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A review of the records of giant squid in the north-eastern Atlantic and severe injuries in *Architeuthis dux* stranded after acoustic explorations

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Abstract

A review of the verified reports to date of *Architeuthis dux* showed that 26% (148 specimens) of worldwide reports were derived from the north-eastern Atlantic. Biological data are presented on fifteen females and two males from Asturian waters (Northern Spain). Both males represent the two first records captured off the Iberian Peninsula. Immature and maturing females ranged from 60 to 140 Kg total weight, whilst mature males weights were 42 and 66 Kg. The peculiar observation of spermatophores embedded in the skin of one of the males near the proximal part of the ventral, ventro-lateral and dorso-lateral arms is discussed. A comparison was undertaken of several morphometric measurements between both sexes and between these two males and five other north-eastern Atlantic males from which data were available. Two incidents of multiple strandings affecting nine specimens in 2001 and 2003 appear to be linked spatially and temporally to geophysical prospecting using air-gun arrays in the Bay of Biscay. Here we present evidence of acute tissue damage in the stranded and surface-floating giant squids. The incidence of such cases during two research cruises contemporary with integrated geological and geophysical studies of the continental margin of the Cantabric Sea indicate that acoustic factors could have caused or contributed to the organ and tissue lesions that probably caused the deaths of these animals. Thus, further environmental regulation of such activity may be warranted to protect animals of the continental slope.

Introduction

The life cycle of the giant squid *Architeuthis* sp. is one of the oceanic enigmas yet to be solved. These animals are often stranded near the coast and they constitute the major food supply for sperm whales in the north Atlantic (Clarke, 1996). Occasionally, pelagic trawling nets catch giant squid but knowledge about the life history, physiology and ecology is fragmented. These studies are based on either dead stranded animals, or those captured alive by nets or from the remains found in the stomach contents of their predators, mainly sperm whales (Roper & Boss, 1982; Clarke, 1996). It has been suggested that the high temperature sensitivity of the O_2 affinity in combination with very low O_2 blood carrying capacity could be the cause of death for giant squid experiencing higher than normal water temperatures along the shores of Newfoundland, Great Britain and Scandinavia in the warm Atlantic current, which can in some years carry large volumes of warm water northwards (Brix, 1983).

The lack of information about this oceanic *Kraken* means that we do not know the number of species of this cosmopolitan genus (Lordan *et al*., 1998). The fascination in giant squids is manifested by the global importance shown by science museums. Thus, the giant squid became one of the main challenges for teuthologists.

To date, there is a remarkable lack of information regarding the giant squid *Architeuthis* spp., especially for males. Teuthologists have not been able to resolve the species composition of this genus. Historically, 21 nominal species have been assigned to this genus (Sweeney & Roper, 2001). However, based strictly on morphological characters related to the head width and the fin shape, these 21 nominal species could be reduced considerably. Furthermore, the material obtained to date, primarily from dead animals or those captured by nets, is from broadly-dispersed locations and generally in such poor condition as to preclude acquisition of precise morphological data.

Sweeney & Roper (2001) noted that among the animals recorded from the North Atlantic, there have only been 17 males (eight from the northwest and nine from the northeast) and only five of these provided detailed information on morphology and some biological characteristics (Aldrich, 1992; Kjennerud, 1958; Knudsen, 1957; Lordan *et al*., 1998).

One aim of this paper is to contribute to the biological and ecological knowledge of the giant squid *Architeuthis* and to remark the possibility of the existence of a resident population of these animals just a few miles off the northern Iberian Peninsula coast. We review the distribution of giant squid location sites in north-eastern Atlantic, report the capture of the two first male specimens of *Architeuthis* in waters off the Spanish Iberian Peninsula, and compare their morphological characteristics with those of other males previously found in the north-eastern Atlantic. Finally, here we present evidence of acute tissue damage in the stranded and surface-floating giant squids.

Material and Methods

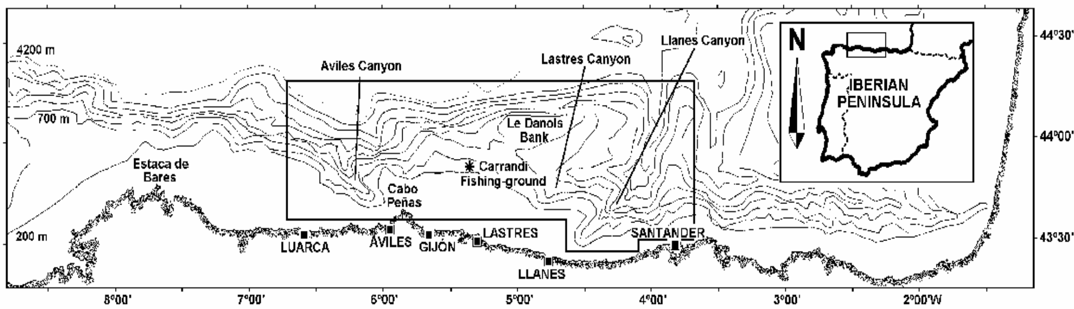
The pair trawler *Minchos V,* which target species was the blue whiting *Micromessistius poutassou,* caught two specimens of *Architeuthis* alive on 9 December, 1999 and 12 January 2001 (Figure 1A) at depths ranging from 300 to 600 m. These specimens of giant squid were collected approximately 34 miles from Gijón (43º52.54'N – 05º18.74'W, Figure 1B) and represented the first records of living specimens collected in the Spanish Atlantic waters.

The specimens were taken on shore and frozen immediately after measurement (Table 1). The animals were defrosted, dissected and immediately “reconstructed”, introduced in a transparent glass case and fixed during 48 h in 4% formalin. At present, they are preserved in 70% alcohol in the Museo-Aula del Mar, property of the Coordinadora para el Estudio de Especies Marinas (CEPESMA) in Luarca (Asturias).

Figure 1. Detail of an immature female of *Architeuthis* sp. caught on 12 January 2001 (**A**) in the northern Spanish Atlantic waters (**B**). Asterisk: place where the specimen was caught (Carrandi fishing ground).

A

B



The statoliths of specimen 2 were removed, mounted in Crystalbond resin and measured to the nearest 0.01 mm. Both sides of the statolith were ground to reveal growth increments. Each increment consisted of two components: an optically translucent ring and a dark ring as previously cited for other cephalopod species (Lipinski *et al*., 1991). Two independent readers made three blind counts each in order to quantify the number of growth increments in the statolith using a semi-automatic image analysis system (TNPC 3.0®). The semi-automatic counting was carried out using a high-resolution digital camera (Sony DCR-PC11E) mounted on a compound microscope (Nikon OPTIPHOT-2). Video images made with 400x magnification were then processed with TNPC 3.0®. Growth increments in each statolith were counted in transects extending outward, from the nucleus (underneath the dorsal dome) to the dorsal dome.

Table 1. Measurements (mm) and weights (g) made to two immature specimens of *Architeuthis* fished in Asturias (Northern Spain) in December 1999 and January 2001.

	SPECIMEN 1	*SPECIMEN 2*
Sex	*F*	*F*
Maturity stage	*Immature*	Immature
Total length	-	8010
Dorsal mantle length	1800	1350
Ventral mantle length	1650	1190
Total weight	1480	810
Maximum mantle width	560	400
Mantle thickness	30	22
Head width	415	260
Arm lengths I (L/R)	n.d. / n.d.	2310 / 2400
II	n.d. / n.d.	1791* / 1900
III	n.d. / n.d.	n.d. / n.d.
IV	2160 / 178*	1870 / 1920
Tentacle length	n.d. / n.d.	6370 / 6530
Tentacular club length	n.d. / n.d.	770 / 790
Dactylus length	n.d. / n.d.	120 / 160
Manus length	n.d. / n.d.	510 / 490
Carpus length	n.d. / n.d.	140 / 140
Upper rostral length	21.0	16.5
Lower rostral length	20.5	15.9
Pen length	1670	1180
Pen width	200	150
Fin length	690	560
Fin width	515	400
Estimated eye diameter	130	120
Gill length	500	370
Nidamental gland length	340	150
Funnel cartilage length	175	135
Funnel cartilage width	41	25
Funnel length	260	160

*, Not intact; -, Not measured; n.d. No data (missing or incomplete)

Three samples of 0.033, 0.027 and 0.023 g from three different parts of the ovary (proximal, medial and distal, respectively) were obtained from specimen 2 to estimate potential fecundity by extrapolation to the total ovary weight (250 g). Total number of oocytes in the sample was counted and measured to the nearest 0.01 mm under a binocular microscope (20 x).

The pair trawlers *Helena María* and *Bautista Pino* captured a male of *Architeuthis* sp. on 13 September 2002 in the proximity of "Pozo de la Vaca" (43º54.26'N-5º29.38'W) at a depth of about 450-475 m. A second male was found moribund and floating at the surface off Gijón (43º53.23'N-5º32.15'W) on 16 October 2003 (Figure 1B). These animals were maintained frozen and delivered to Aula del Mar (CEPESMA) in Luarca (Asturias, Spain), where they were studied after being defrosted at room temperature. Morphometric data were collected in the laboratory according to Roper & Voss (1983). Data from these two males, the only males to date from waters off the Iberian Peninsula, were compared with those of other giant squids reported from the north-eastern Atlantic (Sweeney & Roper, 2001; González *et al*., 2002; unpublished data).

Results and discussion

Immature females

The female specimens of this study were identified as *Architeuthis dux* Steenstrup, 1857. This species reaches a mantle length (ML) up to 3 m (more frequently between 1 and 2 m) and possess very large arms and two large tentacles with a tentacular club with four rows of suckers. The medial rows of the manus much larger than the marginal ones. Furthermore, these animals had a straight simple funnel-locking cartilage, buccal connectives attached to the dorsal border of arm IV and small fins, semiround or semioval. Measurements and weights of the two specimens are summarised in Table 1. The digestive tract of both specimens lacked prey remains.

The counts made independent readers revealed a number of increments ranging from 258 to 318. The periodicity of the deposition is not known, but as indicated by Jackson *et al*. (1991), the increments resemble growth rings that have been laid down in other species. Therefore, assuming the daily formation of growth increments, our specimen would be 9–10 months old, which represents a growth rate ranging from 5.72 to 4.24 mm ML per day. These growth rates surpass those indicated by Gauldie *et al*. (1994) and Lordan *et al*. (1998).

The female Nº2 had a potential fecundity of 9,161,531±487 oocytes. The size of the oocytes ranged from 0.20 to 0.42 mm. This estimation agrees with the estimation made by Boyle (1986) who indicated that the potential fecundity of a submature female of 168 kg, which ovary weighed 7,676 *g* could have been over 10^7 oocytes. On the other hand, in a submature female of 200 kg captured in South Africa, the oocytes measured between 0.5 and 1.4 mm (Pérez Gándaras & Guerra, 1978). Boyle (1986) also indicated that the oocytes of the female studied were very small (all < 1mm diameter, most < 0. 5 mm). In a female of 180 kg stranded in Argentina with a large ovary of 14,024 g, the oocytes measured between 0.65 and 2.52 mm (Ré *et al*., 1998).

Another five specimens were captured alive (Figure 2) and eleven more specimens were collected dead or fragmented during this period. The weights of the animals collected, principally during autumn and early winter, ranged from 75 to 202 kg, However, the most remarkable events were the collection in the same area of another giant squid of 102 kg, a single tentacle measuring 5 m, and another tentacle of 2 m long in the stomach content of the shark *Isurus oxyrhinchus*, from April 1999 to January 2000 in the same area.

The topography of the area where the above mentioned specimens were caught is highlighted by the presence of three canyons (Llanes, Lastres and Avilés) each about 4000 m maximum depth (Figure 1B). Even though primary production in the canyons is not high, there is some production provided by the seasonal upwellings that occurs in the adjacent continental shelf producing an offshore export of organic matter and sedimentation towards the continental margins (Fernández *et al*., 1993). This process sustains important communities of invertebrates, fishes and marine mammals. The most remarkable aspect could be the existence of huge concentrations of juveniles of blue whiting in these canyons, which could constitute an appropriate diet for *Architeuthis*, due to the presence of high quantities of blue whiting in the same trawls and also in the stomach contents of other specimens recently captured (unpublished data).

The capture of these giant squids between 300 and 600 m allows us to assume that these animals inhabit these canyons or surrounded areas, probably close to the bottom or in mid-water, where nets were hauled.

Mature males

Figure 3 illustrates the dissection of the first (September 2002) male. The size of the fresh animal was 42 kg in total body weight (BW), 98 cm in mantle length (ML) and 600 cm in total length (TL). The penis length was 88 cm and it protruded 20 cm outside the mantle (Figure 3A). We found several spermatophores within the Needham's sac, ranging 11-20 cm in length, indicating that the animal was functionally mature. Other spermatophores were observed embedded in the skin near the proximal part of the ventral, ventro-lateral and dorso-lateral arms (Figure 3B). The stomach was completely empty. The second male, also mature, was 66 kg in BW and 122 cm ML. It was not possible to estimate TL because both tentacles were missing. The penis length was 96.5 cm and it protruded 23 cm outside the mantle. There were no spermatophores embedded in the skin. Both males were preserved and are stored in the collection of Aula del Mar. The records of male *Architeuthis* from the north-eastern Atlantic, with morphological data and mean and standard deviation of the morphometric indices are summarised in Table 2.

Figure 2. *Architeuthis dux* immature females of 70 Kg. caught by a pair trawler in the western part of the Carrandi fishing ground in October 2002. (Original by Eloy Alonso).

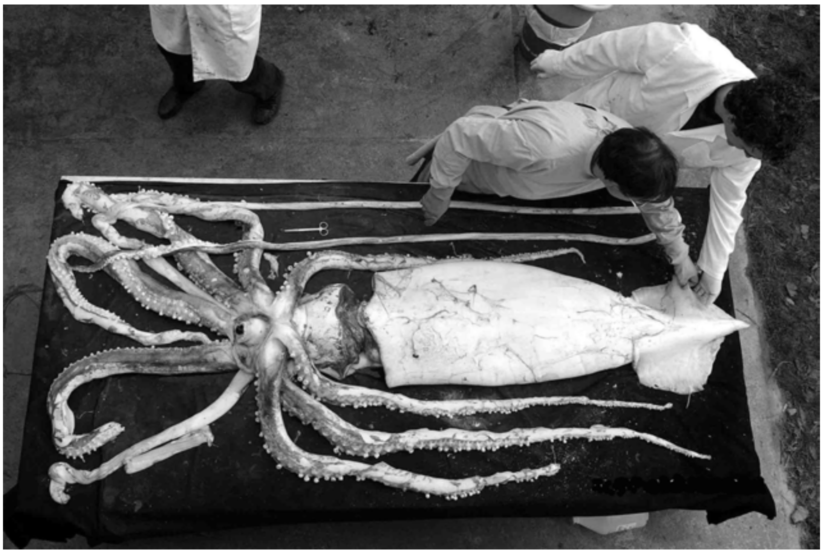
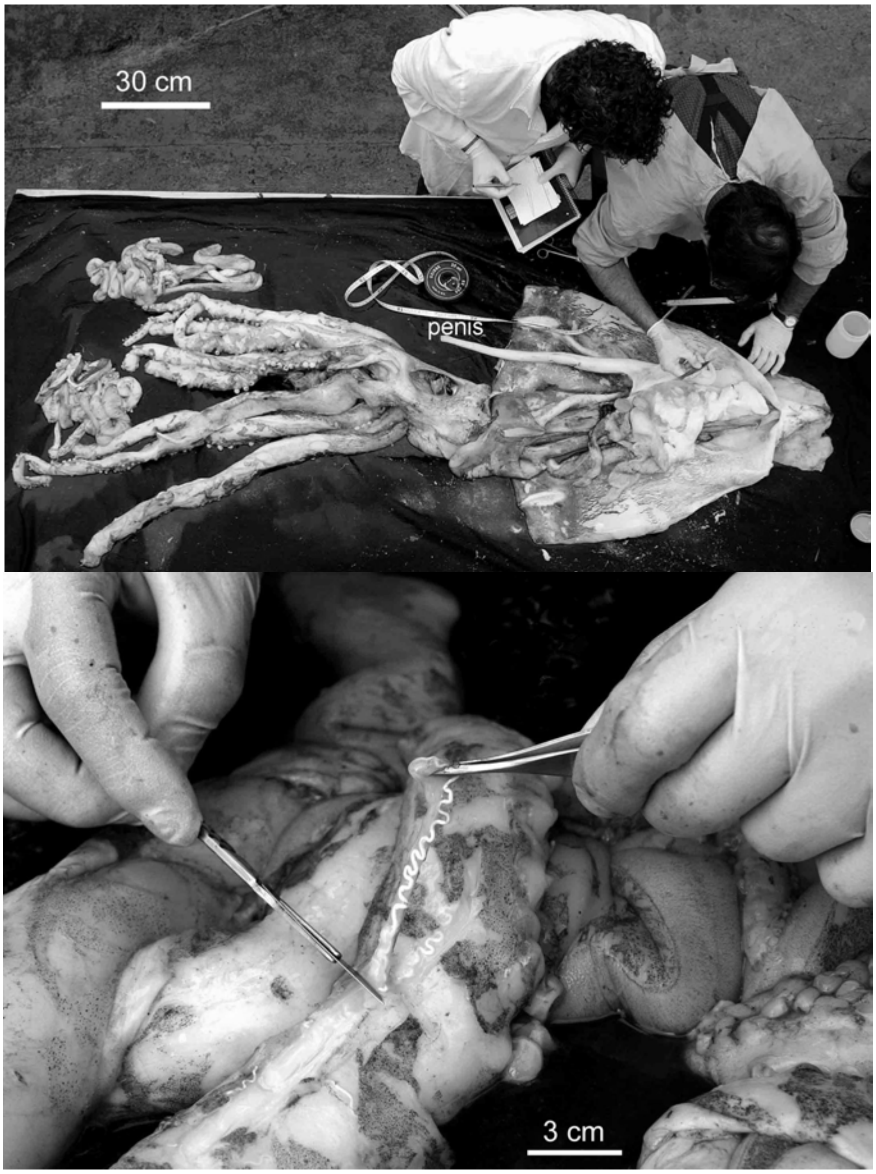


Figure 3. (**A**) Male of giant squid *Architeuthis dux* caught by a pair trawler in September 13th 2002 in 43º54.26'N-5º29.38'W. (**B**) Spermatophores were observed embedded in the skin near by the proximal part of the ventral, ventro-lateral and dorso-lateral arms. (Original by Eloy Alonso).

A

B



For all males compared the total length could be partitioned among the mantle (16.8%), the head (4.5%) and the tentacles (78.3%). The arms represented 24.8% of the total length. The width of the fins represented 26.9% of the mantle length. The mean eye diameter was 8.7% of the mantle length, but was highly variable. The manus, dactylus and carpus represented 51%, 31.7% and 16.9%, respectively of the tentacular club.

Table 2. Male records of *Architeuthis* with morphometric data in the Northeastern Atlantic. Origin: D, Denmark (Knudsen, 1957); N, Norway (Kjennerud, 1958); IR, Ireland (Lordan et al., 1998); AS, Asturias, Spain. Characters: ML, Dorsal mantle length; BW, Body Weight; Mat, Maturity stage; VML, Ventral Mantle Length; Head length; HL, Hectocotylised part of arm; HW, Head width; ALI, ALII, ALIII and ALIV, Arm length; LAL, Longest arm length; HeL, Hectocotylus Part; ACI ACII, ACIII and ACIV, Arm circumference; AF, Arm Formulae, TeL, Tentacle length; ToL, Total length; TC, Tentacular club length; DC, Dactylus club length; MaL, Manus length; CaL, Carpus length, FL, Fin length; FW, Fin width only one fin, both fins between brackets; TaL, Tail length; BC, Body circumference; MW, Max mantle width; ED, Eye diameter; FuL, Funnel length; FuO, Funnel opening diameter; FuCL, Funnel cartilage length; FuCW, Funnel cartilage width; PL, Penis length; SSL, Spermatophore sac length; SoA, Spermatophores on arms; SL, Spermatophore length; LRL, Lower beak rostral length; URL Upper beak rostral length; Sinc, Number of statolits increments; G(W), Daily growth rate (%). Indices: MLI: Mantle Length Index = ML/TL*100; HLI: Head Length Index = HL/ML*100; TELI: Tentacle Length Index = TeL/ML* 100; TCI: Tentacular Club Index = TC/TeL*100; DCI: Dactylus Club Index = DC/CL*100; MaLI: Manus Length Index = MaL/CL*100; CaLI: Carpus Length Index = CaL/CL*100; FLI: Fin Length Index = FL/ML*100; LALI. Longest Arm Length Index = LAL/ML*100; FWI: Fin Width Index = FL/ML*100 (both fins); PLI: Penis Length Index = PL/ML*100; EDI: Eye Diameter Index = ED/ML*100; SLI: Spermatophore Length Index = SL/ML*100; LRLI: Lower Rostral Length Index = LRL/ML*100; URLI: Upper Rostral Length Index = URL/ML*100; S.Inc: Statolith Increments; G(W): Daily growth rate (%). Measures in cm or Kg. S.D. Standard Deviation.

	D (1957)	N (1958)	IR (1998)	IR (1998)	IR (1998)	AS (2002)	AS (2003)	I	Mean	S.D.
ML	101	100	102.8	97.5	108.4	100	122	**MLI**	16.8	0.4
BW	-	48	26.9	22.45	26.5	43	66			
Mat	Mature	Mature	Mature	Mature	Mature	Mature	Mature			
VML	94	87				92	116			
HL	27.5	30-32	27.5	27	28.8	27	28.9	**HLI**	27.6	1.6
HW		25	13.5	13.7	14.4	28	-			
ALI	80	126+	87	-	-	-	-			
ALII		143++	-	-	-	-	-			
ALIII		126+	105.4	-	-	-	-			
ALIV	147/153	164	151.2/146.5	-	111.1	176	-			
LAL	153	164	151.2		111.1	176	-	**LALI**	146.4	21.3
HeL	IV pair (9/13)	IV pair	No obs.	No obs.	No obs.	IV pair (4.5)	-			
ACI		16				15.4	16.2			
ACII		20				19.7	20.4			
ACIII		22				21.6	21.2			
ACIV		22				20+	25.1			
AF		4.3.2.1				4.3.2.1	4.3.2.1			
TeL	490	-	455.5	482.3	-	420/470	-	**TeLI**	462.6	30.8
ToL		-	597	595	-	600	-			
TC		-	50.4	50.0	-	51	-	**TCI**	11.1	0.7
DC		-	15.8	17.2	-	15	-	**DCI**	31.7	2.5
MaL		-	25.2	25.2/23.6	-	29	-	**MaLI**	51.1	4.1
CaL		-	9.4	7.7/9.2	-	7.9	-	**CaLI**	16.9	1.8
FL		28-32	39	35.6	36.8	30	42	**FLI**	33.8	3.3
FW		13 (26)	14.9 (29.8)	13.3 (26.6)	13.2 (26.4)	14 (28)	-	**FWI**	26.9	1.8
TaL		10				8	-			
BC		80				76	82			
MW			37.9	33.5	33.5	33	38			
ED			8	9.5	9.6	8/10	9.5/10.3	**EDI**	8.7	0.9
FuL		14				17	17			
FuO		7				5.5	7.6			
FuCL		12				12	14.5			
FuCW		2.5				3	3.6			
PL	78	92	55.5	57.4	70.2	88	96.5	**PLI**	73.3	14.7
SSL	15.5					30	32.9			
SoA	No obs.	Yes	No obs.	No obs.	No obs.	Yes	No obs.			
SL	11.0-18.0		12.5-15.0	12.0-14.0	13.5-15.5	11.0-20.0	12.3-20.1	**SLI**	11.5-16.3	1.0-2.3
LRL		1.2	1.09	1.27	1.33	1.2	1.34	**LRLI**	1.18	0.09
URL		1.5	1.25	1.2	1.0	1.0	1.2	**URLI**	1.14	0.22
SInc			294	375	422	-	-			
G(W)			4.25	3.29	2.96	-	-			

There have only been six males of *Architeuthis* spp. reported since 1952 in the north-eastern Atlantic (Table 2). All of these males were functionally mature, with two of the eight (Denmark in 1957 and Asturias in 2002) having spermatophores embedded in the skin of several arms and the mantle. The presence of spermatophores in the skin could come from other males or from accidental self-implantation when the animal was attempting to impregnate a female. The latter seems to be the more plausible explanation because males of this species, being much smaller than females, posses a disproportionately large penis (Figure 3A), which allows them to inject the spermatophores under high pressure. Moreover, the great length and flexibility of this organ probably results in delivery of sperm packages in several parts of their own mantle or arms, as well as the arms of a mating female; head-to-head is one of the most common mating positions in teuthoid cephalopods.

Only one mated female of *Architeuthis* (probably *A. sanctipauli*) has been reported to date (Norman & Lu, 1997). That female also had spermatophores embedded within the skin of the two ventral arms, as Knudsen (1957) reported and as we also described in two males.

Males of several species may use their mandibles, tentacular hooks and/or arms to produce these wounds in the skin of arms and/or the mantle of females within which they then deposit their spermatophores (Nesis, 1987). Generally, in these species, the male has a long and muscular penis, which would be adaptive for inserting spermatophores into such wounds. However, these wounds have not been observed in the arms or mantle of *Architeuthis* spp. Therefore, the mechanism of sperm introduction to females of this species, while highly specialised, remains unknown.

The males of *Architeuthis* spp. studied to date were functionally mature at sizes ranging from 97.5 to 122 cm ML and from 22.5 to 66 kg BW (Table 2). Our comparisons revealed morphological differences among males (Table 2), most remarkably that males of similar mantle length showed relative penis lengths ranging from 54% to 64.7% of ML in the Irish specimens and from 77% to 92% of ML in the other specimens. These, and other, measurements and indices should be interpreted with caution, however, because the specimens were preserved under various conditions and for variable periods of time. Furthermore, it is also likely that variable body weight is in part due to loss of body parts (e.g. arms) in some specimens. As noted by Lordan *et al*. (1998), some morphological differences they found may be attributable to damage at capture or to the general plasticity of cephalopod tissue. However, differences in beak morphometry and gill lamellae counts suggest that two different species may inhabit the north Atlantic. In order to resolve species composition of *Architetuthis* spp. in this area, and throughout the world's oceans, it would be necessary to undertake genetic analysis to determine whether morphometric and meristic differences reflect ecotypes or true species.

Records in the north-eastern Atlantic

Table 3 shows the records of *Architetuhis* spp. in the north-eastern Atlantic from published reports updated to October 2003. Among the 148 specimens of giant squids reported in the north-eastern Atlantic, only seven were captured alive, five of them in northern Spanish waters where trawlers targeted blue whiting mainly during summer and autumn. Although sex was undetermined for most of the specimens from the north-eastern Atlantic (69.6%), an unequal sex ratio, strongly favouring females (3:1), was evident among those specimens of known sex.

The north-east Atlantic records of *Architeuthis* spp. represent 26.0% of the animals found worldwide (574 specimens). Most (92.6%) of the animals reported from Norway were found between 1874 and 1977, being almost absent afterwards. This drastic reduction cannot be explained by a reduction in observation effort because the giant squid has received increased attention during recent years. This decrease in sightings could be related to a reduction in the frequency of oceanographic events that cause strandings. The oxygen-carrying capacity of haemocyanin in the blood system of giant squid is lower than that of other pelagic squids. Furthermore, from blood samples obtained from a giant squid captured alive in Bergen, Brix (1983) found more than a fourfold decrease in O_2 affinity when temperature increased from 6.4 to 15ºC, strongly suggesting that giant squids may suffocate from arterial desaturation when ambient temperature increases. This seems to be a plausible explanation for the appearance of moribund or dead animals, floating at the surface or stranded on the shores of several European countries, where giant squids could interact with tropical water masses. It is likely that such interactions occasionally occur during some years in the north Atlantic, as a result of the eastward intensification of current flow in the Gulf Stream affecting the Irminger, Norwegian and Canary warm currents (Thurman, 1993).

It is very difficult to find a realistic explanation of the predominance of females because virtually nothing is known with respect to the life history and demography of *Architeuthis* spp. This unbalanced sex ratio could represent a real predominance of females within the natural population, which seems unlikely, or it could reflect higher vulnerability of females than males to capture or stranding. Such higher vulnerability could be related to spatial segregation of the sexes. Alternatively, that most sightings were in autumn suggests that some seasonal event could be related to the preponderance of females. It is possible, for example, that sexual maturation is much more demanding of somatic resources in females than in males resulting in poorer condition and higher susceptibility of females than males to stress. However, this seems to be an unlikely explanation, as most of the females recorded from the northeast Atlantic were sexually immature or maturing, whereas the few males observed were all mature.

Severe injuries after acoustic explorations

Forty nine strandings, sightings, and captures of giant squids (*A. dux*) in Asturian and Galician coasts (North Spain) have been recorded since 1962 (González *et al*., 2002; Guerra *et al*, 2004). This indicates that an average of one incidence per year is a normal situation. Moreover, 75% of these reports were from live animals caught by trawlers or pair trawlers targeting blue whiting, which is one of the main prey items of giant squids in this area. These catches were from depths of 450-800 m, close to the submarine canyons present in this area (González *et al*., 2002; Guerra *et al*, 2004).

Table 3 Records of *Architeuthis* in the Northeastern Atlantic since 1545.

	1545	1639/80	1790/00	1848/73	1874/99	1900/25	1926/51	1952/77	1978/03	Unknown	Total
Country											
- Iceland		1	1			1		2		1	6
- Ireland		1			2	3			3	1	10
- Norway		1			7	8	5	5	1		27
- Denmark	1	1	1	2			1	2			8
- Scotland				1		5	5	3	4		18
- Canary Islands				1					6		7
- Azores					3			3	2	3	11
- England							1		1		2
- France (Bay of Biscay)							1				1
- Madeira								3			3
- Galicia (Spain)								1	1		2
- Asturias (Spain)								19	30		49
- Huelva (Spain)									2		2
- Portugal									1		1
- Germany						1					1
Year season											
- Spring					2	1	1	1	2		7
- Summer					3	5	1	10	16		35
- Autumn		2	1	2	5	5	4	3	15		37
- Winter		1		2	2	2	5	4	4		20
- Unknown	1	1	1			5	2	20	14	5	49
Sex											
- Male					2	1	1	2	6		12
- Female					2		2	2	27		33
- Sex not determined	1	4	2	4	8	17	10	34	18	5	103
Sampling procedure											
- Stranding	1	1	2	3	6	9	8	1	14	1	46
- Capture					2		2	4	10		18
- Floating on the surface		1		1		1		1	6	1	11
- From predators					2	6		10	4	1	23
- Unknown		2			2	2	3	22	17	2	50

This natural rhythm of standings and sightings has changed in two occasions. Five giant squids were stranded close to the site of an international project by the vessels *Barracuda* and *Nina Hay 502* between the end of September and the middle of October 2001. These vessels used air guns arrays for geophysical prospecting. Other four giant squids were stranded or caught moribund floating at surface from 13 to 17 September 2003 close to the site where a geophysical research cruise of the international project MARCONI (http://www.utm.csic.es/Hesperides/actual/2003/cientifico_tecnica/diario/diario-03-09-01.htm). This cruise held from 30 August to 18 September 2003, also using acoustic air guns arrays. The acoustic pulses produced were low frequency (<100 Hz) and 200 dB of intensity (http://www.utm.csic.es/Hesperides/equipamiento/equipamiento.html).

We necropsied seven of these nine *Architeuthis dux*, which were very fresh. Five were frozen and were examined after thawing and the rest were analysed after fixation in 10% formalin/seawater solution. Six were immature or maturing females and one a maturing male. Females' sizes ranged from 67 Kg and 127 cm mantle length (ML) to 140 Kg and 177 cm ML. The male was 66 kg and 122 cm ML. These animals have been preserved and are stored in the Aula del Mar of CEPESMA in Luarca (Asturias, Spain). One of these animals (an immature female of 140 kg and 153 cm ML) stranded in Colunga beach on 13 September 2003. Though dead, this animal was very fresh and was preserved in 10% formalin two hours after its discovery. Its mantle wall was 3.5 cm thick and showed severe injuries of tissues in a band of 43 cm width located in the central zone of the body (Figure 5A). The mantle epidermis was completely lost, which is common in stranded animals. However, the outer and the inner collagen tunics that sandwich the mantle musculature were intact. The circular and radial muscle fibres of the mantle between the tunics (Shadwich, 1994) were smashed and separated into small pieces (Figure 5B). This injured zone affected a central layer 2 cm thick. This zone included an intact rhomboid reticulation of intermuscular collagen fibres. All the small smashed muscle fibres were embedded in a liquid with high concentration of ammoniacal compounds from the broken vacuoles occurring within the mantle muscles (Voight *et al*., 1994). In this animal, the majority of the internal organs were badly damaged and in many cases unrecognizable. An amorphous mass of tissues and liquid from ruptured organs and coelomic cavities were observed within the mantle cavity. The organs of the digestive tract had long gaps in several regions, especially in the caecum, from which digestive fluids escaped into the mantle cavity. The digestive gland was found completely mangled and its contents, a thick oily brown fluid, scattered over the mass of tissues. The stomach walls were also ripped. The outer layer of columnar epithelium of the two branchial hearts was ripped in several places, giving the impression that they had exploded. The gills were also bruised, with several small blood vessels of its capillary network broken. No signs of vascular congestion or microvascular haemorrhages were observed within these or other vital organs. This could be due to the relative low blood volume (ca 5% of body weight) and to the difficulty of observing haemorrhages in animals in which blood is cell-free (Shadwich, 1994), its respiratory pigment is haemocyanin and the colour of the blood is clear-blue. The ovary was also bruised and the small oocytes had spread into the mantle cavity. In addition to the injuries that are common in standings such as loss of one or several arms and tentacles and burst eye balls, this animal also showed some injuries in its equilibrium receptor system or statocysts (Budelmann, 1996). In the gravity receptors (macula/statolith/statoconia system) the statoliths (small structures made of aragonite with an organic matrix) were not found due to formalin dissolution effect. There were, however, some secondary hair cells of the maculae of both statocysts damaged. There were also some large secondary sensory hair cells damaged in two cristae of the angular acceleration receptors or crista/cupula system (Young, 1989) of both statocysts. No gas-bubble formation was observed in tissues. No parasites were found in the carcase. The lesions found in the remaining five females were not as severe as those found in the mantle wall and internal organs of the specimen described above. All showed, however, similar injuries in the sensory cells of their statocysts and some gill capillaries broken. All structures also showed normal internal coloration and no sign of putrefaction. Analysis for heavy metals in tissues of several organs in frozen samples from two females showed very low levels of contamination. We had the opportunity to examine a male which was found floating 25 metres offshore on 16 September. When it was found it still showed some vital reaction (including changing skin colour, ink ejection, etc.) and was able to move a short distance. The animal was immediately frozen at -20^{o}C after capture.

Figure 5. Injuries in the mantle wall and organs of the biggest female (**A**). The circular and radial muscle fibres between the outer and inner collagen tunics were smashed and separated into small pieces (**B**).

A

B

We necropsied this animal eight days after preservation, which followed defrosting at room temperature. Other than the gills, it appeared to be in excellent health, with all internal organs intact. However, the right gill was practically destroyed whilst the left gill was bruised, with several small blood vessels of its capillary network broken. No signs of vascular congestion or microvascular haemorrhages were observed within vital organs. In the gravity receptor systems the statoliths were found detached from their respective maculae. There were also some secondary hair cells of the maculae and several cristae of both statocysts damaged. No intravascular bubbles were observed in several organs examined. No parasites were found, and no pathogenic bacteria were isolated from this carcase.

The sexual stage of the stranded giant squids demonstrates that death was not due to post-reproductive mortality. There were no indications of other possible natural causes of mortality (e.g., parasites or predation damage). On the contrary, the observed lesions as well as the concurrence of the substantial increase of stranding incidence with presence of vessels using air-gun arrays suggest lethal or sublethal effects of the shock acoustic waves. Low-frequency (<100Hz) acoustic waves at intensity of 150-160 decibels can kill whales, other marine mammals and marine fish by rupturing the membranes surrounding their lungs, swim bladder, brain and auditory space (Green, 2002). A second lethal effect of the shock waves involves the activation of supersaturated gas in marine mammal's blood and their cells, forming small bubbles which can cause embolism in vital organs (Jepson *et al*., 2003).

Very little is currently known about the impact of marine acoustic technology on cephalopods (Anonymous, 2002) and controversy remains on the impact of high-energy acoustics on marine mammals and fishes. It seems, however, that the bubble effect will be greatest in deep-diving animals that will have the highest levels of supersaturated gasses in their blood and cells (Anonymous, 2002; NOAA, 2002). The absence of gas-bubble lesions in stranded giant squids could be due to fact that the animals were never necropsied fresh. It is very difficult to determine definitive evidence of gas embolism *in vivo* after death (Knight, 1996) and even more so when carcasses are examined after preservation. Lack of evidence of embolism could be also due to the differences in composition of cephalopod blood from that of vertebrates. We observed, however, severe injuries in the mantle and viscera of one specimen which were enough to cause its death. To date, we have examined fifteen recent giant squid strandings in Iberian coasts and this is the first time that these kinds of lesions have been observed in an animal. On the other hand, if the lesions were produced during the stranding it should be clear wounds in the outer part of the mantle which was not the case. Therefore, presumably this animal was affected by the impact of acoustic waves. The presence of small injuries in some secondary hair cells of the maculae and also in some large secondary sensory hair cells in several cristae of almost all the animals examined -also observed for first time- allow us to hypothesize an explanatory mechanism for the death and subsequent stranding of these giant squids. These statocyst lesions could easily have caused the animals to become disoriented as it swims through important disruption of sensory information about rotational acceleration which enables the squids to regulate the position of the head, funnel and especially eyes (Hanlon & Messenger, 1996). Besides disorientation from the gravity-receptor system, acoustic waves could also have dazed the squids because cephalopods are quite sensitive to low-frequency vibrations (Hanlon & Messenger, 1996). Disoriented and dazed, these moderately active, buoyant cephalopods (Hanlon & Messenger, 1996) could have floated towards the surface, going from deep cold waters to warmer and shallower waters. A decrease in O_2 affinity of hemocyanin in excess of fourfold has been reported in one live giant squid caught off Radöy near Bergen, Norway when temperature increased from 6.4 to 15ºC. This strongly suggests that giant squids may suffocate from arterial desaturation when increased ambient temperature is experienced (Brix, 1983).

Further investigation is needed into the physical, physiological and behavioural effects of high-intensity acoustic pulses on cephalopods, as well as on other deep-water organisms, including the relationship of these effects to tissue and organ lesions and to strandings. Studies should attempt to determine whether gas-bubble lesions can develop in cephalopods as well as in marine mammals. Research should compare stranded cephalopods suspected of having been exposed to acoustic pulses with results from experimentally exposed and control animals. As has been shown for cetaceans and sonar (Jepson *et al*., 2003), our findings should be considered in a wider conservation sense, to consider whether the regulation and limitation of acoustic pulses is necessary to limit antropogenic acoustic impacts on cephalopods and other deep-sea organisms. Because giant squids float post-mortem and are sufficiently large that their carcasses are not immediately scavenged, they may serve as an indicator of environmental impact to deep-water fauna. They could be the tip of a figurative iceberg indicating that other species, some of them of commercial interest in Asturian fishing grounds, could be also affected. Finally, a precautionary approach would prescribe that until evidence of environmental impact is sufficient for a ban of marine acoustic technology for applications using high energy and low frequency sources, mitigation strategies involving survey design, timing, ramping of source levels and prohibited zones should be used (Anonymous, 2002).

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