

Table S1. *Anguilla anguilla* otolith shape indices (SI) values for the different Minho river habitats and sampling locations. For each SI, overall differences between habitats, tributary vs estuary (ANOVA, $P<0.05$) were shown using an asterisk (*) in the first column. Tributary and estuary sampling locations sharing, respectively, the same letter or number, do not show any statistical difference (Tukey tests, $P>0.05$). Data are presented as mean \pm SE.

| SI | Coura | S. Gonçalo | Insuas | V. Mira | Gadanha | Mouro | Lajes | Caminha | Ponte | Espanha | Marina |
|------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|-----------------------------------|-------------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| FF* | 0.840 \pm 0.005 ^a | 0.856 \pm 0.004 ^a | 0.852 \pm 0.005 ^a | 0.856 \pm 0.004 ^a | 0.845 \pm 0.004 ^a | 0.838 \pm 0.003 ^a | 0.846 \pm 0.009 ^a | 0.860 \pm 0.005 ¹ | 0.858 \pm 0.005 ¹ | 0.847 \pm 0.005 ¹ | 0.853 \pm 0.004 ¹ |
| RO | 0.776 \pm * | 0.792 \pm 0.006 ^a | 0.815 \pm 0.007 ^b | 0.800 \pm 0.007 ^{a,b} | 0.792 \pm 0.006 ^{a,b} | 0.777 \pm 0.005 ^a | 0.797 \pm 0.011 ^{a,b} | 0.729 \pm 0.005 ¹ | 0.735 \pm 0.006 ¹ | 0.730 \pm 0.006 ¹ | 0.743 \pm 0.004 ¹ |
| EL* | 0.100 \pm 0.004 ^a | 0.092 \pm 0.007 ^{a,b} | 0.077 \pm 0.004 ^b | 0.081 \pm 0.004 ^{a,b} | 0.086 \pm 0.004 ^{a,b} | 0.093 \pm 0.003 ^a | 0.081 \pm 0.006 ^{a,b} | 0.133 \pm 0.004 ¹ | 0.126 \pm 0.004 ¹ | 0.129 \pm 0.004 ¹ | 0.121 \pm 0.003 ¹ |
| CI* | 7.475 \pm 0.056 ^a | 7.310 \pm 0.039 ^a | 7.350 \pm 0.042 ^a | 7.305 \pm 0.038 ^a | 7.422 \pm 0.043 ^a | 7.492 \pm 0.035 ^b | 7.419 \pm 0.093 ^a | 7.074 \pm 0.051 ¹ | 7.097 \pm 0.063 ¹ | 7.219 \pm 0.054 ¹ | 7.163 \pm 0.047 ¹ |
| RE* | 0.743 \pm 0.002 ^{a,b} | 0.751 \pm 0.004 ^{a,b} | 0.752 \pm 0.003 ^{a,b} | 0.744 \pm 0.003 ^{a,b} | 0.742 \pm 0.003 ^{a,b} | 0.737 \pm 0.003 ^b | 0.740 \pm 0.004 ^{a,b} | 0.754 \pm 0.003 ¹ | 0.749 \pm 0.003 ¹ | 0.749 \pm 0.003 ¹ | 0.750 \pm 0.002 ¹ |

Table S2. *Anguilla anguilla* otolith elliptical Fourier descriptors (EFD) values for the different Minho river habitats and sampling locations. For each EFD, overall differences between habitats, tributary vs estuary (ANOVA, $P<0.05$) were shown using an asterisk (*) in the first column. Tributary and estuary sampling locations sharing respectively the same letter or number, do not show any statistical difference (Tukey tests, $P>0.05$). Data are presented as mean \pm SE.

| EFD | Coura | S. Gonçalo | Insuas | V. Mira | Gadanha | Mouro | Lajes | Caminha | Ponte | Espanha | Marina |
|-----|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|
| d1* | 0.799 \pm 0.007 ^a | 0.810 \pm 0.009 ^a | 0.818 \pm 0.007 ^a | 0.817 \pm 0.007 ^a | 0.791 \pm 0.008 ^a | 0.812 \pm 0.007 ^a | 0.805 \pm 0.008 ^a | 0.761 \pm 0.006 ¹ | 0.744 \pm 0.008 ² | 0.754 \pm 0.006 ^{1,2} | 0.757 \pm 0.005 ^{1,2} |
| a2 | -0.000 \pm 0.002 ^a | -0.000 \pm 0.003 ^a | -0.005 \pm 0.002 ^a | -0.001 \pm 0.002 ^a | -0.001 \pm 0.003 ^a | -0.002 \pm 0.001 ^a | -0.009 \pm 0.003 ^a | -0.000 \pm 0.002 ¹ | -0.0015 \pm 0.003 ¹ | -0.001 \pm 0.003 ¹ | -0.002 \pm 0.002 ¹ |
| b2* | -0.012 \pm 0.002 ^a | -0.011 \pm 0.002 ^a | -0.017 \pm 0.002 ^a | -0.012 \pm 0.001 ^a | -0.014 \pm 0.002 ^a | -0.012 \pm 0.001 ^a | -0.018 \pm 0.003 ^a | -0.016 \pm 0.002 ¹ | -0.022 \pm 0.002 ^{1,2} | -0.013 \pm 0.002 ² | -0.018 \pm 0.002 ^{1,2} |
| c2* | -0.013 \pm 0.002 ^a | -0.013 \pm 0.002 ^a | -0.018 \pm 0.002 ^a | -0.014 \pm 0.002 ^a | -0.017 \pm 0.002 ^a | -0.014 \pm 0.002 ^a | -0.022 \pm 0.003 ^a | -0.015 \pm 0.002 ¹ | -0.021 \pm 0.002 ¹ | -0.020 \pm 0.003 ¹ | -0.021 \pm 0.002 ¹ |
| d2 | -0.011 \pm 0.002 ^a | -0.008 \pm 0.002 ^a | -0.009 \pm 0.003 ^a | -0.011 \pm 0.002 ^a | -0.013 \pm 0.002 ^a | -0.010 \pm 0.001 ^a | -0.007 \pm 0.003 ^a | -0.017 \pm 0.002 ¹ | -0.009 \pm 0.003 ¹ | -0.009 \pm 0.002 ¹ | -0.010 \pm 0.002 ¹ |
| a3* | 0.033 \pm 0.002 ^a | 0.031 \pm 0.002 ^a | 0.029 \pm 0.002 ^a | 0.032 \pm 0.002 ^a | 0.034 \pm 0.002 ^a | 0.030 \pm 0.002 ^a | 0.040 \pm 0.003 ^a | 0.038 \pm 0.002 ¹ | 0.035 \pm 0.002 ¹ | 0.037 \pm 0.002 ¹ | 0.035 \pm 0.001 ¹ |
| b3* | -0.014 \pm 0.002 ^a | -0.016 \pm 0.003 ^a | -0.018 \pm 0.002 ^a | -0.017 \pm 0.001 ^a | -0.020 \pm 0.002 ^a | -0.017 \pm 0.001 ^a | -0.021 \pm 0.002 ^a | -0.020 \pm 0.001 ¹ | -0.011 \pm 0.002 ^{1,2} | -0.015 \pm 0.002 ^{1,2} | -0.014 \pm 0.001 ² |
| c3* | -0.010 \pm 0.002 ^a | -0.015 \pm 0.002 ^a | -0.014 \pm 0.001 ^a | -0.013 \pm 0.001 ^a | -0.016 \pm 0.001 ^a | -0.012 \pm 0.001 ^a | -0.013 \pm 0.002 ^a | -0.019 \pm 0.001 ¹ | -0.014 \pm 0.002 ¹ | -0.018 \pm 0.001 ¹ | -0.016 \pm 0.001 ¹ |
| d3* | 0.019 \pm 0.001 ^a | 0.020 \pm 0.002 ^a | 0.016 \pm 0.002 ^a | 0.016 \pm 0.001 ^a | 0.019 \pm 0.001 ^a | 0.019 \pm 0.001 ^a | 0.011 \pm 0.003 ^a | 0.026 \pm 0.002 ¹ | 0.027 \pm 0.002 ¹ | 0.024 \pm 0.002 ¹ | 0.024 \pm 0.001 ¹ |
| a4 | 0.006 \pm 0.002 ^a | 0.006 \pm 0.001 ^a | 0.005 \pm 0.002 ^a | 0.006 \pm 0.002 ^a | 0.005 \pm 0.001 ^a | 0.005 \pm 0.001 ^a | 0.001 \pm 0.002 ^a | 0.007 \pm 0.001 ¹ | 0.002 \pm 0.002 ¹ | 0.001 \pm 0.002 ¹ | 0.003 \pm 0.001 ¹ |
| b4* | -0.011 \pm 0.002 ^a | -0.010 \pm 0.003 ^a | -0.013 \pm 0.002 ^a | -0.010 \pm 0.002 ^a | -0.012 \pm 0.001 ^a | -0.012 \pm 0.001 ^a | -0.015 \pm 0.002 ^a | -0.014 \pm 0.001 ¹ | -0.018 \pm 0.002 ¹ | -0.015 \pm 0.002 ¹ | -0.017 \pm 0.001 ¹ |
| c4 | 0.002 \pm 0.001 ^a | 0.004 \pm 0.001 ^a | 0.007 \pm 0.001 ^a | 0.005 \pm 0.001 ^a | 0.007 \pm 0.001 ^a | 0.005 \pm 0.001 ^a | 0.007 \pm 0.002 ^a | 0.005 \pm 0.001 ¹ | 0.006 \pm 0.001 ¹ | 0.005 \pm 0.001 ¹ | 0.006 \pm 0.001 ¹ |
| d4* | -0.006 \pm 0.001 ^a | -0.006 \pm 0.001 ^a | -0.007 \pm 0.001 ^a | -0.005 \pm 0.001 ^a | -0.003 \pm 0.001 ^a | -0.006 \pm 0.001 ^a | -0.009 \pm 0.001 ^a | -0.006 \pm 0.001 ¹ | -0.002 \pm 0.001 ¹ | -0.003 \pm 0.001 ¹ | -0.003 \pm 0.001 ¹ |
| a5 | 0.010 \pm 0.001 ^a | 0.011 \pm 0.002 ^a | 0.009 \pm 0.002 ^a | 0.010 \pm 0.001 ^a | 0.012 \pm 0.001 ^a | 0.009 \pm 0.001 ^a | 0.14 \pm 0.003 ^a | 0.011 \pm 0.001 ¹ | 0.010 \pm 0.002 ¹ | 0.011 \pm 0.001 ¹ | 0.012 \pm 0.001 ¹ |

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|------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
| b5 | -0.012 ± 0.001 ^a | -0.012 ± 0.002 ^a | -0.016 ± 0.001 ^a | -0.014 ± 0.001 ^a | -0.018 ± 0.002 ^a | -0.014 ± 0.001 ^a | -0.016 ± 0.002 ^a | -0.018 ± 0.001 ¹ | -0.013 ± 0.002 ¹ | -0.016 ± 0.002 ¹ | -0.014 ± 0.001 ¹ |
| c5* | 0.005 ± 0.001 ^a | 0.009 ± 0.002 ^b | 0.006 ± 0.001 ^a | 0.007 ± 0.001 ^a | 0.007 ± 0.000 ^a | 0.007 ± 0.001 ^a | 0.007 ± 0.001 ^a | 0.008 ± 0.001 ¹ | 0.004 ± 0.001 ¹ | 0.005 ± 0.001 ¹ | 0.006 ± 0.001 ¹ |
| d5* | -0.003 ± 0.001 ^a | -0.003 ± 0.002 ^a | -0.003 ± 0.001 ^a | -0.002 ± 0.001 ^a | -0.003 ± 0.000 ^a | -0.003 ± 0.001 ^a | -0.001 ± 0.002 ^a | -0.002 ± 0.001 ¹ | -0.001 ± 0.001 ¹ | -0.001 ± 0.001 ¹ | -0.003 ± 0.001 ¹ |
| a6 | 0.006 ± 0.001 ^a | 0.007 ± 0.001 ^a | 0.006 ± 0.002 ^a | 0.006 ± 0.001 ^a | 0.006 ± 0.000 ^a | 0.006 ± 0.001 ^a | 0.005 ± 0.002 ^a | 0.008 ± 0.001 ¹ | 0.004 ± 0.001 ¹ | 0.005 ± 0.001 ¹ | 0.006 ± 0.001 ¹ |
| b6* | -0.006 ± 0.001 ^a | -0.003 ± 0.002 ^a | -0.003 ± 0.001 ^a | -0.005 ± 0.001 ^a | -0.005 ± 0.001 ^a | -0.005 ± 0.002 ^a | -0.006 ± 0.001 ¹ | -0.006 ± 0.002 ¹ | -0.007 ± 0.001 ¹ | -0.008 ± 0.002 ¹ | -0.006 ± 0.001 ¹ |