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Double-yolked pheasant eggs provide an insight into the control of albumen secretion in bird eggs

Charles Deeming

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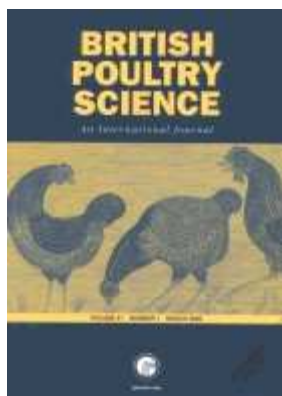
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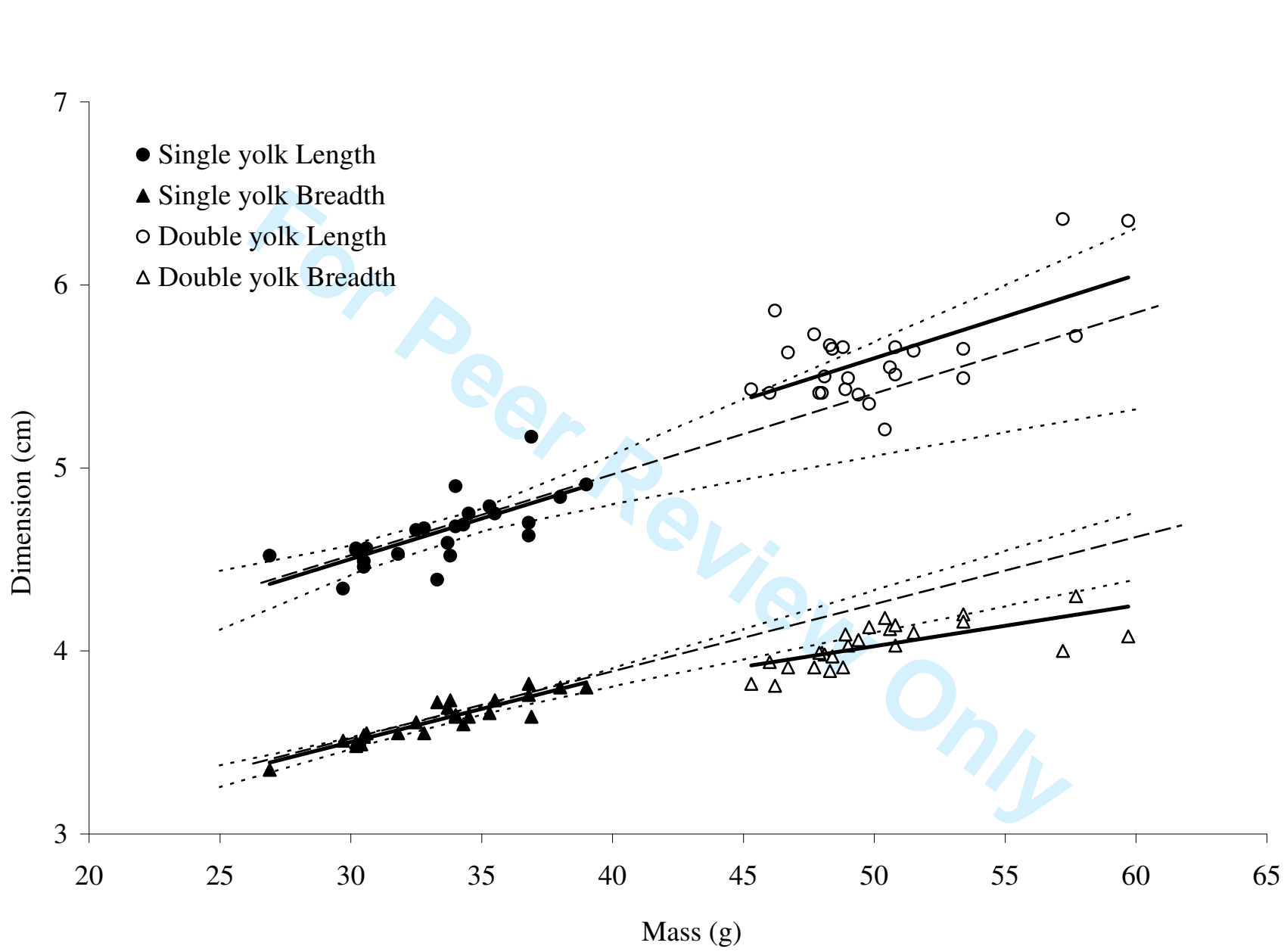
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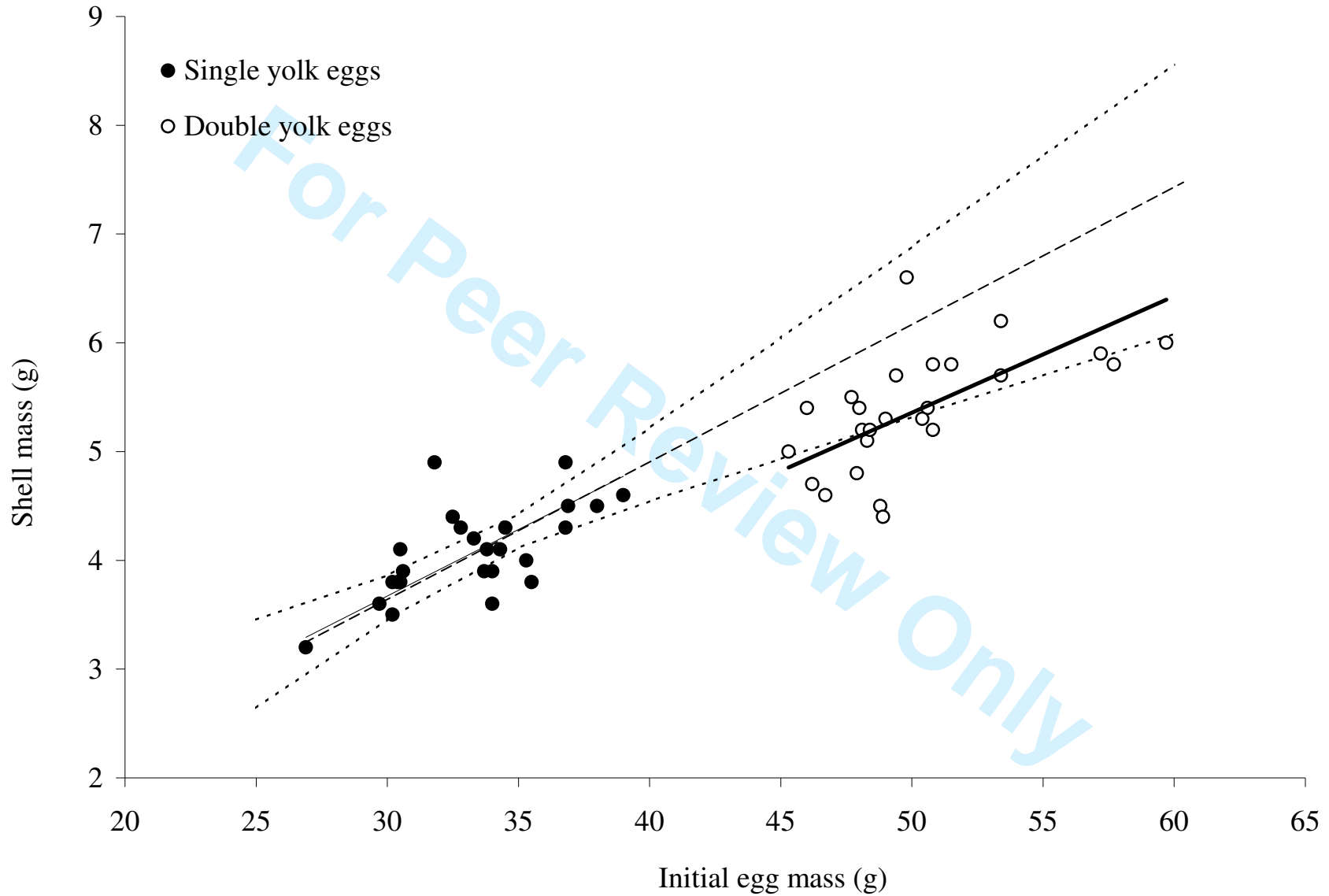


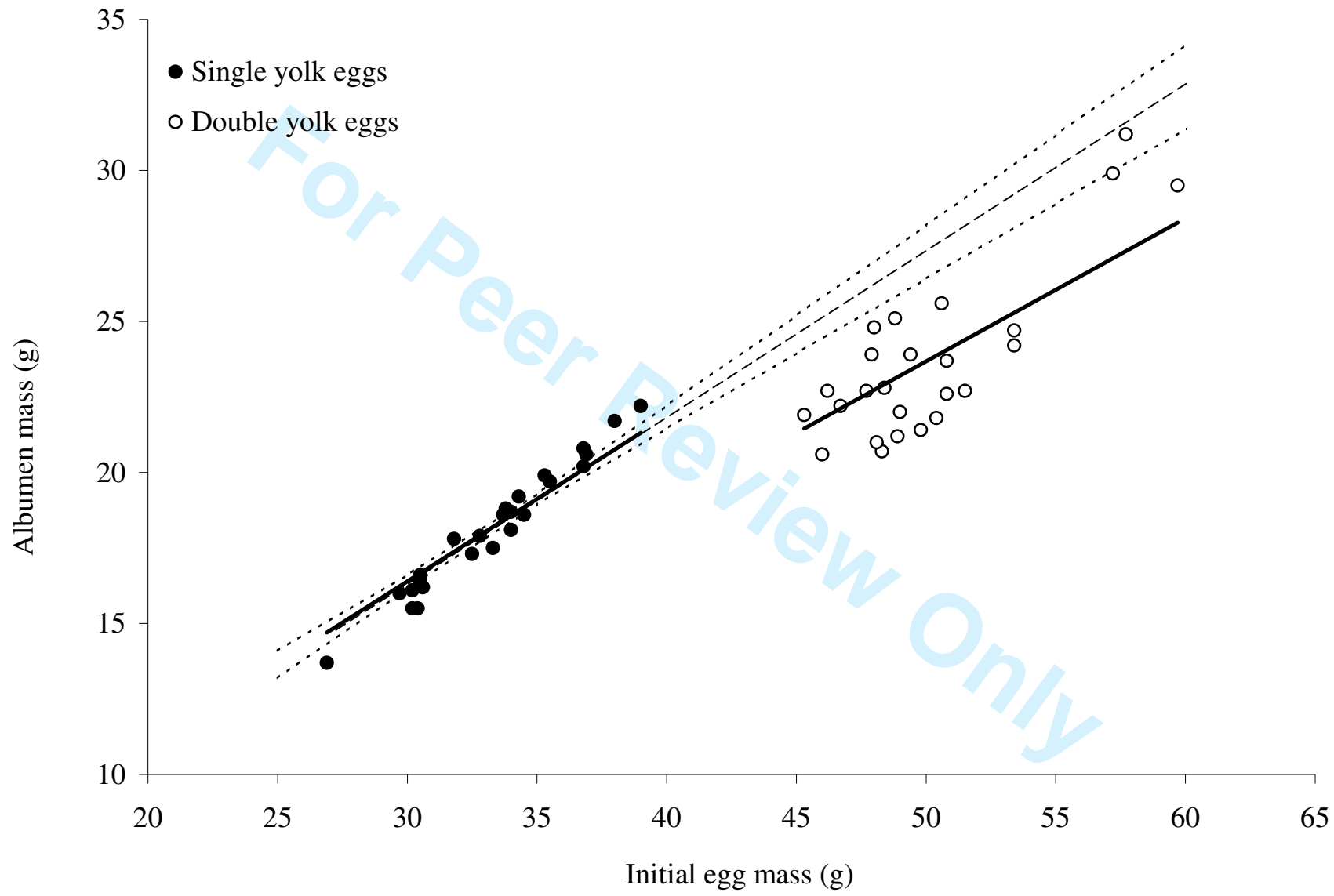
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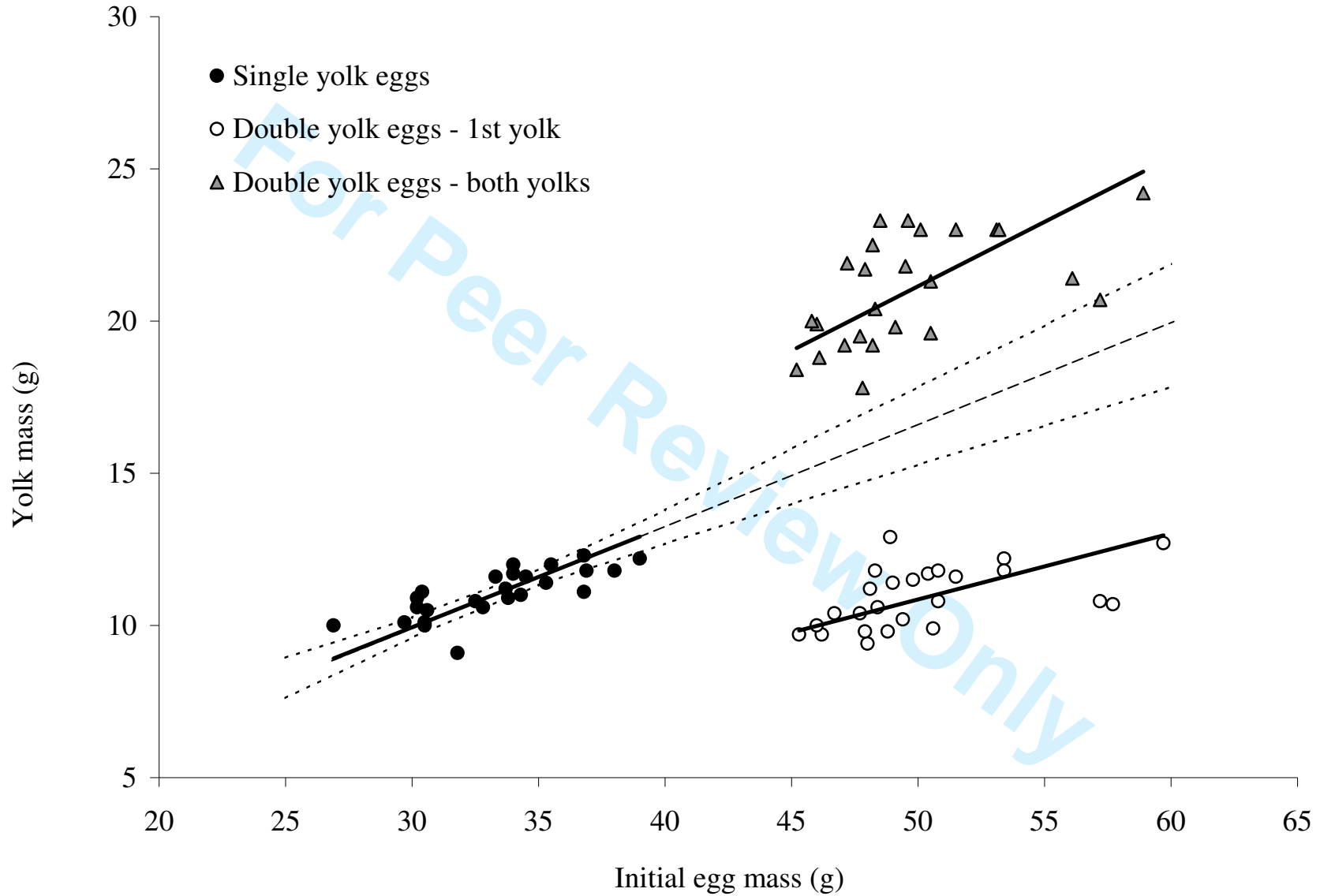
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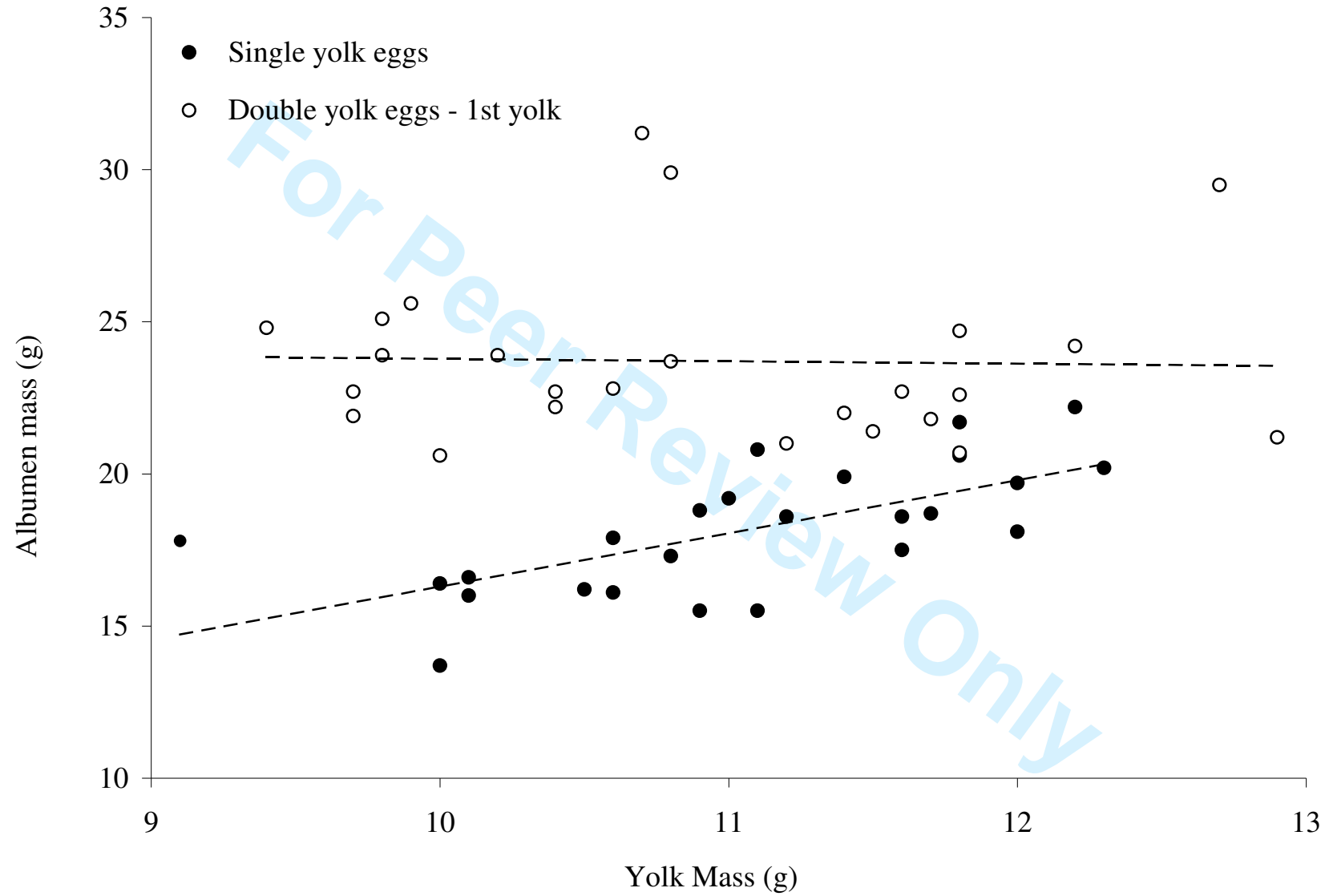








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Normal	Number	Length (cm)	Breadth (cm)	e	Mass (g)	IEM (g)	Shell mass (g)
		4.65	3.63	1.284	33.0	33.4	4.1
		0.18	0.12	0.050	3.0	3.0	0.4
1	4	4.49	3.53	1.272	30.5	30.5	4.1
1	6	4.66	3.61	1.291	32.5	32.5	4.4
1	2	4.53	3.55	1.276	31.4	31.8	4.9
1	10	4.52	3.35	1.349	26.9	26.9	3.2
1	15	4.91	3.8	1.292	38.8	39	4.6
1	17	4.56	3.55	1.285	30.6	30.6	3.9
1	20	4.68	3.64	1.286	33.9	34	3.6
1	22	5.17	3.64	1.420	36.7	36.9	4.5
1	24	4.67	3.55	1.315	32.7	32.8	4.3
1	26	4.34	3.51	1.236	29.3	29.7	3.6
1	30	4.7	3.76	1.250	36.1	36.8	4.9
1	32	4.75	3.73	1.273	35.9	35.5	3.8
1	34	4.9	3.65	1.342	33.1	34	3.9
1	36	4.52	3.73	1.212	33.7	33.8	4.1
1	38	4.55	3.48	1.307	29.6	30.2	3.5
1	40	4.59	3.69	1.244	33.3	33.7	3.9
1	42	4.39	3.72	1.180	32.8	33.3	4.2
1	45	4.79	3.66	1.309	34.8	35.3	4
1	47	4.63	3.82	1.212	36.6	36.8	4.3
1	49	4.75	3.64	1.305	34.2	34.5	4.3
1	52	4.69	3.6	1.303	33	34.3	4.1
1	54	4.56	3.5	1.303	29.8	30.2	3.8
1	56	4.84	3.8	1.274	37.4	38	4.5
1	58	4.53	3.49	1.298	29.3	30.4	3.8
1	60	4.46	3.54	1.260	30.3	30.5	3.8

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18.2	11.1			0.122	0.544
2.1	0.8			0.010	0.018

Albumen mass (g)	Yolk mass (g)	Yolk #1 cm ³		Shell mass (g)	Albumen mass (g)
16.4	10	9.718173	2.65	0.134	0.538
17.3	10.8	10.49563	2.72	0.135	0.532
17.8	9.1	8.843537	2.57	0.154	0.560
13.7	10	9.718173	2.65	0.119	0.509
22.2	12.2	11.85617	2.83	0.118	0.569
16.2	10.5	10.20408	2.69	0.127	0.529
18.7	11.7	11.37026	2.79	0.106	0.550
20.6	11.8	11.46744	2.80	0.122	0.558
17.9	10.6	10.30126	2.70	0.131	0.546
16	10.1	9.815355	2.66	0.121	0.539
20.8	11.1	10.78717	2.74	0.133	0.565
19.7	12	11.66181	2.81	0.107	0.555
18.1	12	11.66181	2.81	0.115	0.532
18.8	10.9	10.59281	2.72	0.121	0.556
16.1	10.6	10.30126	2.70	0.116	0.533
18.6	11.2	10.88435	2.75	0.116	0.552
17.5	11.6	11.27308	2.78	0.126	0.526
19.9	11.4	11.07872	2.77	0.113	0.564
20.2	12.3	11.95335	2.84	0.117	0.549
18.6	11.6	11.27308	2.78	0.125	0.539
19.2	11	10.68999	2.73	0.120	0.560
15.5	10.9	10.59281	2.72	0.126	0.513
21.7	11.8	11.46744	2.80	0.118	0.571
15.5	11.1	10.78717	2.74	0.125	0.510
16.6	10.1	9.815355	2.66	0.125	0.544

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3 0.333
4 0.020
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6 Yolk mass (g)
7 0.328
8 0.332
9 0.286
10 0.372
11 0.313
12 0.343
13 0.344
14 0.320
15 0.323
16 0.340
17 0.302
18 0.338
19 0.353
20 0.322
21 0.351
22 0.332
23 0.348
24 0.323
25 0.334
26 0.336
27 0.321
28 0.361
29 0.311
30 0.365
31 0.331
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5.61	4.03	1.390	49.7	50.2	5.4
0.27	0.12	0.083	3.6	3.7	0.5

Double yolk	Number	Length (cm)	Breadth (cm)	e	Mass (g)	IEM (g)	Shell mass (g)
2	3	5.73	3.91	1.465	47.7	47.7	5.5
2	5	5.4	4.06	1.330	49.1	49.4	5.7
2	7	5.55	4.12	1.347	50.5	50.6	5.4
2	9	5.43	3.82	1.421	45.2	45.3	5
2	11	5.63	3.91	1.440	46	46.7	4.6
2	14	5.86	3.81	1.538	46.1	46.2	4.7
2	21	5.35	4.13	1.295	49.5	49.8	6.6
2	25	5.72	4.3	1.330	57.2	57.7	5.8
2	27	5.64	4.1	1.376	51.5	51.5	5.8
2	29	5.49	4.2	1.307	53.1	53.4	6.2
2	31	5.51	4.14	1.331	50.5	50.8	5.8
2	33	5.67	3.89	1.458	48.2	48.3	5.1
2	35	6.35	4.08	1.556	58.9	59.7	6
2	37	5.66	3.91	1.448	48.2	48.8	4.5
2	39	5.41	3.94	1.373	45.8	46	5.4
2	41	5.41	3.99	1.356	47.8	48	5.4
2	44	5.49	4.03	1.362	47.9	49	5.3
2	46	5.5	3.98	1.382	47.2	48.1	5.2
2	48	6.36	4	1.590	56.1	57.2	5.9
2	51	5.65	4.16	1.358	53.2	53.4	5.7
2	53	5.43	4.09	1.328	48.5	48.9	4.4
2	55	5.65	3.97	1.423	48.3	48.4	5.2
2	57	5.21	4.18	1.246	49.6	50.4	5.3
2	59	5.66	4.03	1.404	50.1	50.8	5.2
2	61	5.41	3.99	1.356	47.1	47.9	4.8

23.7	10.9	10.2	21.1	0.93		
2.8	1.0	0.9	1.8	0.0		
Albumen mass (g)	Yolk #1 mass (g)	Yolk #2 mass (g)	Total yolk		Yolk #1 cm3	
22.7	10.4	9.1	19.5	0.875	10.1069	8.843537
23.9	10.2	9.6	19.8	0.941176	9.912536	9.329446
25.6	9.9	9.7	19.6	0.979798	9.620991	9.426628
21.9	9.7	8.7	18.4	0.896907	9.426628	8.45481
22.2	10.4	9.5	19.9	0.913462	10.1069	9.232264
22.7	9.7	9.1	18.8	0.938144	9.426628	8.843537
21.4	11.5	10.3	21.8	0.895652	11.1759	10.00972
31.2	10.7	10	20.7	0.934579	10.39845	9.718173
22.7	11.6	11.4	23	0.982759	11.27308	11.07872
24.2	12.2	10.8	23	0.885246	11.85617	10.49563
23.7	10.8	10.5	21.3	0.972222	10.49563	10.20408
20.7	11.8	10.7	22.5	0.90678	11.46744	10.39845
29.5	12.7	11.5	24.2	0.905512	12.34208	11.1759
25.1	9.8	9.4	19.2	0.959184	9.52381	9.135083
20.6	10	10	20	1	9.718173	9.718173
24.8	9.4	8.4	17.8	0.893617	9.135083	8.163265
22	11.4	10.3	21.7	0.903509	11.07872	10.00972
21	11.2	10.7	21.9	0.955357	10.88435	10.39845
29.9	10.8	10.6	21.4	0.981481	10.49563	10.30126
24.7	11.8	11.2	23	0.949153	11.46744	10.88435
21.2	12.9	10.4	23.3	0.806202	12.53644	10.1069
22.8	10.6	9.8	20.4	0.924528	10.30126	9.52381
21.8	11.7	11.6	23.3	0.991453	11.37026	11.27308
22.6	11.8	11.2	23	0.949153	11.46744	10.88435
23.9	9.8	9.4	19.2	0.959184	9.52381	9.135083

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						0.107	0.472
						0.009	0.033
						Shell mass (g)	Albumen mass (g)
	2.68	2.57	5.25	18.95044	3.31	0.115	0.476
	2.67	2.61	5.28	19.24198	3.32	0.115	0.484
	2.64	2.62	5.26	19.04762	3.31	0.107	0.506
	2.62	2.53	5.15	17.88144	3.24	0.110	0.483
	2.68	2.60	5.29	19.33916	3.33	0.099	0.475
	2.62	2.57	5.19	18.27017	3.27	0.102	0.491
	2.77	2.67	5.45	21.18562	3.43	0.133	0.430
	2.71	2.65	5.36	20.11662	3.37	0.101	0.541
	2.78	2.77	5.55	22.3518	3.49	0.113	0.441
	2.83	2.72	5.55	22.3518	3.49	0.116	0.453
	2.72	2.69	5.41	20.69971	3.41	0.114	0.467
	2.80	2.71	5.51	21.86589	3.47	0.106	0.429
	2.87	2.77	5.64	23.51798	3.55	0.101	0.494
	2.63	2.59	5.22	18.65889	3.29	0.092	0.514
	2.65	2.65	5.30	19.43635	3.34	0.117	0.448
	2.59	2.50	5.09	17.29835	3.21	0.113	0.517
	2.77	2.67	5.44	21.08844	3.43	0.108	0.449
	2.75	2.71	5.46	21.2828	3.44	0.108	0.437
	2.72	2.70	5.42	20.79689	3.41	0.103	0.523
	2.80	2.75	5.55	22.3518	3.49	0.107	0.463
	2.88	2.68	5.56	22.64334	3.51	0.090	0.434
	2.70	2.63	5.33	19.82507	3.36	0.107	0.471
	2.79	2.78	5.57	22.64334	3.51	0.105	0.433
	2.80	2.75	5.55	22.3518	3.49	0.102	0.445
	2.63	2.59	5.22	18.65889	3.29	0.100	0.499

	0.218	0.203	0.421	27.387	0.864	17.895	5.817	0.337
	0.018	0.015	0.031	2.000	0.060	1.740	3.354	0.204
							0.670864	0.040886
	Yolk #1 mass (g)	Yolk #2 mass (g)	Total yolk	pred AM				
	0.218	0.191	0.409	26.044	0.872	16.9984	5.7016	0.33542
	0.206	0.194	0.401	26.972	0.886	16.6482	7.2518	0.435591
	0.196	0.192	0.387	27.628	0.927	16.1229	9.4771	0.587804
	0.214	0.192	0.406	24.734	0.885	15.7727	6.1273	0.388475
	0.223	0.203	0.426	25.498	0.871	16.9984	5.2016	0.306005
	0.210	0.197	0.407	25.225	0.900	15.7727	6.9273	0.439196
	0.231	0.207	0.438	27.191	0.787	18.9245	2.4755	0.130809
	0.185	0.173	0.359	31.504	0.990	17.5237	13.6763	0.780446
	0.225	0.221	0.447	28.119	0.807	19.0996	3.6004	0.188507
	0.228	0.202	0.431	29.156	0.830	20.1502	4.0498	0.200981
	0.213	0.207	0.419	27.737	0.854	17.6988	6.0012	0.339074
	0.244	0.222	0.466	26.372	0.785	19.4498	1.2502	0.064278
	0.213	0.193	0.405	32.596	0.905	21.0257	8.4743	0.403045
	0.201	0.193	0.393	26.645	0.942	15.9478	9.1522	0.573885
	0.217	0.217	0.435	25.116	0.820	16.298	4.302	0.263959
	0.196	0.175	0.371	26.208	0.946	15.2474	9.5526	0.626507
	0.233	0.210	0.443	26.754	0.822	18.7494	3.2506	0.173371
	0.233	0.222	0.455	26.263	0.800	18.3992	2.6008	0.141354
	0.189	0.185	0.374	31.231	0.957	17.6988	12.2012	0.68938
	0.221	0.210	0.431	29.156	0.847	19.4498	5.2502	0.269936
	0.264	0.213	0.476	26.699	0.794	21.3759	-0.1759	-0.008229
	0.219	0.202	0.421	26.426	0.863	17.3486	5.4514	0.314227
	0.232	0.230	0.462	27.518	0.792	19.2747	2.5253	0.131016
	0.232	0.220	0.453	27.737	0.815	19.4498	3.1502	0.161966
	0.205	0.196	0.401	26.153	0.914	15.9478	7.9522	0.498639

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X	Y	XY	X ²	Y ²
			0	0
			0	0
30.5	16.4	500.2	930.25	268.96
32.5	17.3	562.25	1056.25	299.29
31.8	17.8	566.04	1011.24	316.84
26.9	13.7	368.53	723.61	187.69
39	22.2	865.8	1521	492.84
30.6	16.2	495.72	936.36	262.44
34	18.7	635.8	1156	349.69
36.9	20.6	760.14	1361.61	424.36
32.8	17.9	587.12	1075.84	320.41
29.7	16	475.2	882.09	256
36.8	20.8	765.44	1354.24	432.64
35.5	19.7	699.35	1260.25	388.09
34	18.1	615.4	1156	327.61
33.8	18.8	635.44	1142.44	353.44
30.2	16.1	486.22	912.04	259.21
33.7	18.6	626.82	1135.69	345.96
33.3	17.5	582.75	1108.89	306.25
35.3	19.9	702.47	1246.09	396.01
36.8	20.2	743.36	1354.24	408.04
34.5	18.6	641.7	1190.25	345.96
34.3	19.2	658.56	1176.49	368.64
30.2	15.5	468.1	912.04	240.25
38	21.7	824.6	1444	470.89
30.4	15.5	471.2	924.16	240.25
30.5	16.6	506.3	930.25	275.56

SE	0.674435333
s ² _r	0.133703836
SP _{x,y}	148.702
(SP _{x,y}) ²	22112.2848
SS _y	107.2016
SS _x	212.36
n	25
Σx	832.0
mean x	33.28
Σy	453.6
Mean y	18.144
Σxy	15244.51
ΣxΣy	377395.2
Σy ²	8337.32
(Σy) ²	205752.96
Σx ²	27901.32
(Σx) ²	692224

x-value					
X	SE	Pred Y		Min	Max
			t(n-2)	2.069	
	60				
	25	0.22	13.65	0.45518	13.19482 14.10518
	30	0.11	16.38	0.22759	16.15241 16.60759
	35	0.085	19.11	0.175865	18.93414 19.28587
	40	0.184	21.84	0.380696	21.4593 22.2207
	45	0.303	24.57	0.626907	23.94309 25.19691
	50	0.426	27.3	0.881394	26.41861 28.18139
	55	0.55	30.03	1.13795	28.89205 31.16795
	60	0.674	32.76	1.394506	31.36549 34.15451

- 1) Input data into B & C
- 2) Work out pred Y
- 3) Work out SE by putting x-value in L2
- 4) Input SE value into appropriate row from II

	X	Y	XY	X ²	Y ²
1					
2					
3					
4					
5				0	0
6				0	0
7					
8	30.5	10	305	930.25	100
9					
10	32.5	10.8	351	1056.25	116.64
11	31.8	9.1	289.38	1011.24	82.81
12					
13	26.9	10	269	723.61	100
14	39	12.2	475.8	1521	148.84
15	30.6	10.5	321.3	936.36	110.25
16	34	11.7	397.8	1156	136.89
17	36.9	11.8	435.42	1361.61	139.24
18	32.8	10.6	347.68	1075.84	112.36
19					
20	29.7	10.1	299.97	882.09	102.01
21					
22	36.8	11.1	408.48	1354.24	123.21
23	35.5	12	426	1260.25	144
24					
25	34	12	408	1156	144
26	33.8	10.9	368.42	1142.44	118.81
27	30.2	10.6	320.12	912.04	112.36
28	33.7	11.2	377.44	1135.69	125.44
29	33.3	11.6	386.28	1108.89	134.56
30	35.3	11.4	402.42	1246.09	129.96
31	36.8	12.3	452.64	1354.24	151.29
32	34.5	11.6	400.2	1190.25	134.56
33	34.3	11	377.3	1176.49	121
34	30.2	10.9	329.18	912.04	118.81
35	38	11.8	448.4	1444	139.24
36	30.4	11.1	337.44	924.16	123.21
37	30.5	10.1	308.05	930.25	102.01
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SE	0.319034237
s ² _r	0.280516799
SP _{x,y}	44.128
(SP _{x,y}) ²	1947.280384
SS _y	15.6216
SS _x	212.36
n	25
Σx	832.0
mean x	33.28
Σy	276.4
Mean y	11.056
Σxy	9242.72
ΣxΣy	229964.8
Σy ²	3071.5
(Σy) ²	76396.96
Σx ²	27901.32
(Σx) ²	692224

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x-value		t(n-2)				
	25		2.069			
X	SE	Pred Y		Min	Max	
	25	0.319	8.275	0.660011	7.614989	8.935011
	30	0.159	9.93	0.328971	9.601029	10.25897
	35	0.123	11.585	0.254487	11.33051	11.83949
	40	0.266	13.24	0.550354	12.68965	13.79035
	45	0.439	14.895	0.908291	13.98671	15.80329
	50	0.617	16.55	1.276573	15.27343	17.82657
	55	0.796	18.205	1.646924	16.55808	19.85192
	60	0.977	19.86	2.021413	17.83859	21.88141

- 1) Input data into B & C
- 2) Work out pred Y
- 3) Work out SE by putting x-value in L2
- 4) Input SE value into appropriate row from I1

X	Y	XY	X ²	Y ²
			0	0
			0	0
30.5	4.1	125.05	930.25	16.81
32.5	4.4	143	1056.25	19.36
31.8	4.9	155.82	1011.24	24.01
26.9	3.2	86.08	723.61	10.24
39	4.6	179.4	1521	21.16
30.6	3.9	119.34	936.36	15.21
34	3.6	122.4	1156	12.96
36.9	4.5	166.05	1361.61	20.25
32.8	4.3	141.04	1075.84	18.49
29.7	3.6	106.92	882.09	12.96
36.8	4.9	180.32	1354.24	24.01
35.5	3.8	134.9	1260.25	14.44
34	3.9	132.6	1156	15.21
33.8	4.1	138.58	1142.44	16.81
30.2	3.5	105.7	912.04	12.25
33.7	3.9	131.43	1135.69	15.21
33.3	4.2	139.86	1108.89	17.64
35.3	4	141.2	1246.09	16
36.8	4.3	158.24	1354.24	18.49
34.5	4.3	148.35	1190.25	18.49
34.3	4.1	140.63	1176.49	16.81
30.2	3.8	114.76	912.04	14.44
38	4.5	171	1444	20.25
30.4	3.8	115.52	924.16	14.44
30.5	3.8	115.9	930.25	14.44

SE	0.195546848
s ² _r	0.10538673
SP _{x,y}	19.53
(SP _{x,y}) ²	381.4209
SS _y	4.22
SS _x	212.36
n	25
Σx	832.0
mean x	33.28
Σy	102
Mean y	4.08
Σxy	3414.09
ΣxΣy	84864
Σy ²	420.38
(Σy) ²	10404
Σx ²	27901.32
(Σx) ²	692224

X	SE	Pred Y	Min	Max
25	0.196	3.05	2.644476	3.455524
30	0.098	3.66	3.457238	3.862762
35	0.075	4.27	4.114825	4.425175
40	0.163	4.88	4.542753	5.217247
45	0.269	5.49	4.933439	6.046561
50	0.378	6.1	5.317918	6.882082
55	0.488	6.71	5.700328	7.719672
60	0.599	7.32	6.080669	8.559331

- 1) Input data into B & C
- 2) Work out pred Y
- 3) Work out SE by putting x-value in L2
- 4) Input SE value into appropriate row from II

X	Y	XY	X ²	Y ²
			0	0
			0	0
30.5	4.49	136.945	930.25	20.1601
32.5	4.66	151.45	1056.25	21.7156
31.8	4.53	144.054	1011.24	20.5209
26.9	4.52	121.588	723.61	20.4304
39	4.91	191.49	1521	24.1081
30.6	4.56	139.536	936.36	20.7936
34	4.68	159.12	1156	21.9024
36.9	5.17	190.773	1361.61	26.7289
32.8	4.67	153.176	1075.84	21.8089
29.7	4.34	128.898	882.09	18.8356
36.8	4.7	172.96	1354.24	22.09
35.5	4.75	168.625	1260.25	22.5625
34	4.9	166.6	1156	24.01
33.8	4.52	152.776	1142.44	20.4304
30.2	4.55	137.41	912.04	20.7025
33.7	4.59	154.683	1135.69	21.0681
33.3	4.39	146.187	1108.89	19.2721
35.3	4.79	169.087	1246.09	22.9441
36.8	4.63	170.384	1354.24	21.4369
34.5	4.75	163.875	1190.25	22.5625
34.3	4.69	160.867	1176.49	21.9961
30.2	4.56	137.712	912.04	20.7936
38	4.84	183.92	1444	23.4256
30.4	4.53	137.712	924.16	20.5209
30.5	4.46	136.03	930.25	19.8916

SE	0.238547079
s ² _r	0.016726745
SP _{x,y}	9.3876
(SP _{x,y}) ²	88.12703376
SS _y	0.799704
SS _x	212.36
n	25
Σx	832.0
mean x	33.28
Σy	116.18
Mean y	4.6472
Σxy	3875.858
ΣxΣy	96661.76
Σy ²	540.7114
(Σy) ²	13497.7924
Σx ²	27901.32
(Σx) ²	692224

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x-value		t(n-2)				
	60		2.069			
X	SE	Pred Y		Min	Max	
	25	0.078	4.276	0.161382	4.114618	4.437382
	30	0.039	4.496	0.080691	4.415309	4.576691
	35	0.03	4.716	0.06207	4.65393	4.77807
	40	0.065	4.936	0.134485	4.801515	5.070485
	45	0.107	5.156	0.221383	4.934617	5.377383
	50	0.151	5.376	0.312419	5.063581	5.688419
	55	0.194	5.596	0.401386	5.194614	5.997386
	60	0.239	5.816	0.494491	5.321509	6.310491

- 1) Input data into B & C
- 2) Work out pred Y
- 3) Work out SE by putting x-value in L2
- 4) Input SE value into appropriate row from II

X	Y	XY	X ²	Y ²
			0	0
			0	0
30.5	3.53	107.665	930.25	12.4609
32.5	3.61	117.325	1056.25	13.0321
31.8	3.55	112.89	1011.24	12.6025
26.9	3.35	90.115	723.61	11.2225
39	3.8	148.2	1521	14.44
30.6	3.55	108.63	936.36	12.6025
34	3.64	123.76	1156	13.2496
36.9	3.64	134.316	1361.61	13.2496
32.8	3.55	116.44	1075.84	12.6025
29.7	3.51	104.247	882.09	12.3201
36.8	3.76	138.368	1354.24	14.1376
35.5	3.73	132.415	1260.25	13.9129
34	3.65	124.1	1156	13.3225
33.8	3.73	126.074	1142.44	13.9129
30.2	3.48	105.096	912.04	12.1104
33.7	3.69	124.353	1135.69	13.6161
33.3	3.72	123.876	1108.89	13.8384
35.3	3.66	129.198	1246.09	13.3956
36.8	3.82	140.576	1354.24	14.5924
34.5	3.64	125.58	1190.25	13.2496
34.3	3.6	123.48	1176.49	12.96
30.2	3.5	105.7	912.04	12.25
38	3.8	144.4	1444	14.44
30.4	3.49	106.096	924.16	12.1801
30.5	3.54	107.97	930.25	12.5316

SE	0.029086197
s ² _r	0.002331622
SP _{x,y}	7.6988
(SP _{x,y}) ²	59.27152144
SS _y	0.332736
SS _x	212.36
n	25
Σx	832.0
mean x	33.28
Σy	90.54
Mean y	3.6216
Σxy	3020.87
ΣxΣy	75329.28
Σy ²	328.2324
(Σy) ²	8197.4916
Σx ²	27901.32
(Σx) ²	692224

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x-value		t(n-2)			
X	SE	Pred Y		Min	Max
25	0.029	3.315	0.060001	3.254999	3.375001
30	0.015	3.495	0.031035	3.463965	3.526035
35	0.011	3.675	0.022759	3.652241	3.697759
40	0.024	3.855	0.049656	3.805344	3.904656
45	0.04	4.035	0.08276	3.95224	4.11776
50	0.056	4.215	0.115864	4.099136	4.330864
55	0.073	4.395	0.151037	4.243963	4.546037
60	0.089	4.575	0.184141	4.390859	4.759141

- 1) Input data into B & C
- 2) Work out pred Y
- 3) Work out SE by putting x-value in L2
- 4) Input SE value into appropriate row from I1

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4 1 **Double-yolked pheasant eggs provide an insight into the control of albumen secretion in**
5 2 **bird eggs**

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9 4 D.C. DEEMING

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22 Barry: I have failed to insert the Figures for this MS from Excel after they were edited
23 by the author post PDL correction. I have uploaded the Excel file with the 5 figures in
24 the first 5 worksheets. I hope you are able to insert them correctly – my computer
25 simply screws them up (I hate Excel graphs and cannot use them) Note that the legend
26 on Figure one has capital letters for the measurements but again I cannot change this.
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36 **Running title: Control of albumen secretion**
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58 **Accepted for publication: 3rd Aug 2010**
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4 11 **Abstract** 1. The possible role of the presence of the yolk in stimulating secretion of albumen
5 12 was investigated.
6
7 13 2. Double-yolked and single-yolked pheasant (*Phasianus colchinus*) eggs were opened to
8
9 14 determine the masses of the shell, albumen and yolk(s).
10
11 15 3. In double-yolked eggs, the two yolk masses were not significantly different. Albumen
12 16 mass was increased above that expected from an egg with a single-yolk of comparable size
13 17 but below that expected from an egg having a mass of the combined yolks. The mass of shell
14 18 per unit area reflected the mass of the initial mass of the egg irrespective of the number of
15 19 yolks.
16 20
17 21 4. The additional mass of albumen is unrelated to yolk or initial egg mass. It is postulated that
18 22 in double-yolked eggs the oviduct is mechanically stimulated by the presence of both yolks,
19 23 which empties the stores of water-soluble albumen proteins in the magnum wall. Such stores
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24 INTRODUCTION

25 Absolute and relative masses of the shell, albumen and yolk vary between bird species and
26 are associated with the degree of hatchling maturity (Carey *et al.*, 1980; Sotherland and Rahn,
27 1987; Deeming, 2007). There is also allometric scaling of these components with initial egg
28 mass between species (Deeming, 2007) and within species (*e.g.*, Anderson and Deeming,
29 2002; Fernández and Reboreda, 2008). In general, as egg mass increases it is associated with
30 a bigger increase in albumen mass rather than the yolk or shell (Hill, 1995; Deeming 2007).
31 Intra-clutch variation in egg composition appears to be small although within a clutch the first
32 egg laid tends to have a higher proportion of albumen (Romanoff and Romanoff, 1949). For
33 many bird species between-clutch variation in egg composition is the greatest between
34 individuals. For example, 50-80% of the variation in the composition of duck eggs is related
35 to the female that laid them (Rohwer, 1986; Hepp *et al.*, 1987; Flint and Grand, 1999). In
36 addition, variation in albumen mass is also explained more by between-female differences
37 than by differences among eggs in yolk mass (Rohwer, 1986; Hepp *et al.*, 1987). How can all
38 of this variation in egg composition be explained in terms of the mechanism of egg
39 production?

40 The physiological and biochemical processes of ovulation, fertilisation and then deposition
41 of the albumen proteins, shell membranes and the calcitic shell are well described, at least in
42 poultry species (Gilbert, 1971; Palmer & Guilette, 1991), but there are still aspects of these
43 processes that are unclear.

44 Whilst the process and timing of yolk production is relatively well understood (*e.g.*,
45 McIndoe, 1971; Grau, 1982; Astheimer and Grau, 1985; Astheimer, 1986; Warham, 1990)
46 the same is not true for the deposition of albumen. The synthesis and the process of secretion
47 of albumen proteins are well described (see Gilbert, 1971; Edwards *et al.*, 1976; Palmer and
48 Gillette, 1991) but the mechanisms that control the secretion, and in particular, the quantity of

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3 49 albumen proteins to be secreted, are undefined. This is important because published data
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5 50 imply that, for an individual female, the composition of the eggs she lays in a clutch will
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7 51 exhibit relatively more variation in albumen mass than in yolk mass (Rohwer, 1986; Hepp *et*
8
9 52 *al.*, 1987).

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11
12 53 Double-yolked eggs are common in the commercial production of poultry, waterfowl and
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14 54 game birds, where they are considered as a loss to overall egg production during the laying
15
16 55 season. Double-yolked eggs tend to be more common during the start of a laying period and
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18 56 decrease in incidence as birds mature (*e.g.*, Lewis *et al.*, 1997). The absolute quantities of the
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20 57 egg components are greater in the double-yolked eggs as they become larger but their relative
21
22 58 proportions also differ from those in single-yolked eggs (Romanoff and Romanoff, 1949).
23
24 59 The internal structure of double-yolked eggs varies according to where the yolks meet in the
25
26 60 oviduct and the relative sizes of the two yolks (Romanoff and Romanoff, 1949).

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29 61 Despite the fact that double-yolked eggs are common there has been little interest in
30
31 62 considering how such an egg is formed and how this process would differ from that for a
32
33 63 single-yolked egg. Differences in composition of double-yolked and single-yolked eggs of
34
35 64 differing sizes may provide an insight into mechanisms underlying the formation of the egg
36
37 65 and in particular, secretion of albumen. To date our understanding of albumen secretion
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39 66 suggest that mechanical stimulation by the yolk may be crucial for controlling the secretion
40
41 67 of proteins, though there may be neuronal or endocrine contributions (Palmer and Guillette,
42
43 68 1991).

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45
46 69 Within this context, double-yolked eggs present a means to elucidate the mechanism for
47
48 70 control of albumen secretion. If, in single-yolked eggs the quantity of albumen is fixed, then
49
50 71 it will vary very little between eggs in a clutch and a double-yolked egg should contain the
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52 72 same mass of albumen as that produced in a single-yolked egg. Alternatively, the quantity of
53
54 73 albumen in a double-yolked egg may be equivalent to that produced for an egg with a single

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3 74 yolk of the combined masses of the two yolks and will be much greater than in a single-
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5 75 yolked egg. However, in the fowl, production of a single-yolked egg uses approximately two-
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8 76 thirds of the available proteins in the magnum wall (Edwards *et al.*, 1976) and it is predicted
9
10 77 that double-yolked eggs can only obtain approximately 50% more albumen than in a single-
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12
13 78 yolked egg.

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15 79 These hypotheses were tested in this study by comparison of the allometric relationships
16
17 80 between the mass of the whole egg and the shell, yolk and albumen in single- and double-
18
19
20 81 yolked eggs of the pheasant (*Phasianus colchinus*).
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MATERIALS AND METHODS

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27 84 Eggs were collected from semi-domesticated pheasants reared on a commercial game farm
28
29 85 during the spring of 2010. Half of the eggs were large and assumed to be double-yolked,
30
31 86 which, on opening, proved to be the case. The other half were of a size range typical for
32
33 87 pheasant eggs. In the laboratory, the maximum length (L) and breadth (B) of each egg was
34
35 88 measured using electronic callipers to 0.01 cm prior to weighing to the nearest 0.1 g. As the
36
37 89 eggs were not freshly laid, a small hole was made in the blunt of the egg and water was
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39 90 introduced into the air space using a pipette to fill the air space within. The egg was then re-
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41 91 weighed to determine the initial egg mass (IEM, g) as described by Rahn *et al.* (1976). The
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43 92 egg was opened around the blunt end using curved forceps to expose the contents. For single-
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45 93 yolked eggs, the yolk was separated from the albumen and weighed to the nearest 0.1 g. For
46
47 94 double-yolked eggs the two yolks were abutted with no albumen between them (Type I
48
49 95 described by Romanoff and Romanoff, 1949). They were first removed from the surrounding
50
51 96 albumen before being manually separated prior to weighing to determine the mass of each
52
53 97 yolk (to the nearest 0.1 g). If the yolk was broken during separation from the albumen or
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55 98 from each other the egg was discarded. For all eggs, the shell was dried with a tissue and
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3 99 weighed to the nearest 0.1 g. Albumen mass (AM, g) was calculated by subtracting shell and
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6 100 yolk masses from the value for IEM. For analysis, the heavier yolk in the double-yolked eggs,
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8 101 the 'first yolk', was considered to be the yolk that would have formed the basis of a single-
9
10 102 yolked egg and would have been next in the laying sequence. The other yolk is referred to as
11
12 103 the 'second yolk'.

14
15 104 Data were analysed using PASW Statistics (SPSS Inc.). Given that an egg with no mass
16
17 105 will also have components with no mass, linear regression analysis that forced the line
18
19 106 through the origin was used to determine the relationships between egg mass and the
20
21 107 components. Slopes of regression estimates were compared using the method of Bailey
22
23 108 (1981). A 95% confidence interval (Fowler *et al.*, 1995) was calculated for each of these
24
25 109 regression estimates to ease comparison between the two types of egg when the lines were
26
27 110 extrapolated to accommodate the range of egg sizes for double-yolked eggs. Pearson
28
29 111 correlation analysis was used to compare the relationships between yolk mass (YM) and
30
31 112 albumen mass (AM) because it was unclear which component should be considered as
32
33 113 independent. Analysis of covariance (ANCOVA) was used to determine the effect of the
34
35 114 number of yolks on the mass of the albumen and shell in an egg, with total yolk mass as a
36
37 115 covariate.
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43 44 45 46 117 RESULTS

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48 118 Double-yolked eggs were approximately 1.5 times heavier than single-yolked eggs (n = 25
49
50 119 for each group; Table 1). In all measurements, the double-yolked eggs were significantly
51
52 120 different from the single-yolked eggs. The only exception was for the mass of the larger yolk
53
54 121 in each of the double-yolked eggs, which was not significantly different from the single yolk
55
56 122 in more typical eggs (Table 1). On average, weights of the two yolks in the double-yolked
57
58 123 eggs differed by 0.7 g (Table 1).
59
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Insert Table 1 here

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3 124 Double-yolked eggs were longer and broader than single-yolked eggs, although the
4
5
6 125 proportional increase in mean length over that of single-yolked eggs was greater than the
7
8 126 increase in mean breadth (1.21 times versus 1.11 times, respectively). This meant that the
9
10 127 ratio of length to breadth (L/B) was significantly greater in double-yolked eggs (Table 1).
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12
13 128 Extrapolation of regression analyses for the relationships between the maximum linear
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15 129 dimensions and mass showed that double-yolked eggs were longer than predicted, but two-
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17 130 thirds of the values were within the 95% confidence interval. By contrast, double-yolked eggs
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19 131 were narrower than predicted on the basis of a single-yolked egg of the same mass, with most
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22 132 values lying outside of the 95% confidence interval (Figure 1).
23

24
25 133 The shell formed 0.122 of the initial mass of the single-yolked egg which was
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27 134 significantly greater than the 0.107 proportion of the IEM formed by the shell of double-
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29 135 yolked eggs (Table 1). For a given IEM, double-yolked eggs had lighter eggshells than would
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31 136 be predicted from a single-yolked egg of an equivalent mass (Figure 2; Table 2). Although
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34 137 there was some overlap with the lower part of the 95% confidence interval, the slopes of the
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36 138 regression lines shown in Figure 2 were significantly different from each other (Table 2).
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38
39 139 However, when the surface area of the eggs was calculated ($SA = 4.835IEM^{0.662}$; Paganelli *et*
40
41 140 *al.*, 1974) the masses of shell per cm² were not significantly different (0.083 ± 0.007 g/cm²
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43 141 versus 0.083 ± 0.007 g/cm² for single- and double-yolked eggs respectively). Moreover,
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45 142 ANCOVA for shell mass showed no significant effect of either the number of yolks as a fixed
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48 143 factor, the yolk mass as a covariate or the interaction. Therefore, when the analysis was
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50 144 controlled for yolk size, shell mass was unaffected by the number of yolks.
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53 145 Not unsurprisingly the combined mass of two yolks was significantly greater than a single
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55 146 yolk (Table 1). For any given IEM for double-yolked eggs, the larger yolk was smaller than
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57
58 147 would be predicted on the basis of a single-yolked egg of an equivalent IEM (Table 2)
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60 148 whereas the combined yolk mass was far higher than expected from extrapolation of

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3 149 regression estimates with both sets of values falling well outside of the 95% confidence
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5 150 interval (Figure 4). All the slopes of the lines shown in Figure 4 are significantly different
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8 151 from each other (Table 2). Insert Table 2 here

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10 152 Double-yolked eggs had significantly more albumen mass than single-yolked eggs but
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12 153 this formed a significantly smaller proportion of their IEM (Table 1). Regression analysis
13
14 154 showed that, for a given IEM, albumen mass in double-yolked eggs was less than predicted
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16 155 on the basis of the regression relationship for single-yolked eggs with almost all values
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18 156 falling well below the 95% confidence interval around the extrapolated regression (Figure 3;
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20 157 Table 2). The slope for the relationship between IEM and AM was significantly smaller for
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22 158 double-yolked eggs than for single-yolked eggs (Table 2). ANCOVA for AM showed
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24 159 significant effects of the number of yolks as a fixed factor, the yolk mass as a covariate and
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26 160 the interaction of these factors. Therefore, when analysis was controlled for yolk mass there
27
28 161 were significant effects of number and mass of yolks on albumen mass.

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34 162 There was a significant positive correlation between yolk mass and albumen for the
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36 163 single-yolked eggs but no correlation for double-yolked eggs (Figure 5; $r_{25} = 0.668$, $P <$
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38 164 0.001 and $r_{25} = 0.015$, $P > 0.05$ respectively). In double-yolked eggs, the mass of albumen did
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40 165 not exhibit any significant relationship with the mass of either of the individual yolks (Figure
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42 166 5). For double-yolked eggs, predicted albumen mass was calculated on the basis of yolk mass
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44 167 of single-yolked eggs ($AM = 1.751YM - 1.212$; Figure 5), which was subtracted from the
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46 168 observed albumen mass. On average, this additional albumen in double-yolked eggs weighed
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48 169 5.82 g (SE = 0.67) and was 33.7% (SE = 4.0) of the albumen mass predicted on the basis of
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50 170 yolk size).

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DISCUSSION

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60 173 The two yolks in the doubled-yolked eggs were abutted in a manner comparable to Type I

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3 174 defined by Romanoff and Romanoff (1949), who suggested that such yolks come together
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5 175 prior to the magnum. This interpretation is supported by the fact that the two yolks had no
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8 176 albumen between them and were of comparable size with the smaller yolk being on average
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10 177 93% of the largest yolk. This suggests that the second yolk was next in the ovulation
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12 178 sequence, was released from the ovary prematurely and entered the infundibulum closely
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15 179 behind the first yolk.

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18 180 Composition of single-yolked eggs was comparable to published values (Kirikçi *et al.*,
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20 181 2005). However, contrary to prediction, double-yolked pheasant eggs did not have a
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22 182 composition that was simply associated with the greater amount of yolk present. Instead,
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24 183 albumen mass was increased together with an increase in shell mass, but both had reduced
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27 184 values compared with those predicted on the basis of the relationship for a single-yolked egg
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29 185 of the same IEM (Figures 2 and 3, respectively). The quantity of albumen in the doubled-
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31 186 yolked eggs showed no correlation with the mass of the larger yolk or the combined yolk
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34 187 masses (Figure 5). Moreover, the mass of additional albumen, compared with that expected
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36 188 from the largest yolk mass, was around one third of that in a single-yolked egg rather than the
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39 189 50% predicted from data for fowl eggs (Edwards *et al.*, 1976).

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41 190 For double-yolked eggs, the mass of the two yolks can be explained by premature
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43 191 ovulation of the second yolk, which then travels down the oviduct with the first yolk to be
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46 192 ovulated. The calcitic shell is deposited on the external surface of the outer shell membrane
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48 193 deposited in the isthmus and its size is presumably a function of the combined quantities of
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51 194 yolk and albumen proteins deposited in the magnum (Sparks and Board, 1991). Therefore,
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53 195 the mass of the shell in double-yolked eggs is simply a reflection of the large structure that it
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56 196 encloses and shell deposition per unit area does not differ between eggs types. By contrast,
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58 197 the difference in the masses of albumen between the two types of eggs is harder to explain.

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60 198 These results for double-yolked eggs firstly indicate that the avian oviduct is capable of

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3 199 secreting considerably more albumen that would be normally expected during typical egg
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5 200 formation, the quantity of albumen in any egg laid by the same bird is not fixed, and
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8 201 secondly, there seems to be an upper limit to the amount of additional albumen mass that can
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10 202 be incorporated into the egg.
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13 203 Albumen is secreted in two discrete processes: the first involves secretion of water-
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15 204 soluble proteins from the magnum and the second involves the absorption of water by these
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17 205 proteins during 'plumping' (Gilbert, 1971; Palmer and Guillette, 1991). The final mass of
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19 206 albumen at oviposition is a function of the amount of water-soluble proteins secreted around
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21 207 the yolk. In the domestic fowl, the yolk spends around 3 h in the magnum (Melek *et al.*,
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23 208 1973), during which period water-soluble proteins are secreted by the wall of the magnum
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25 209 and accumulate as albumen proteins around the yolk.
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29 210 This process of secretion does not, however, lead to the depletion of the albumen proteins
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31 211 in the magnum wall (Edwards *et al.*, 1976). The process of accumulation of water-soluble
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33 212 proteins in readiness for the next yolk in the laying cycle starts from a level that is around a
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35 213 third of the maximum reached prior to secretion (Edwards *et al.*, 1976). It would seem that
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37 214 the production of double-yolked eggs is possible because the oviduct releases an additional,
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39 215 albeit limited, amount of albumen proteins. The mechanism behind this process may either be
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41 216 dependent on the time spent in the magnum or be the effect of a mechanical stimulus.
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45 217 The time spent in the magnum is important because increasing the length of the effective
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47 218 daylength for domestic fowl to 27 h leads to significant increases in albumen mass (by 1.5 g
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49 219 in fowl eggs) and shell mass but with no significant effect on yolk mass (Morris, 1973). The
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51 220 explanation for this lies in the prolonged period of egg formation and, in particular, the time
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53 221 the yolk spends in the magnum and isthmus, which increases by 30 min (Melek *et al.*, 1973).
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55 222 In general, however, longer formation times for whole eggs are associated with increases in
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57 223 the three different components (reviews by Shanawany, 1982, 1990; see also Siopes and
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3 224 Neely, 1997). In double-yolked eggs, the two yolks are abutted against each other when they
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5 225 enter the magnum and, as a consequence, are longer than a single yolk. This means that the
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8 226 yolks will spend more time in the magnum because as the leading yolk enters the isthmus the
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10 227 second yolk is still present. This may lead to a longer period of protein secretion. However,
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12 228 this cannot be sole explanation for the additional secretion of albumen proteins because long
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15 229 daylengths extend the period spent in the magnum by 16%, but only increase the mass of
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17 230 albumen by 4% (Morris, 1973; Melek *et al.*, 1973).

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20 231 Introduction of foreign bodies into the magnum will stimulate secretion (Palmer and
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22 232 Guillette, 1991), supporting the hypothesis that mechanical stimulation by the yolk in the
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24 233 lumen stretching the wall of the magnum plays a key role in the process of albumen
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27 234 secretion, although the process may be under neuronal control. If, under normal
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29 235 circumstances, secretion is uniformly spread along the length of the magnum then as the yolk
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31 236 leaves any particular section of magnum there should remain in the walls approximately a
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33 237 third of the initial reserves of water-soluble proteins (Edwards *et al.*, 1976).

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36 238 In double-yolked eggs this process is supplemented by the second yolk that is following
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38 239 close behind the first yolk. In the eggs studied here, because it is of comparable size, the
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40 240 second yolk may present a comparable mechanical stimulus to that presented by the first
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42 241 yolk. As the second yolk moves through the magnum it could stimulate the wall to continue
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44 242 the process of protein secretion at a near normal rate. However, the reserves of protein are
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46 243 such that they are quickly depleted before the second yolk can accumulate a mass of protein
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48 244 equivalent to that secreted for the first yolk. Although it is possible that the second yolk does
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50 245 not provide a comparable mechanical stimulus as the first yolk, the similarity in yolk sizes in
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52 246 this instance makes this unlikely. Accumulation of protein in the magnum takes hours
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54 247 (Edwards *et al.*, 1976) and so it is more likely that the yolks have moved on down the oviduct
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56 248 before more albumen proteins can be manufactured by the magnum wall.
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3 249 Thus, when two yolks in close proximity enter the magnum, secretion of water-soluble
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6 250 proteins is stimulated but the second yolk leads to the depletion of reserves and limits the
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8 251 quantity of albumen that is deposited in the developing egg. Contrary to the suggestion by
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10 252 Solomon (1991), the magnum does not have sufficient reserves of albumen proteins for two
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13 253 eggs. Ahemeral lighting programmes may increase albumen content of single-yolked eggs
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15 254 because the yolk spends more time in the magnum (Morris, 1973). In the isthmus, deposition
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17 255 of the shell membranes around the albumen proteins presumably reflects the combined
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20 256 quantities of albumen protein and yolk and physically constrains the volume of the egg once
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22 257 plumping is complete. Such a restraint implies that it is secretion of albumen proteins rather
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25 258 than plumping that determines final IEM.

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27 259 In conclusion, double-yolked eggs provide an insight into the control of albumen
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29 260 secretion in the avian oviduct. The presence of a second yolk stimulates additional secretion
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31 261 of water-soluble albumen proteins by the magnum wall. The short time delay between the
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34 262 first and second yolk means that the wall is unable to produce sufficient new proteins to
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36 263 match the ratio between yolk and albumen and the reserves in the magnum wall are depleted
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38 264 by the presence of the second yolk. This means that egg size is effectively limited by the
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41 265 amount of albumen proteins that the magnum wall can produce. Whether there is neuronal
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43 266 control of the rate of secretion following mechanical stimulation remains unclear.
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56
57 272 manuscript.
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3 349 **Legends for Figures**
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7 351 **Figure 1.** *Maximum linear dimension (cm) as a function of initial egg mass (g) for single-*
8 *and double-yolked pheasant eggs (n = 25 for both cases). Solid lines illustrate regression*
9 *equations (see Table 2). The dashed lines indicate relationships extrapolated from the linear*
10 *regressions for single-yolked eggs and the dotted lines the upper and lower limits of the 95%*
11 *confidence interval around these lines.*
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17 357 **Figure 2.** *Shell mass (g) as a function of initial egg mass (g) for single- and double-yolked*
18 *pheasant eggs (n = 25 for both cases). Solid lines illustrate regression equations (see Table*
19 *2). The dashed line indicates the relationship extrapolated from the linear regression for*
20 *single-yolked eggs and the dotted lines the upper and lower limits of the 95% confidence*
21 *interval around this line.*
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28 363 **Figure 3.** *Albumen mass (g) as a function of initial egg mass (g) for single- and double-*
29 *yolked pheasant eggs (n = 25 for both cases). Solid lines illustrate regression equations*
30 *(Table 2). The dashed line indicates the relationship extrapolated from the linear regression*
31 *for single-yolked eggs and the dotted lines the upper and lower limits of the 95% confidence*
32 *interval around this line.*
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38 369 **Figure 4.** *Yolk mass (g) as a function of initial egg mass (g) for single- and double-yolked*
39 *pheasant eggs (n = 25 for both cases). For double-yolked eggs data for the 1st yolk (heavier)*
40 *and the mass of the two yolks combined are shown. Solid lines illustrate regression equations*
41 *(Table 2). The dashed line indicates the relationship extrapolated from the linear regression*
42 *for single-yolked eggs and the dotted lines the upper and lower limits of the 95% confidence*
43 *interval around this line.*
44
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50 376 **Figure 5.** *Albumen mass (g) as a function of yolk mass (g) for single- and double-yolked*
51 *pheasant eggs (n = 25 for both cases). See text for regression estimates.*
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Table 1. Summary statistics for the mass and composition of single- and double-yolked pheasant eggs. Comparisons of means in any row were by independent two-sample *t*-tests unless indicated. Sample size is 25 in both groups

Trait	Single-yolked		Double-yolked		Comparison between single- and double-yolked eggs	
	Mean (\pm SD)	Range	Mean (\pm SD)	Range	t_{48}	<i>P</i>
Length (cm)	4.65 \pm 0.18	4.34–5.17	5.61 \pm 0.27	5.21–6.36	-14.799	< 0.001
Breadth (cm)	3.63 \pm 0.12	3.35–3.82	4.03 \pm 0.12	3.81–4.30	-12.074	< 0.001
Length / Breadth	1.284 \pm 0.050	1.180–1.420	1.390 \pm 0.083	1.246–1.590	-5.666	< 0.001
Initial egg mass (IEM, g)	33.4 \pm 3.0	26.9–39.0	50.2 \pm 3.7	45.3–57.7	-17.887	< 0.001
Shell mass (g)	4.1 \pm 0.4	3.2–4.9	5.4 \pm 0.5	4.4–6.6	-9.490	< 0.001
Shell mass as proportion of IEM	0.122 \pm 0.010	0.106–0.154	0.107 \pm 0.009	0.090–0.117	5.804	< 0.001
Albumen mass	18.2 \pm 2.1	13.7–22.2	23.7 \pm 2.8	20.6–31.2	-7.900	< 0.001
Albumen mass as proportion of IEM	0.544 \pm 0.018	0.510–0.569	0.472 \pm 0.033	0.429–0.541	9.699	< 0.001
1st yolk mass (g)	11.1 \pm 0.8	10.0–12.3	10.9 \pm 1.0	9.4–12.9	0.563	> 0.05
2nd yolk mass	-	-	10.2 \pm 0.9	8.7–11.6	-	-
Total yolk mass	-	-	21.1 \pm 1.8	17.8–24.2	-25.377 ¹	< 0.001
Total yolk mass as proportion of IEM	0.333 \pm 0.020	0.286–0.372	0.421 \pm 0.031	0.359–0.476	-11.942	< 0.001

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 386 ¹Two sample *t*-test indicated here compared the total yolk masses in the single- and double-
 387 yolked eggs.

388 **Table 2.** *Regression estimates of the slope of the relationships between initial egg mass (g)*
 389 *and component mass (g) as shown in Figures 1-4*

Component	Single-yolked		Double-yolked		Comparison of slopes	
	Slope (SE)	R ²	Slope (SE)	R ²	t ₄₈	P value
Shell mass	0.122 (0.002)	0.994	0.107 (0.002)	0.993	-5.303	< 0.001
Albumen mass	0.546 (0.003)	0.999	0.474 (0.007)	0.995	9.454	< 0.001
1st yolk mass	0.331 (0.004)	0.997	0.217 (0.004)	0.993	20.153	< 0.001
Total yolk mass	-	-	0.419 (0.006)	0.994	-12.203	< 0.001

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391 ¹Two sample *t*-test indicated here compared total yolk masses in the single- and double-
 392 yolked eggs.