water

Article

Monitoring Skeletal Anomalies in Big-Scale Sand Smelt, *Atherina boyeri*, as a Potential Complementary Tool for Early Detection of Effects of Anthropic Pressure in Coastal Lagoons

Chiara Leone, Francesca De Luca *, Eleonora Ciccotti, Arianna Martini and Clara Boglione

Supplementary Materials

The following Supplementary Material contains additional information to MM section and is organised in two sections: Section S1. Environmental quality assessment; S2. Skeletal anomalies.

Section S1. Environmental quality assessment (from [1])

A methodology based on expert judgement [2] was used, following an approach under development [3] for the ecological evaluation of transitional waters [4] with special reference to lagoon ecosystems in the Mediterranean area [5]. Such approach has already been proposed for the ecological quality evaluation of streams and rivers [6] and to assess marine environmental status [7]. The rationale behind this method is that the evaluation of the ecological status is a notion intrinsically subjective, and hence the approach tries to limit as much as possible this subjectivity, confining it to an initial phase, while the subjective steps of the evaluation procedure are as objective as possible [6]. Therefore, expert judgments by scientists provide for the selection of relevant metrics in the earliest steps, also combining metrics into a score and defining thresholds between ecological status classes in the scoring scale.

The methodology followed to perform a comprehensive environmental quality assessment of the lagoons, therefore, has foreseen a first step of selection of the metrics by expert judgment, i.e. the choice of several environmental indicators of direct and indirect human pressures acting on lagoon ecosystems. The choice was based on the scheme proposed by [8] and [9], modified to adapt to the specificity of the lagoons under study.

Specific pressure indicators have been chosen, grouped under three categories of anthropogenic pressures (CP) related to three main aspects influencing lagoon environmental quality: changes over time in lagoon morphology and hydrology (Morpho), use of surrounding landscape and lagoon resources (Use) and lagoon water quality (Qual). The pressure indicators contributing to each CP, detailed in Table S1 along with the time and scale of the evaluation and the data source used, were selected based on extensive background information gathered from scientific databases (Web of Science, Scopus) for publications, as well as by grey literature, Agencies databases and Reports (Table S1). Such material was also used for their quantification, based on the type, rate or interval of variation, and scored on a range scale (from 0-not present to 5-very high, as detailed in Table S2).

The scores of the pressure indicators contribute to the scoring of values for three Category Pressure Indexes, CPIs: “Changes in morphology and hydrology” (CPI-Morpho), “Landscape and use of resources” (CPI-Use) and “Water quality” (CPI-Qual). For each category, a specific CPI value is in fact calculated as the sum of the scores of each pressure indicator (i), as indicated in the formula (1)

$$CPI = \sum_{i=1}^{n} f(i) \quad (1)$$

which can grade from 0 to 20 or 25, depending on the number of pressure indicators contributing to the CPI.

Category pressure indices finally contribute to the calculation of the value of a Final Pressure Index FPI, according to the formula (2)

$$FPI = \sum_{c=1}^{3} CPI \quad (2)$$

the final value of which can score from 0 to 65.

Table S1. Single pressure indicators that contribute to three categories of anthropogenic pressures: changes over time in lagoon morphology and hydrology (CPI-Morpho), use of surrounding landscape and lagoon resources (CPI-Use) and lagoon water quality (CPI-Qual). Time and spatial scales of evaluation used for the quantification of the anthropogenic pressures are reported, as well as reference and data sources.

Pressure Category	**Indicator**	**Time Scale of Evaluation**	**Spatial Scale of Evaluation**	**Reference**	**Source of data**
CPI-Morpho	EXCHANGE – Geomorphic types according to mean water renewal time	Fixed in time	Whole lagoon	[10] [11] [12]	In situ observations Field observations GIS- instruments [4,11,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28]
	BANKS — Percentage of natural banks	Fixed in time	Sectors	Expert judgement	
	INLET — Status and efficiency	Fixed in time	Whole lagoon	Expert judgement	
	FW SUPPLY — Surface freshwater tributaries	Fixed in time	Sectors	Expert judgement	
CPI-Use	LANDSCAPE — Percentage of anthropogenically affected land	Fixed in time	Sectors	[29]	[30,31,32]
	AQUACULTURE — Shellfish farming production	Annual	Whole lagoon	Expert judgement	
	FISHERY — Production	Annual	Whole lagoon	Expert judgement	
	FIXED BARRIER — Closed days/year	Annual	Whole lagoon	Expert judgement	
CPI-Qual	DO - Dissolved Oxygen	Seasonal	Whole lagoon	Legislative Decree (L.D) no. 152/06; [33]	[34,35,36,37,38,39]
	Chl-α - Chlorophyll	Seasonal	Whole lagoon	L.D. no. 152/06	
	DIN - Dissolved Inorganic Nitrogen	Seasonal	Whole lagoon	L.D. no. 152/06	
	RP - Reactive Phosphorus	Seasonal	Whole lagoon	L.D. no. 152/06	
				L.D. no. 152/06	
	Contaminants - (Directive 2013/39/EU)	Annual	Whole lagoon	L.D. no. 152/06, M.D. no. 260/2010, L.D. 172/2015	

Table S2. Pressure indicators, description and degree levels of change (impact score) proposed for the quantification of the anthropogenic pressures (modified by [8,9]).

		IMPACT SCORE					
INDICATOR	**Description**	**(0)**	**(1)**	**(2)**	**(3)**	(4)	(5)
		CHANGES IN MORPHOLOGY AND HYDROLOGY (Morpho)					
Exchange	Geomorphic types according to mean water renewal time (days)			>100 <250	<100	>250	
Banks	% of natural banks	0-5% natural	5-10%	10-25%	25-50%	50-75%	>75 % modified
Inlet	Status and efficiency	High efficiency, natural functioning		A functional tidal inlet, maintenance of secondary canals	Maintenance on tidal channel	Poor efficiency due to no maintenance	
FW Supply	Surface freshwater tributaries and their functionality	Original tributaries network		Natural functionality and/or partially managed (water pump)	Tributaries diverted, possible freshwater revenue sources	Total freshwater inputs diversion	
		LANDSCAPE AND USE OF RESOURCE (Use)					
Landscape	% of anthropogenically affected land	100% Natural	80%	60%	30%	10%	0%
Aquaculture	Production (t/year)	0 Absent	<100	>100 <500	> 500 < 1000	>1000 <5000	>5000
Fisheries	Production (t/year)	0 Absent	<100	>100 <500	> 500 < 1000	>1000 <5000	>5000
Fixed Barrier	Closed months per year (e.g. sluice, fishing barrier)	0 Absent	1	2	4	6	> 6
		WATER QUALITY (Qual)					
DO	Dissolved Oxygen saturation> 80% for 95% of the time	Not applicable	> 80 %	70 < % < 80	50 < % ≤ 70	20 < % ≤ 50	≤ 20 %
CHL -*α*	90th percentile of the seasonal values of Chlorophyll-*α* concentration	Not applicable	< 2.45 $\mu g\ l^{-1}$	2.45 < $\mu g\ l^{-1}$ < 3.65	3.65 < $\mu g\ l^{-1}$ < 10.06	10.06 < $\mu g\ l^{-1}$ < 19.94	> 20 $\mu g\ l^{-1}$
DIN	Seasonal average of Dissolved Inorganic Nitrogen	Not applicable	< 95.46 $\mu g\ l^{-1}$	95.46 < $\mu g\ l^{-1}$ < 254.49	254.49 < $\mu g\ l^{-1}$ < 394.23	394.23 < $\mu g\ l^{-1}$ < 601.47	> 601.47 $\mu g\ l^{-1}$
RP	Seasonal average of Reactive Phosphorus			[RP] < 0.48 µM (15 $\mu g\ l^{-1}$ c.a.)	[RP] > 0.48 µM (15 $\mu g\ l^{-1}$ c.a.)		

Contaminants	Water chemical quality (Directive 2013/39/EU)	Chemical substance below the limit of detection	100% compliance of samples with EQSs for all priority substances	One List II priority substance fails to comply with EQS AND no significant increase in the concentration of this substance	One List II priority substance fails to comply with EQS AND significant increase in the concentration of this substance OR (ii) More than one List II priority substances fail to comply with EQSs AND no significant increase in the concentration of these substances	More than one List II priority substances fail to comply with EQSs AND significant increase in the concentration of these substances OR (ii) one List I priority substance fails to comply with EQSs	More than one List I priority substance from WFD Chemical status fails to comply with EQSs

EQS Environmental Quality Standards

Section S2. Skeletal anomalies.

Table S3. Frequencies (%) of individual affected by each anomaly. In red the severe anomalies, highlighted in grey the 21 anomalies used for the CA and in bold the 6 anomalies used for the MCA.

	ORB	CAB	FOG	CAP	SAB	LES	COM	GOR	GRA
	102	116	118	114	102	125	109	139	97
A5	1	0	2	1	1	2	1	0	5
B3	0	0	0	0	0	0	1	0	0
B3*	0	1	1	0	0	0	0	0	0
B4	0	0	1	1	1	0	1	0	0
B4z	0	48	1	2	11	0	6	6	2
B5	5	72	41	18	37	36	23	40	41
B5*	1	6	2	0	3	2	1	1	0
B6	1	31	23	12	14	2	57	55	1
B7	0	2	0	1	1	0	0	0	0
B7*	0	0	0	0	1	0	0	0	0
B26	0	1	0	1	0	0	0	0	0
B28	0	0	0	0	0	0	1	0	0
C3*	0	0	1	0	0	0	1	1	0
C4	1	0	0	1	0	0	1	1	0
C5	4	19	14	13	18	2	3	2	1
C5#	2	1	0	0	1	1	0	0	0
C5*	1	1	0	0	0	0	2	3	0
C6	50	92	71	62	81	92	79	77	94
C6(T)	32	1	13	11	12	78	74	85	96
C6#	0	1	0	0	1	0	0	0	0
C6*	0	1	0	0	0	0	1	1	0
C26	0	0	2	1	0	0	1	0	0
C28	1	0	0	0	0	0	0	0	0
D3	1	1	0	0	0	1	0	0	0
D3*	2	2	1	2	2	1	4	1	0
D4	4	5	5	2	3	1	6	4	1
D5	3	12	14	18	9	1	9	5	8
D5#	0	3	3	0	2	1	0	0	0
D5*	12	16	10	16	7	23	10	13	21
D6	6	8	11	10	11	1	5	3	3
D6*	7	8	3	3	1	5	5	4	5
D6#	0	1	1	0	0	0	0	1	0
D26	0	0	0	0	1	0	0	0	0
D27	1	0	0	0	0	0	4	0	0
D28	1	0	0	0	0	0	1	1	0
E11L	**0**	**4**	**4**	**2**	**10**	**1**	**6**	**5**	**0**
E11R	0	2	3	0	6	0	5	3	0
E30L	4	1	1	2	4	0	5	1	0
E30R	1	1	2	2	1	0	2	0	1
F8	1	16	13	12	11	2	6	5	4
F11	0	5	1	1	8	2	0	0	0
F20	0	1	0	1	3	0	0	0	2
G9	12	14	16	6	15	15	3	1	2
G9*	2	3	0	1	1	0	0	0	0
G10	7	4	8	7	8	4	10	4	6
G10*	51	86	87	95	83	58	94	98	75
G19	88	85	38	81	96	86	100	99	93
G19*	14	66	71	55	70	10	28	32	6
G11	**0**	**8**	**5**	**3**	**5**	**20**	**0**	**0**	**0**
H8	1	53	53	33	29	14	5	9	11
H11	0	0	0	2	0	0	0	0	0
H20	0	0	2	2	5	2	0	0	1
I8	0	59	42	25	23	6	5	13	14
I11	0	3	6	1	7	3	0	1	1
I20	0	0	0	1	1	0	0	0	0
I26	0	0	1	0	0	0	0	0	0

L8	0	1	0	0	0	0	0	0	0
L11L	0	1	0	0	0	0	0	1	0
L11R	0	1	0	0	0	0	0	1	0
M8*	**26**	**48**	**43**	**52**	**48**	**28**	**74**	**49**	**20**
SB	0	0	2	0	0	0	0	0	0
SC	1	0	0	1	0	0	0	0	0

Table S4. Frequencies (%) of each type of anomaly on the total number of anomalies. In red the severe anomalies, highlighted in grey the 21 anomalies used for the CA and in bold the 6 anomalies used for the MCA.

	ORB	CAB	FOG	CAP	SAB	LES	COM	GOR	GRA
A5	0.3	0.0	0.1	0.1	0.1	0.2	0.1	0.0	0.5
B3	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
B3*	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
B4	0.0	0.0	0.1	0.1	0.1	0.0	0.3	0.0	0.0
B4z	**0.0**	**7.5**	**0.1**	**0.4**	**2.1**	**0.0**	**1.0**	**1.6**	**0.4**
B5	**2.0**	**13.9**	**8.5**	**2.8**	**8.4**	**8.2**	**4.3**	**10.0**	**8.4**
B5*	0.3	0.5	0.1	0.0	0.3	0.2	0.1	0.1	0.0
B6	0.2	1.6	1.7	1.0	0.8	0.2	4.5	4.0	0.1
B7	0.0	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0
B7*	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
B26	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
B28	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
C3*	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.2	0.0
C4	0.5	0.0	0.0	0.3	0.0	0.0	0.1	0.4	0.0
C5	0.9	1.9	1.6	1.9	2.4	0.3	0.3	0.4	0.2
C5#	0.5	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0
C5*	0.2	0.0	0.0	0.0	0.0	0.0	0.1	0.6	0.0
C6	28.2	16.5	15.9	15.1	22.1	23.5	13.1	12.4	17.5
C6(T)	**17.5**	**0.1**	**3.0**	**3.9**	**2.9**	**24.7**	**19.8**	**23.0**	**32.3**
C6#	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
C6*	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.0
C26	0.0	0.0	0.3	0.3	0.0	0.0	0.1	0.0	0.0
C28	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
D3	0.2	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
D3*	0.3	0.1	0.1	0.1	0.2	0.1	0.3	0.2	0.0
D4	1.2	0.4	0.5	0.3	0.3	0.1	1.0	0.6	0.2
D5	0.5	0.7	1.2	2.0	0.7	0.1	1.0	0.4	1.6
D5#	0.0	0.1	0.2	0.0	0.1	0.1	0.0	0.0	0.0
D5*	2.0	0.8	0.7	1.2	0.4	2.5	0.8	0.9	2.0
D6	1.1	0.4	1.0	0.9	0.8	0.1	0.3	0.2	0.6
D6#	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0
D6*	1.1	0.4	0.2	0.2	0.1	0.5	0.3	0.4	0.5
D26	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
D27	0.2	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0
D28	0.2	0.0	0.0	0.0	0.0	0.0	0.1	0.3	0.0
E11L	**0.0**	**0.3**	**0.6**	**0.9**	**0.9**	**0.1**	**2.0**	**0.8**	**0.0**
E11R	0.0	0.1	0.7	0.0	0.6	0.0	1.0	0.3	0.0
E30L	0.8	0.0	0.1	0.2	0.4	0.0	0.3	0.1	0.0
E30R	0.2	0.0	0.1	0.1	0.1	0.0	0.1	0.0	0.1
F8	0.2	1.3	1.4	1.9	0.9	0.2	0.4	0.4	0.4
F11	0.0	0.5	0.1	0.1	1.5	0.3	0.0	0.0	0.0
F20	0.0	0.0	0.0	0.5	0.2	0.0	0.0	0.0	0.2
G9	1.8	0.8	1.2	0.5	0.9	1.7	0.2	0.1	0.2
G9*	0.3	0.2	0.0	0.1	0.1	0.0	0.0	0.0	0.0
G10	1.2	0.3	0.6	0.8	0.9	0.4	1.0	0.3	0.7
G10*	9.5	8.2	11.9	13.9	9.8	9.5	13.9	14.0	10.7
G19	19.6	8.7	5.4	12.4	11.8	14.6	14.1	14.5	16.5
G19*	2.1	6.9	10.4	8.7	8.4	1.2	2.4	2.5	0.6
G11	**0.0**	**1.6**	**0.9**	**0.5**	**0.8**	**4.1**	**0.0**	**0.0**	**0.0**
H8	0.2	6.5	6.0	3.8	3.7	1.6	0.6	1.3	1.0
H11	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
H20	0.0	0.0	0.2	0.5	0.7	0.3	0.0	0.0	0.1
I8	0.0	4.3	4.2	2.6	2.1	0.6	0.3	0.9	1.3
I11	0.0	0.5	1.0	0.1	0.6	0.3	0.0	0.2	0.1
I20	0.0	0.0	0.0	0.3	0.5	0.0	0.0	0.0	0.0
I26	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
L8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
L11L	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.1	0.0
L11R	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.1	0.0

M8*	**6.4**	**13.7**	**19.5**	**21.2**	**13.4**	**4.3**	**15.5**	**8.8**	**4.0**
SB	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
SC	0.2	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0

Correspondence Analysis

The CA performed on the RM (1023 fish x 63 variables, i.e. 62 anomalies and the variable ABS, to take into account specimens devoid of anomalies) gave an ordination model in which 20.7% of the variance was explained by the first three axes (Figure S1).

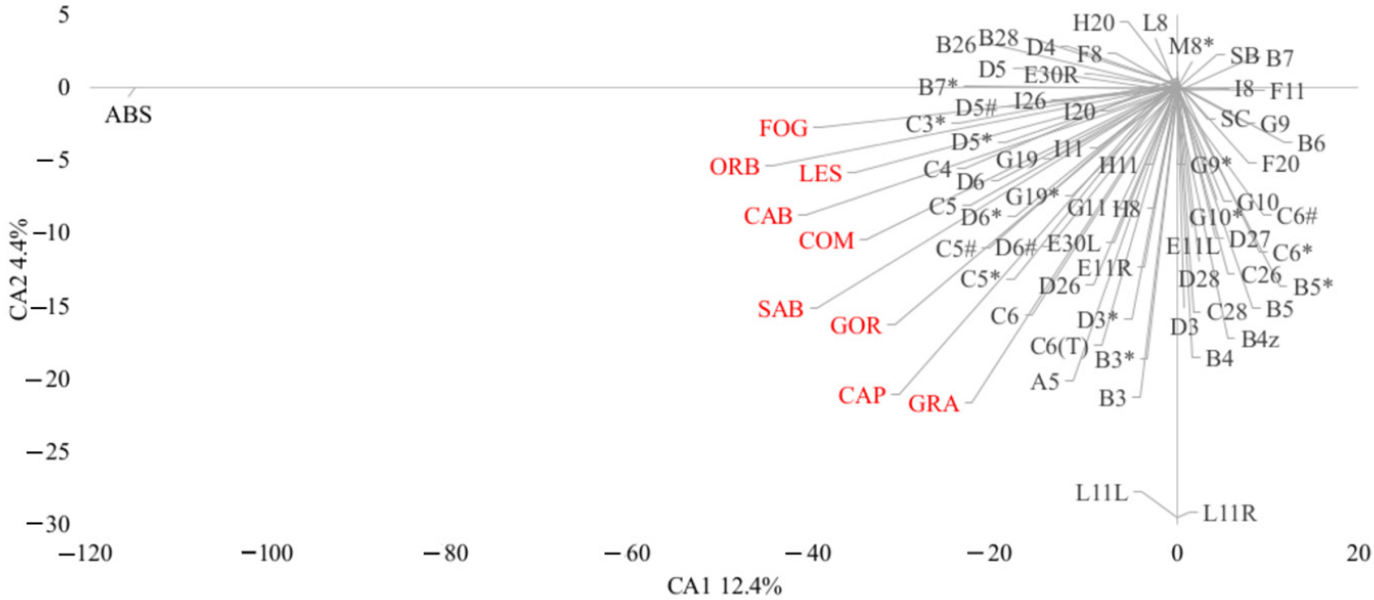

Figure S1. Correspondence analysis (CA) (1023 fish x 63 variables). Ordination of samples in the first two factorial axes (CA1 and CA2) plane. These axes account for 16.8% of total variance. Please, refer to Table 2 for anomalies' code. Sites: (ORB) Orbetello, (CAB) Cabras, (FOG) Fogliano, (CAP) Caprolace, (SAB) Sabaudia, (LES) Lesina, (COM) Comacchio, (GOR) Sacca di Goro, (GRA) Grado Marano.

Given the low variance obtained by this ordination model and the unclear distributions of the variables (i.e. skeletal anomalies) with respect to the sites of origin, a second CA was performed on a subset of data (Figure S2).

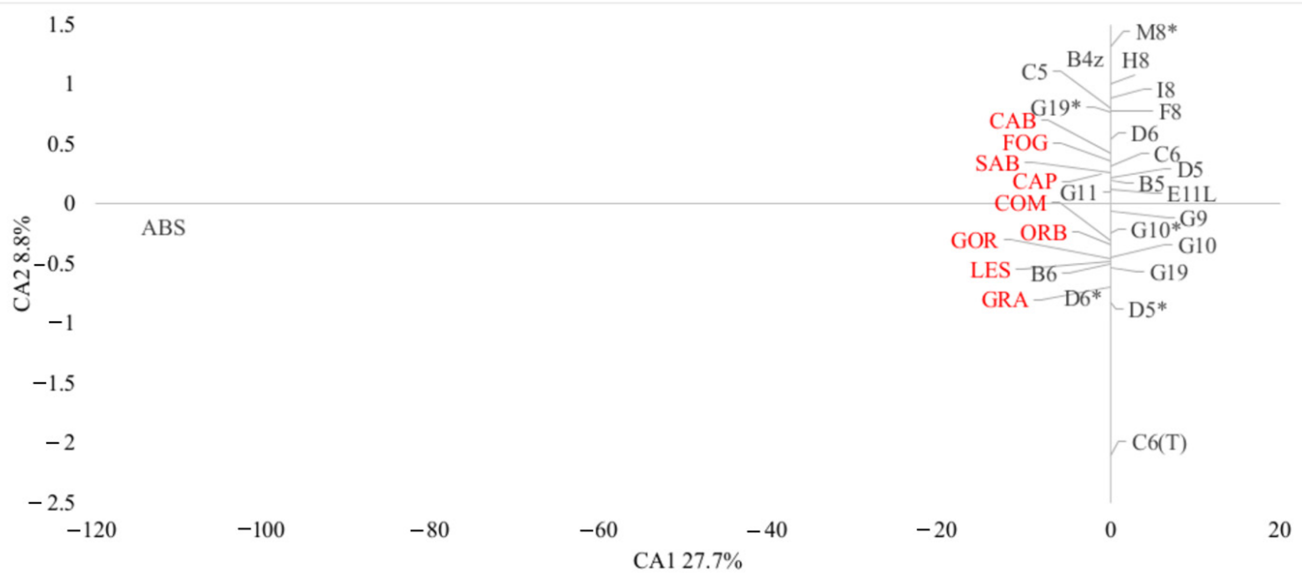

Figure S2. Correspondence analysis (CA) *(1023 specimens and 22 variables)*. Ordination of samples in the first two factorial axes (CA1 and CA2) plane. These axes account for 36.5% of total variance. Please, refer to Table 2 for anomalies' code. Sites: (ORB) Orbetello, (CAB) Cabras, (FOG) Fogliano, (CAP) Caprolace, (SAB) Sabaudia, (LES) Lesina, (COM) Comacchio, (GOR) Sacca di Goro, (GRA) Grado Marano.

The CA was performed on a matrix (1023 specimens and 22 variables, i.e. 21 anomalies and the ABS variable) that were retained, excluding the rarest anomalies (frequency < 4% in all the specimens). This CA gave an ordination model in which 43.4% of the total variance was explained by the first three axes. Despite the higher explained variance, the ordination model was difficult to be interpreted, because of the weight of the ABS variable, which forces the sites of origin to aggregate close to the origin of the axes.

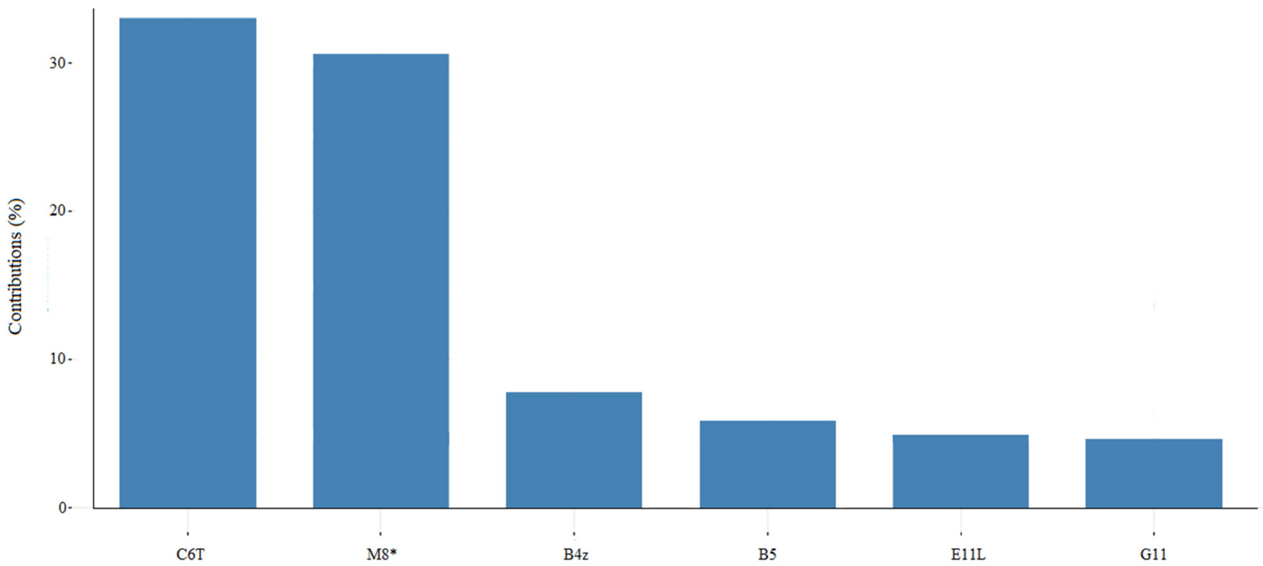

Figure S3. R output for the analysis of the variable contribution for the matrix 1022x21. The bar plot of variable contributions shows the six variables with the highest percentage contribution to CA1 and CA2. Skeletal anomalies: malformed zygapophysis of abdominal vertebrae (B4z), malformed neural arches or spine of abdominal vertebrae (B5), Tulip-like shaped haemal arches of the hemal vertebrae (C6T), malformed left pectoral fin rays (E11L), malformed caudal fin rays (G11) and malformed interdorsal element (M8*).

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