\_Palanka | 45.8 | | 791 687 | | 4 263 | | 0.538 | |
| Danube | Downstream\_Pancevo | 4.2 | | 539 619 | | 1 619 | | 0.300 | |
| Danube | Upstream\_Tsiza | 4.2 | | 613 641 | | 1 468 | | 0.239 | |
| Danube | Ilok\_Backa\_Palanka | 4.2 | | 330 505 | | 222 | | 0.067 | |
| Danube | Hercegszanto | 4.2 | | 525 255 | | 1 464 | | 0.279 | |
| Danube | Dunafoldvar |  | | 428 756 | |  | |  | |
| Danube | US\_Budapest | 8.3 | | 221 956 | | 1 752 | | 0.789 | |
| Danube | Szob | 29.2 | | 748 290 | | 3 153 | | 0.421 | |
| Danube | Gonyu | 75.0 | | 390 920 | | 3 683 | | 0.942 | |
| Danube | Hainburg\_Upstream\_Morava |  | | 520 969 | |  | |  | |
| Danube | Wien | 4.2 | | 910 450 | | 470 | | 0.052 | |
| Danube | Oberloiben |  | | 548 731 | |  | |  | |
| Danube | Enghagen\_US\_dam |  | | 976 985 | |  | |  | |
| Danube | Jochenstein |  | | 449 314 | |  | |  | |
| Danube | Niederaltaich\_Muhlau | 4.2 | | 407 345 | | 22 | | 0.005 | |
| Danube | Kelheim |  | | 333 438 | |  | |  | |
| Danube | Bittenbrunn |  | | 218 906 | |  | |  | |
| Danube | Böfinger\_Halde |  | | 461 478 | |  | |  | |
| Danube | Gutenstein |  | | 782 934 | |  | |  | |
| Danube | Geisingen |  | | 924 809 | |  | |  | |
| Arges |  |  | | 1 046 962 | |  | |  | |
| Drava |  |  | | 1 139 978 | |  | |  | |
| Enns |  |  | | 482 171 | |  | |  | |
| Hron |  |  | | 1 075 347 | |  | |  | |
| Inn |  | 4.2 | | 249 920 | | 259 | | 0.104 | |
| Ipel |  |  | | 824 533 | |  | |  | |
| Isar |  |  | | 785 668 | |  | |  | |
| Lech |  |  | | 997 406 | |  | |  | |
| Morava |  |  | | 1 164 765 | |  | |  | |
| Olt |  |  | | 824 855 | |  | |  | |
| Prut |  |  | | 1 219 468 | |  | |  | |
| Raab |  |  | | 949 793 | |  | |  | |
| Russenski Lom | | |  | | 767 755 | |  | |  | |
| Sava |  |  | | 852 953 | |  | |  | |
| Siret |  |  | | 1 071 696 | |  | |  | |
| Traun |  |  | | 802 042 | |  | |  | |
| Tsiza |  | 4.2 | | 1 255 702 | | 928 | | 0.074 | |
| Velika Morava | | |  | | 1 110 492 | |  | |  | |