

Appendices

Appendix 1: Taxonomy and naming

Pilot whales are classified into two species: Long-finned (Globicephala melas) and Short-finned (Globicephala macrorhynchus). The short-finned pilot whale was described, from skeletal materials only, by John Edward Gray in 1846. He presumed from the skeleton that the whale had a large beak. The long-finned pilot whale was described by Thomas Stewart Traill in 1809 as Delphinus melas. Its scientific name was eventually changed to Globicephala melena. Since 1986, the specific epithet of the long-finned pilot whale was changed to its original form melas. Other species classifications have been proposed but only two have been accepted. There exist geographic forms of short-finned pilot whales off the east coast of Japan, which comprise genetically isolated stocks. Fossils of an extinct relative, Globicephala baereckeii, have been found in Pleistocene deposits in Florida. Another Globicephala dolphin was discovered in Pliocene strata in Tuscany, Italy, and was named G. eturia. The pilot whales were also close relatives of the extinct blunt-snouted dolphin Platalearostrum hoekmani. Close living relatives of the pilot whales are the melon-headed whale Peponocephala electra, the pygmy killer whale Feresa attenuata, the false killer whale Pseudorca crassidens, and Risso's dolphin Grampus griseus.

Appendix 2: Statistical Analysis

Statistics was done using the SPSS software.

Variables calculated:

For each individual and each variable, differences between Right and Left sides (Di) were calculated: Di=(Ri-Li).

Standardized differences Dstand were calculated: Di/(Ri+Li)/2

Variables assumption analyses:

The assumption of unimodal distribution of data was verified by plotting the frequency distributions of each variable.

The assumption of normal distribution of data was verified by the One-Sample Kolmogorov-Smirnov Test.

Data Analysis: When assumptions of data normality were met, parametric tests, such as a Paired Samples T-test, were used, otherwise non-parametric tests were used, such as Sign Test.

Reduced Major Axis (RMA) regression was calculated for each pair of variables using the Excel software: The RMA regression line is the regression line that usually represents the most useful relationship between the X and Y axes. It assumes that both axes are equally error prone. The RMA line assumes that neither axis depends on the other and is very nearly halfway between the first two lines. It minimizes the error at right angles to the line. The ER, or Error Ratio line, minimizes the error on both X and Y directions. There was not usually much difference between the RMA and ER lines. All four lines intersected at the centroid of the data.

Mass of ossicles
 Mass Malleus (M = Malleus mass)
 34 individuals
 At the population level:

Frequency distribution of weights measured was not different from normal neither in the right ear (P = 0.311) nor in the left ear (P = 0.782). Frequency distribution of Di was not different from normal (P = 0.992).



22 individuals in which left M was heavier (M = 0.164; Std = 0.015); 12 individuals in which right M was heavier (M = 0.162; Std = 0.015).

Binomial Test

Category			N	Observed Prop.	Test Prop.	Exact Sig. (2-tailed)
	Group 1	<= 0 22		0.65	0.5	0.121
Di_MW	Group 2	> 0	12	0.35		
	Total		34	1		

Left M was significantly different from Right M (paired-t33 = 2.302 Ptwo tailed = 0.028).



Regressing R vs L (RMA), the intercept did not significantly differ from zero (b = -0.015, SE = 0.008, t = 1.778, p = 0.085), suggesting no significant constant difference between the two sides. Moreover, the slope was not significantly different from 1 (a = 1.104, SE = 0.052, t = 1.986, p = 0.056), suggesting no allometric effect. Although, T-test showed asymmetry the Regression method did not. We can try to resolve these differences by considering the fact that in the regression analysis, the modeled constant and slope might have diverted each other's effect, and thus neither was significantly different from 0 and 1, respectively. Thus, as a conclusion there was asymmetry, in M weight, manifested in the T-test only.

There was no age effect to the asymmetry (Pearson correlation between the standard difference between Right and Left); R = 0.066, p = 0.743. Actually, there was no age effect whatsoever on Malleus weight (R = -0.015, p = 0.94)..

Mass Incus (I)

33 individuals

At the population level: Frequency distribution of weights measured was not different from normal neither in the right ear (P = 0.772) nor in the left ear (P = 0.811). Frequency distribution of Di was not different from normal (P = 0.877).



0.035 0.04 0.045 0.05 0.055 0.06

22 individuals in which the left I was heavier (I= 0.048; Std = 0.005); 11 individuals in which the right I was heavier (I= 0.047; Std = 0.004).

The Left I was significantly different from the Right I (paired-t32 = 2.248 Ptwo tailed = 0.032).

Regressing R vs L (RMA), the intercept significantly differed from zero (b = -0.0056, SE = 0.002, t = 2.473, P = 0.019) suggesting a significant constant difference between the two sides. Furthermore, the slope was significantly different from 1 (a = 1.128, SE = 0.048, t = 2.691, P = 0.011), suggesting an allometric effect.

Mass Stapes (S)

33 individuals

At the population level: Frequency distribution of weights measured was not different from normal neither in the right ear (P = 0.622) nor in the left ear (P = 0.988). The frequency distribution of Di was marginally different from normal (P = 0.048).



23 individuals in which left S is heavier (S = 0.0158; Std = 0.0018); 10 individuals in which right S is heavier (S = 0.0155; Std = 0.0020).

Left S is not significantly different, on average, from Right S (paired-t32 = 1.135 Ptwo tailed = 0.265). On the other hand, the number of individuals with heavier left S was significantly larger than the number of individuals with heavier right S (sign test, P = 0.035).

Regressing R vs L (RMA), the intercept did not significantly differ from zero (b = 0.002, SE = 0.002, t = 1.053, P = 0.301) suggesting no significant constant difference between the two sides. Furthermore, the slope was not significantly different from 1 (a = 0.876, SE = 0.138, t = 0.895, P = 0.378), suggesting no allometric effect.

There was no age effect on the asymmetry (Pearson correlation between the standard difference between Right and Left); R = 0.010, p = 0.961.

Length of Ossicles

Malleus length (M)

34 individuals

At the population level: The frequency distribution of lengths measured was not different from normal neither in the right ear (P = 0.683) nor in the left ear (P = 0.926). The frequency distribution of Di was not different from normal (P = 0.048).



22 individuals in which the left M was longer than the right M (M = 6.579; Std = 0.227); 12 individuals in which right M was longer (M = 6.557; Std = 0.231).

The Left M was not significantly different, on average, from the Right M (paired-t33 = 0.993 Ptwo tailed = 0.328). In accordance, the number of individuals with longer left M (22) was not significantly larger than the number of individuals with longer right M (12) (sign test, P = 0.121).



Regressing R vs L (RMA), the intercept did not significantly differ from zero (b = 0.252, SE = 0.627, t = 0.401, P = 0.691) suggesting no significant constant difference between the two sides. Furthermore, the slope was not significantly different from 1 (a = 0.965, SE = 0.095, t = 0.366, P = 0.717), suggesting no allometric effect.

Length Incus (I)

33 individuals

At the population level: Frequency distribution of lengths measured was not different from normal neither in the right ear (P = 0.943) nor in the left ear (P = 0.715). The frequency distribution of Di was not different from normal (P = 0.899).



The Left I was not significantly different, on average, from the Right I (paired-t(32) = 1.816 Ptwo tailed = 0.079). In accordance, the number of individuals with longer left I (20) was not significantly larger than individuals with longer right I (13) (sign test, P = 0.296).



Regressing R vs L (RMA), the intercept did not significantly differ from zero (b = 1.67, SE = 0.194, t = 1.675, P = 0.104) suggesting no significant constant difference between the two sides. Furthermore, the slope did not significantly differ from 1 (a = 0.916, SE = 0.053, t = 0.1.605, P = 0.119), suggesting no allometric effect.

Length Stapes (S)

32 individuals

At the population level: The frequency distribution of lengths measured was not different from normal neither in the right ear (P = 0.192) nor in the left ear (P = 0.927). Yet, the frequency distribution of Di was different from normal (P = 0.024).



The Left S was not significantly different, on average, from the Right S (paired-t33 = 0.993 Ptwo tailed = 0.328). In accordance, the number of individuals with longer left S (20) was not significantly larger than individuals with longer right S (12) (sign test, P = 0.215).



Regressing R vs L (RMA), the intercept significantly differed from zero (b = -2.055, SE = 0.621, t = 3.310, P = 0.003) suggesting a constant difference between the two sides. Also the slope significantly differed from 1 (a = 1.736, SE = 0.221, t = 7.856, P = 0.000), suggesting an allometric effect.

Inter Ossicular Angles

29 individuals: We define: Left = the angle is wider on the left ear. Right = the angle is wider on the right ear. **At the population level:** The frequency distribution of lengths measured was not different from normal neither in the right ear (P = 0.815) nor in the left ear (P = 0.986). The frequency distribution of Di was not different from normal (P = 0.828). The average deviation (Right-Left) was -°3.476; SD of the average is °8.062



The left Angle was significantly different, on average, from the Right Angle (paired-t(28) = 2.276 Ptwo tailed = 0.031). This is in contrast to the observation that the number of individuals with larger left Angle (19) was not significantly different from the number of individuals with larger right Angle (10) (sign test, P = 0.136).



Regressing R vs L (RMA), the intercept did not significantly differ from zero (b = 0.959, SE = 17.21, t = 0.056, P = 0.956) suggesting no difference between the two sides. The slope did not significantly differ from 1 (a = 1.027, SE = 0.195, t = 0.140, P = 0.890), suggesting no allometric effect

Stapes Footplate Area

33 individuals

At the population level: Frequency distribution of areas measured was not different from normal neither in right the ear (P = 0.617) nor in the left ear (P = 0.960). The frequency distribution of Di was not different from normal (P = 0.196).



The left SFP area was not significantly different, on average, from the Right SFP area (paired-t(32) = -0.848 Ptwo tailed = 0.403). In accordance, the number of individuals with larger left SFP area (17) was not significantly larger than the number of individuals with greater right SFP area (16) (sign test, P = 1.000).



Regressing R vs L (RMA), the intercept significantly differed from zero (b = -10.612, SE = 2.062, t = 5.146, P = 0.000) suggesting a constant difference between the two sides. Also, the slope significantly differed from 1 (a = 2.214, SE = 0.238, t = 5.093, P = 0.000), suggesting an allometric effect.

"Effective Pressure"

We defined "Effective Pressure" at each ear as EP = (Malleus weight + Incus weight) / (Stapedial footplate area)

33 individuals

At the population level: The frequency distribution of Pressure was not significantly different from normal neither in the right ear (P = 0.638) nor in the left ear (P = 0.534) (K-S test of Normality).





22 individuals in which the Ep on the left ear was greater than on the right one; 12 individuals in which right pressure was greater. Statistically, this is not a significant difference (sign test, P = 0.215).

Testing the difference in mean pressure between the left ear (M = 0.079; Std = 0.010) and the right ear (M = 0.077; Std = 0.0086), there was no significant difference between them (paired-t31 = 1.254 Ptwo tailed = 0.219).



Regressing R vs L (RMA), the intercept significantly differed from zero (b = -0.03, SE = 0.01, t = 2.303, P = 0.028) suggesting significant constant difference between the two sides. Furthermore, the slope also significantly differed from 1 (a = 1.453, SE = 0.184, t = 2.460, P = 0.020), suggesting an allometric effect. Due to suspicion, the residuals from the above regression were analyzed and found to be normally distributed (Kolmogorov-Smirnov test, P = 0.567)

Dependent Left Pressure

Model Summary

Model	R	R Squareb	Adjusted R Square	Std. Error of the Estimate			
1	.994a	0.989	0.988	0.0085519			

Predictors: Right Pressure; b. For regression through the origin (the no-intercept model), R Square measures the proportion of the variability in the dependent variable about the origin explained by regression. This cannot be compared to R Square for models that include an intercept.

Coefficients a, b.

Model.		Unstand	lardized Coefficients	Standardized Coefficients	+	C:~
		В	Std. Error	Beta	L	Sig.
1	Right Pressure1.020.022		0.994	46.94	0	

a. Dependent Variable: Left Pressure; b. Linear Regression through the Origin.

Excluded Variables a,b.

Model		Beta In	t	Sig.	Partial Correlation	Collinearity Statistics
						Tolerance
1	Gender1	.016c	0.43	0.67	0.087	0.308
	RpressureBYgender	.008c	0.19	0.85	0.04	0.301

a Dependent Variable: Left Pressure; b. Linear Regression through the Origin; c. Predictors in the Model: Right Pressure.



Constant=0 (If forced)



When the constant is not forced: Green line = males; blue line = females; black line = all the data combined.



Histograms











Tsur I, et al. Dolphin (*Globicephala Macrorhynchus*) Middle Ear: Can Ossicle Asymmetry Aid Locating the Source of Incoming Sounds?. Otolaryngol Open Access J 2023, 8(2): 000276.

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Effect of Age and Gender

Effect of Age and sex on auditory bones: For the age effect N=26 (Both sexes). For the effect of sex N = 28 (Seven males and twenty one females).

There was no correlation between auditory bones weight and age (Table 1). This result is not affected when analyzing for the effect of sex on auditory bone weight with age as covariate (ANCOVA) in any of the bones (F= 19; M= 7) (Table 2; illustrated in figures 1a-d). Using independent t-test, the Malleus was found significantly (though not strongly) heavier in males compared to females. So did, yet trivially, the sum of the three auditory bones. No difference between males and females was found in Stapes and Incus weight (Table 3).

		Stapes	Malleus	Incus	Sum Weight of Auditory bones
	Pearson Correlation	0.048	-0.028	-0.177	-0.061
Age (yr)	P. (1-tailed)	0.407	0.446	0.189	0.381
	N	27	27	27	27

Table 1: Correlations between Auditory Bone Weight and Age.

**. Correlation is significant at the 0.01 level (1-tailed).

		N	F	Р
	Age		0.25	0.62
Stapes weight	Sex	26	0.01	0.94
	Age*Sex		1.07	0.31
	Age		0.14	0.72
Malleus weight	Sex	26	0.06	0.81
	Age*Sex		1.88	0.18
	Age		0.8	0.38
Incus weight	Sex	26	0.68	0.42
	Age*Sex		0	0.96

Table 2: ANCOVA Auditory Bone Weight by Sex (Factor) and Age (Covariate).



Figure 1: A: Stapes Weight



b. Incus Weight

Total Auditory Bone Weight

	sex	N	Mean	Std. Deviation	t	df	p (1-tailed)
Stanes Weight Avg	F	21	0.016	0.0016	-1.17	26	0.253
Stapes weight Avg	М	7	0.016	0.0012			
Malleus Weight Avg	F	21	0.16	0.0116	-2 17	26	0.039
Huneus Weight Hvg	М	7	0.174	0.0201	2.17		
Incus Weight Avg	F	21	0.047	0.0046	-1.48	26	0 151
incus tronghority	М	7	0.05	0.0038		10	01101
Total Weight of auditory hones	F	21	0.223	0.0165	-2.18	26	0.039
Total Weight of dualtery bones	М	7	0.24	0.0215	20	0.009	

Table 3: Auditory Bone Weight in Females and Males.