

## Article

# Stock Discrimination of Gilthead Seabream (*Sparus aurata* Linnaeus, 1758) through the Examination of Otolith Morphology and Genetic Structure

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## Supplementary Materials:

**Table S1.** Sample origin, year and month of collection. n: number of fish analyzed.

| Geographic group        | Sampling location  | Month/Year                | n   |
|-------------------------|--------------------|---------------------------|-----|
| Northwestern Aegean Sea | Maliakos gulf      | 09/2015                   | 18  |
|                         | Kavala             | 11/2015, 08–10/2017       | 57  |
| East Aegean Sea         | Kalymnos           | 10/2015, 09/2017          | 65  |
|                         | Corfu              | 10/2014, 05/2015, 10/2017 | 95  |
| Ionian Sea              | Central Ionian     | 10/2017                   | 64  |
|                         | Patraikos gulf     | 10/2017, 02/2018          | 46  |
| Messolonghi lagoon      | Messolonghi lagoon | 09–10/2017                | 50  |
| Total                   |                    |                           | 396 |

**Table S2.** Sampling information per area, year, month and SRD group. n: number of fish analyzed.  $SL_{mean}$ : average standard length ( $\pm SD$ , cm).

| Area        | Year | Month     | SRD   | n  | $SL_{mean}$ |
|-------------|------|-----------|-------|----|-------------|
| NW. Aegean  | 2015 | November  | L30   | 2  | 20.7(0.3)   |
|             |      |           | 31–60 | 4  | 20(0.4)     |
|             |      |           | M60   | 22 | 20.9(1)     |
|             |      | September | L30   | 11 | 17.6(1.5)   |
|             |      |           | 31–60 | 5  | 18.6(2.2)   |
|             |      |           | M60   | 2  | 17.5(2.4)   |
|             | 2017 | August    | L30   | 11 | 16.2(1.2)   |
|             |      |           | 31–60 | -  | -           |
|             |      |           | M60   | 2  | 16.3(0.6)   |
|             |      | October   | L30   | 4  | 20(1.1)     |
|             |      |           | 31–60 | 8  | 20.1(0.5)   |
|             |      |           | M60   | 4  | 19.7(0.4)   |
| E. Aegean   | 2015 | October   | L30   | 14 | 19.4(0.9)   |
|             |      |           | 31–60 | 12 | 19.2(1)     |
|             |      |           | M60   | 4  | 18.8(0.8)   |
|             | 2017 | September | L30   | 21 | 17.6(0.8)   |
|             |      |           | 31–60 | 9  | 17.8(1.3)   |
|             |      |           | M60   | 5  | 17.9(0.9)   |
| Ionian      | 2014 | October   | L30   | 31 | 17.7(2)     |
|             |      |           | 31–60 | 13 | 17.6(1.6)   |
|             |      |           | M60   | 1  | 17.3        |
|             | 2015 | May       | L30   | 10 | 17.3(1)     |
|             |      |           | 31–60 | 18 | 18.1(1.7)   |
|             |      |           | M60   | 9  | 18.7(2)     |
|             | 2017 | October   | L30   | 49 | 17(1.6)     |
|             |      |           | 31–60 | 27 | 17.3(2.2)   |
|             |      |           | M60   | 12 | 19.3(3.1)   |
|             | 2018 | February  | L30   | 9  | 18.5(0.6)   |
|             |      |           | 31–60 | 13 | 18.2(0.5)   |
|             |      |           | M60   | 13 | 18.7(0.4)   |
| Messolonghi | 2017 | October   | L30   | 15 | 17(0.3)     |
|             |      |           | 31–60 | 12 | 17(0.5)     |
|             |      |           | M60   | 8  | 16.9(0.7)   |
|             |      | September | L30   | 7  | 16.9(0.4)   |
|             |      |           | 31–60 | 5  | 17.2(0.7)   |
|             |      |           | M60   | 3  | 17.2(0.3)   |

**Table S3.** Results of pairwise tests (t-tests) for differences in otolith shape between wild-caught fish with different SRD (degree of scale regeneration) levels (Right otolith). L30: wild-caught fish with SRD  $\leq 30\%$ ; 31–60: fish with SRD 31–60%; M60: fish with SRD  $> 60\%$ . Number of permutations = 9999. ns,  $P(\text{perm}) > 0.05$ . \*  $P(\text{perm}) < 0.05$ . \*\*  $P(\text{perm}) < 0.01$ . \*\*\*  $P(\text{perm}) < 0.001$ . Number of fish otoliths per group is shown in Table S5.

| NW. Aegean  | L30   | 31–60 | M60 |
|-------------|-------|-------|-----|
| L30         | -     | ns    | ns  |
| 31–60       | 1.468 | -     | **  |
| M60         | 1.257 | 1.936 | -   |
| E. Aegean   | L30   | 31–60 | M60 |
| L30         | -     | ns    | ns  |
| 31–60       | 1.194 | -     | ns  |
| M60         | 0.696 | 0.898 | -   |
| Ionian      | L30   | 31–60 | M60 |
| L30         | -     | ns    | **  |
| 31–60       | 1.265 | -     | ns  |
| M60         | 1.699 | 1.018 | -   |
| Messolonghi | L30   | 31–60 | M60 |
| L30         | -     | ns    | ns  |
| 31–60       | 1.313 | -     | ns  |
| M60         | 1.46  | 0.869 | -   |

**Table S4.** Average scores along the first two CAP axes (CAP1, CAP2) for wild-caught fish of different geographical origin. L30: wild-caught fish with SRD (degree of scale regeneration degree)  $\leq 30\%$ ; 31–60: wild-caught fish with SRD 31–60%; M60: wild-caught fish with SRD  $> 60\%$ .

| Left otolith |                                      |        |        | Right otolith |                                      |        |        |
|--------------|--------------------------------------|--------|--------|---------------|--------------------------------------|--------|--------|
| L30          | REGION                               | CAP1   | CAP2   | L30           | REGION                               | CAP1   | CAP2   |
|              | NW. Aegean                           | 0.080  | -0.051 |               | NW. Aegean                           | 0.062  | 0.023  |
|              | E. Aegean                            | 0.034  | 0.003  |               | E. Aegean                            | 0.004  | 0.014  |
|              | Ionian                               | -0.015 | 0.033  |               | Ionian                               | -0.026 | 0.004  |
|              | Messolonghi                          | -0.097 | -0.071 |               | Messolonghi                          | 0.023  | -0.067 |
|              | Canonical correlation ( $\delta_1$ ) | 0.88   | 0.71   |               | Canonical correlation ( $\delta_1$ ) | 0.67   | 0.59   |
| 31–60        | REGION                               | CAP1   | CAP2   | 31–60         | REGION                               | CAP1   | CAP2   |
|              | NW. Aegean                           | -0.203 | -0.004 |               | NW. Aegean                           | 0.135  | 0.073  |
|              | E. Aegean                            | 0.028  | 0.011  |               | E. Aegean                            | -0.030 | 0.013  |
|              | Ionian                               | 0.034  | -0.039 |               | Ionian                               | -0.042 | 0.009  |
|              | Messolonghi                          | 0.028  | 0.138  |               | Messolonghi                          | 0.063  | -0.126 |
|              | Canonical correlation ( $\delta_1$ ) | 0.82   | 0.61   |               | Canonical correlation ( $\delta_1$ ) | 0.86   | 0.81   |
| M60          | REGION                               | CAP1   | CAP2   | M60           | REGION                               | CAP1   | CAP2   |
|              | NW. Aegean                           | 0.010  | 0.102  |               | NW. Aegean                           | -0.058 | 0.079  |
|              | E. Aegean                            | -0.072 | 0.015  |               | E. Aegean                            | 0.049  | -0.028 |
|              | Ionian                               | -0.057 | -0.074 |               | Ionian                               | -0.029 | -0.084 |
|              | Messolonghi                          | 0.219  | -0.071 |               | Messolonghi                          | 0.192  | 0.034  |
|              | Canonical correlation ( $\delta_1$ ) | 0.81   | 0.70   |               | Canonical correlation ( $\delta_1$ ) | 0.83   | 0.78   |

**Table S5.** Cross validation re-classification matrix of the canonical analysis of principal coordinates (CAP) on otolith elliptic Fourier data to reclassify wild-caught fish to their origin. L30: wild-caught fish with SRD (degree of scale regeneration)  $\leq 30\%$ ; 31–60: wild-caught fish with SRD 31–60%; M60: wild-caught fish with SRD  $> 60\%$ . Different tests were performed for each body side (left, right).

| Left otolith |            |           |        |             |       |           | Right otolith |           |        |             |       |           |  |
|--------------|------------|-----------|--------|-------------|-------|-----------|---------------|-----------|--------|-------------|-------|-----------|--|
| L30          | NW. Aegean | E. Aegean | Ionian | Messolonghi | Total | % correct | NW. Aegean    | E. Aegean | Ionian | Messolonghi | Total | % correct |  |
| NW. Aegean   | 15         | 10        | 3      | 0           | 28    | 53.6      | 18            | 3         | 2      | 5           | 28    | 64.3      |  |
| E. Aegean    | 10         | 11        | 14     | 0           | 35    | 31.4      | 6             | 11        | 14     | 4           | 35    | 31.4      |  |
| Ionian       | 5          | 18        | 64     | 1           | 88    | 72.7      | 7             | 16        | 52     | 15          | 90    | 57.8      |  |
| Messolonghi  | 0          | 0         | 1      | 21          | 22    | 95.5      | 1             | 0         | 0      | 21          | 22    | 95.5      |  |
| Total        | 30         | 39        | 82     | 22          |       | 64.2      | 32            | 30        | 68     | 45          |       | 58.3      |  |
|              |            |           |        |             |       |           |               |           |        |             |       |           |  |
| 31–60        | NW. Aegean | E. Aegean | Ionian | Messolonghi | Total | % correct | NW. Aegean    | E. Aegean | Ionian | Messolonghi | Total | % correct |  |
| NW. Aegean   | 10         | 3         | 0      | 3           | 16    | 62.5      | 13            | 3         | 1      | 0           | 17    | 76.5      |  |
| E. Aegean    | 0          | 7         | 8      | 5           | 20    | 35        | 0             | 10        | 10     | 1           | 21    | 47.6      |  |
| Ionian       | 1          | 8         | 44     | 11          | 64    | 68.8      | 2             | 18        | 41     | 4           | 65    | 63.1      |  |
| Messolonghi  | 1          | 6         | 3      | 7           | 17    | 41.2      | 1             | 0         | 1      | 15          | 17    | 88.2      |  |
| Total        | 12         | 24        | 55     | 26          |       | 58.1      | 16            | 31        | 53     | 20          |       | 65.8      |  |
|              |            |           |        |             |       |           |               |           |        |             |       |           |  |
| M60          | NW. Aegean | E. Aegean | Ionian | Messolonghi | Total | % correct | NW. Aegean    | E. Aegean | Ionian | Messolonghi | Total | % correct |  |
| NW. Aegean   | 20         | 2         | 5      | 1           | 28    | 71.4      | 21            | 0         | 6      | 1           | 28    | 75        |  |
| E. Aegean    | 2          | 4         | 3      | 0           | 9     | 44.4      | 1             | 1         | 4      | 2           | 8     | 12.5      |  |
| Ionian       | 6          | 7         | 18     | 1           | 32    | 56.3      | 5             | 6         | 17     | 1           | 29    | 58.6      |  |
| Messolonghi  | 0          | 0         | 1      | 9           | 10    | 90        | 1             | 3         | 0      | 7           | 11    | 63.6      |  |
| Total        | 28         | 13        | 27     | 11          |       | 64.6      | 28            | 10        | 27     | 11          |       | 60.5      |  |

**Table S6.** Summary statistics for genetic variation of Gilthead seabreams per fish group. n: number of fish; A: average number of alleles; Ae: effective number of alleles; Ar: allelic richness; Ho and He: observed and expected heterozygosity; Fis: fixation index. NWA: wild-caught fish from the northwestern Aegean Sea; EA: wild-caught fish from the East Aegean Sea; I: wild-caught fish from both the Ionian Sea and Messolonghi lagoon; L30: wild-caught fish with SRD (degree of scale regeneration)  $\leq 30\%$ ; 31–60: wild-caught fish with SRD 31–60%; M60: wild-caught fish with SRD  $>60\%$ . NWA: reared fish from the northwestern Aegean Sea; IR: reared fish from the Ionian Sea.

|                  | Fish group | n           | A            | Ae           | Ar          | Ho          | He          | Fis         |       |
|------------------|------------|-------------|--------------|--------------|-------------|-------------|-------------|-------------|-------|
| Wild-caught fish | NWA        | L30         | 26           | 13.89 ± 5.88 | 8.16 ± 4.53 | 8.70 ± 2.77 | 0.87 ± 0.02 | 0.85 ± 0.04 | −0.03 |
|                  |            | 31–60       | 17           | 12.11 ± 5.23 | 7.98 ± 3.98 | 8.92 ± 3.22 | 0.88 ± 0.03 | 0.86 ± 0.03 | −0.02 |
|                  |            | M60         | 21           | 12.44 ± 5.98 | 8.39 ± 5.24 | 8.71 ± 3.19 | 0.85 ± 0.03 | 0.86 ± 0.03 | 0.01  |
|                  |            | sub-total*  | 64           | 12.81 ± 5.70 | 8.18 ± 4.58 | 8.78 ± 3.06 | 0.87 ± 0.03 | 0.86 ± 0.03 | −0.02 |
|                  |            | sub-total** | 64           | 16.67 ± 7.00 | 9.30 ± 5.76 | 8.78 ± 3.06 | 0.87 ± 0.01 | 0.85 ± 0.03 | −0.01 |
|                  | EA         | L30         | 33           | 14.56 ± 5.55 | 9.42 ± 4.92 | 9.08 ± 2.62 | 0.92 ± 0.02 | 0.87 ± 0.03 | −0.05 |
|                  |            | 31–60       | 21           | 12.67 ± 5.02 | 7.56 ± 3.35 | 8.77 ± 2.57 | 0.91 ± 0.02 | 0.86 ± 0.03 | −0.06 |
|                  |            | M60         | 9            | 8.67 ± 2.78  | 6.70 ± 2.77 | 8.67 ± 2.78 | 0.88 ± 0.04 | 0.88 ± 0.02 | 0.00  |
|                  |            | sub-total*  | 63           | 11.97 ± 4.45 | 7.89 ± 3.68 | 8.84 ± 2.66 | 0.90 ± 0.03 | 0.87 ± 0.03 | −0.04 |
|                  |            | sub-total** | 63           | 16.33 ± 6.61 | 9.82 ± 5.66 | 8.84 ± 2.66 | 0.91 ± 0.01 | 0.87 ± 0.03 | −0.05 |
|                  | I          | L30         | 70           | 16.11 ± 6.83 | 8.88 ± 5.01 | 8.54 ± 2.58 | 0.85 ± 0.01 | 0.86 ± 0.03 | 0.01  |
|                  |            | 31–60       | 53           | 15.44 ± 6.31 | 9.09 ± 5.05 | 8.67 ± 2.55 | 0.87 ± 0.02 | 0.86 ± 0.03 | −0.01 |
|                  |            | M60         | 36           | 14.22 ± 6.51 | 8.85 ± 4.30 | 8.86 ± 2.75 | 0.90 ± 0.02 | 0.87 ± 0.02 | −0.04 |
|                  |            | sub-total*  | 159          | 15.26 ± 6.55 | 8.94 ± 4.79 | 8.69 ± 2.63 | 0.87 ± 0.02 | 0.86 ± 0.03 | −0.01 |
|                  |            | sub-total** | 159          | 18.00 ± 7.63 | 9.57 ± 5.59 | 8.68 ± 2.63 | 0.87 ± 0.01 | 0.86 ± 0.03 | −0.05 |
|                  | Overall*   | 286         | 13.35 ± 5.57 | 8.34 ± 4.35  | 8.77 ± 2.78 | 0.88 ± 0.02 | 0.86 ± 0.03 |             |       |
|                  | Overall**  | 286         | 19.44 ± 8.09 | 9.92 ± 6.04  | 8.73 ± 2.63 | 0.88 ± 0.01 | 0.86 ± 0.03 |             |       |
| Reared fish      | NWAR       | 27          | 10.44 ± 4.33 | 6.46 ± 2.99  | 7.39 ± 2.24 | 0.84 ± 0.02 | 0.83 ± 0.03 | 0.01        |       |
|                  | IR         | 28          | 9.78 ± 3.60  | 5.43 ± 2.43  | 7.08 ± 2.03 | 0.80 ± 0.03 | 0.79 ± 0.03 | −0.01       |       |
|                  | Overall*   | 55          | 10.11 ± 3.97 | 5.95 ± 2.71  | 7.24 ± 2.14 | 0.82 ± 0.03 | 0.81 ± 0.03 |             |       |
|                  | Overall**  | 55          | 11.78 ± 4.66 | 6.26 ± 2.90  | 7.24 ± 2.14 | 0.82 ± 0.02 | 0.81 ± 0.03 |             |       |

\*average genetic diversity

\*\*analyzed as an entire population

**Table S7.** Pairwise  $F_{ST}$  (lower triangle) and the respective levels of statistical significance (upper triangle) among wild-caught fish of different geographical origin. L30: wild-caught fish with SRD (degree of scale regeneration)  $\leq 30\%$ ; 31–60: wild-caught fish with SRD 31–60%; M60: wild-caught fish with SRD  $>60\%$ . ns:  $p > 0.05$ . Number of fish per group is shown in Table S6.

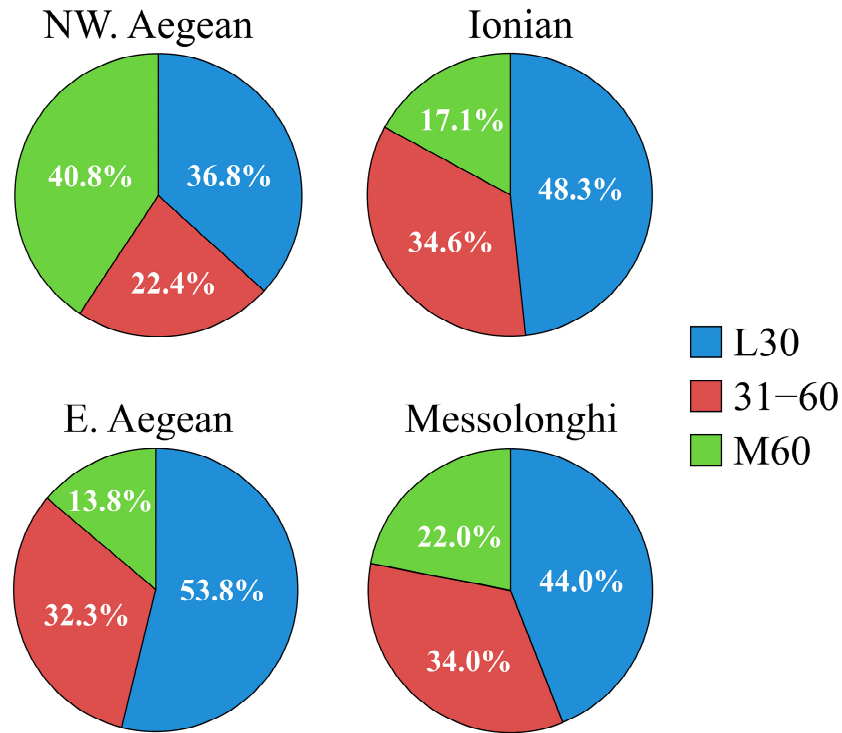
|                     | L30     | NW. Aegean | E. Aegean | Ionian <sup>1</sup> |
|---------------------|---------|------------|-----------|---------------------|
| NW. Aegean          |         | -          | ns        | ns                  |
| E. Aegean           | –0.0051 |            | -         | ns                  |
| Ionian <sup>1</sup> | –0.0015 | –0.0032    |           | -                   |
|                     | 31–60   | NW. Aegean | E. Aegean | Ionian <sup>1</sup> |
| NW. Aegean          |         | -          | ns        | ns                  |
| E. Aegean           | 0.0026  |            | -         | ns                  |
| Ionian <sup>1</sup> | 0.0007  | 0.0048     |           | -                   |
|                     | M60     | NW. Aegean | E. Aegean | Ionian <sup>1</sup> |
| NW. Aegean          |         | -          | ns        | ns                  |
| E. Aegean           | –0.0064 |            | -         | ns                  |
| Ionian <sup>1</sup> | –0.0015 | –0.0072    |           | -                   |

<sup>1</sup>Ionian group includes wild-caught fish from both the Ionian Sea and Messolonghi lagoon.

**Table S8.** Pairwise  $F_{ST}$  (lower triangle) and the respective levels of statistical significance (upper triangle) among wild-caught fish with different possibility to include aquaculture escapees. L30: wild-caught fish with SRD (degree of scale regeneration)  $\leq 30\%$ ; 31–60: wild-caught fish with SRD 31–60%; M60: wild-caught fish with SRD  $>60\%$ . ns:  $p > 0.05$ . Number of fish per group is shown in Table S6.

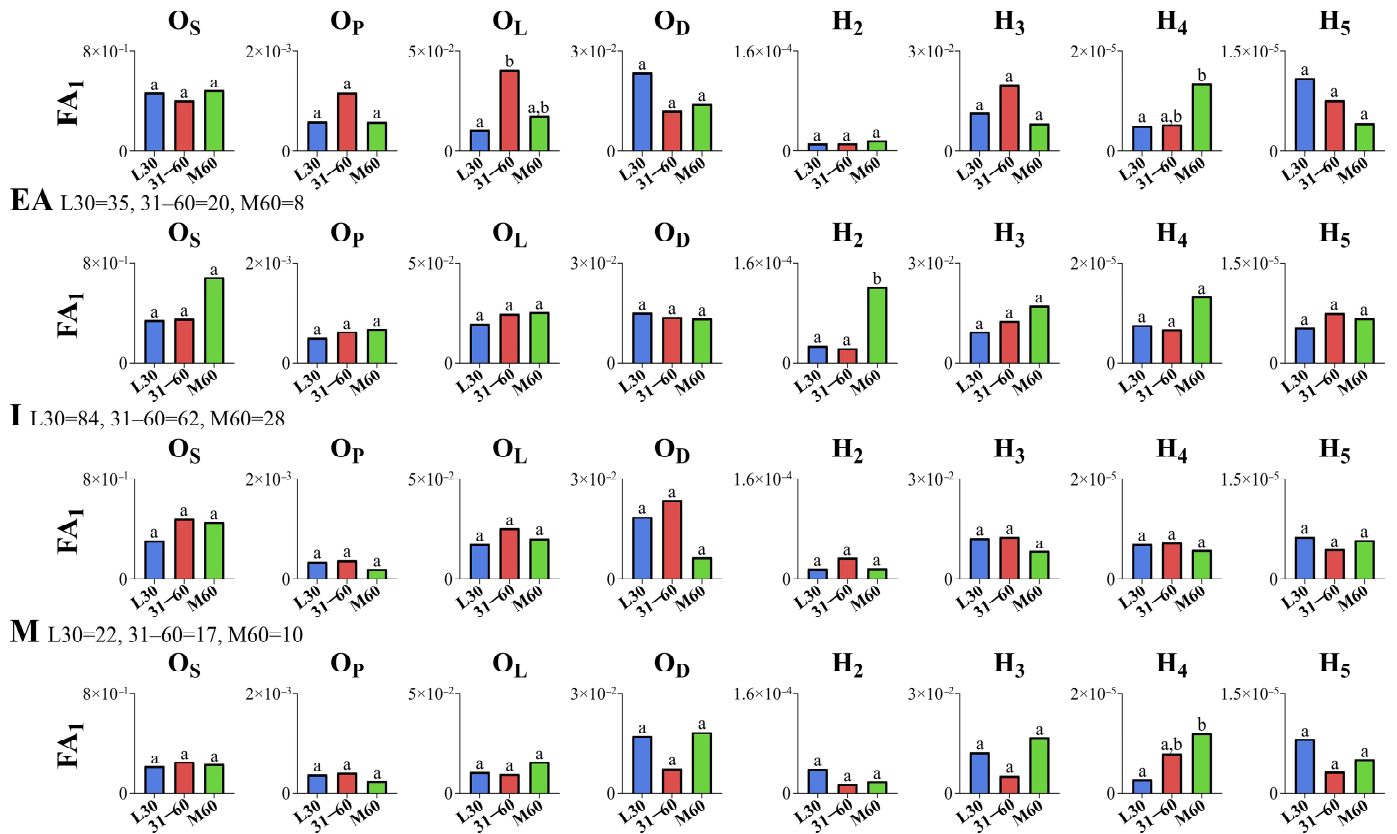
| <b>Northwestern Aegean</b> | L30     | 31–60   | M60 |
|----------------------------|---------|---------|-----|
| L30                        | -       | ns      | ns  |
| 31–60                      | 0.0021  | -       | ns  |
| M60                        | -0.0075 | 0.0015  | -   |
| <b>East Aegean</b>         | L30     | 31–60   | M60 |
| L30                        | -       | ns      | ns  |
| 31–60                      | 0.0040  | -       | ns  |
| M60                        | -0.0091 | 0.0034  | -   |
| <b>Ionian<sup>1</sup></b>  | L30     | 31–60   | M60 |
| L30                        | -       | ns      | ns  |
| 31–60                      | 0.0006  | -       | ns  |
| M60                        | 0.0001  | -0.0016 | -   |

<sup>1</sup>*Ionian group includes wild-caught fish from both the Ionian Sea and Messolonghi lagoon.*



**Figure S1.** Pie charts illustrating the percentages of wild-caught fish with different SRD (degree of scale regeneration) levels. L30: wild-caught fish with SRD  $\leq 30\%$ ; 31-60: wild-caught fish with SRD 31-60%; M60: wild-caught fish with SRD  $> 60\%$ . Number of fish per group is shown in Table 1.

**NWA** L30=28, 31-60=16, M60=26



**Figure S2.** Comparison of the index  $FA_1$  among wild-caught fish of different SRD (degree of scale regeneration) levels. L30: wild-caught fish with SRD  $\leq 30\%$ ; 31-60: wild-caught fish with SRD 31-60%; M60: wild-caught fish with SRD  $> 60\%$ . NWA, northwestern Aegean Sea; EA, East Aegean

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Sea; I, Ionian Sea; M, Messolonghi lagoon. OL: maximum otolith length; OD: maximum otolith depth; OS: otolith surface area; OP: otolith perimeter; H<sub>2</sub>-H<sub>5</sub>: harmonics 2-5. Values without a common letter are statistically different ( $p < 0.05$ , F-test). Numerical values next to the geographic groups represent the number of fish otolith pairs per SRD category.