

Dwarf males of giant warty squid *Kondakovia longimana* and a description of their spermatophores

V. Laptikhovsky¹ J. C. Xavier^{2,3}

1- CEFAS, Pakefield Road, Lowestoft, Suffolk, NR33 0HT, UK. Email: vladimir.laptikhovsky@cefas.co.uk

2- Marine and Environmental Sciences Centre (MARE), Department of Life Sciences, University of Coimbra, 3004-517, Coimbra, Portugal

3- British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley Road, Cambridge, CB3 0ET, UK

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Abstract

Antarctic squid are widely known to play an important role in the diet of various Antarctic predators, although the biology of those squid and how that relates to their availability to their predators is poorly known. We assessed the reproductive system of the deep-sea giant warty Antarctic squid *Kondakovia longimana* under a predator-prey context. The spermatophores of male *K. longimana* are described, based on two specimens foraged by albatrosses at Bird Island, South Georgia (54°S, 38°W). Spermatophore length (SL) was 42–90 mm, head occupied mean = 1.8–2.4% SL, ejaculatory tube = 17–22% SL, cement body = 19–22% SL, seminal reservoir = 56–59% SL and rear empty part = 0.4–0.8% SL. All spermatophores of *K. longimana* in our study were normal and functional, and very dissimilar to those of other spent squid, in which the spermatophores have short, semi-transparent seminal reservoirs and a large empty rear part. Senescent male *K. longimana* could become available to predation by albatrosses (presumably being surfaced) with spermatophoric sacs (Needham's sacs) containing at least a hundred of normal spermatophores having no evidence of reproductive system degeneration. From known relation between beak and body size all known adult males in *K. longimana* were dwarfs in respect to females. Our data also show that they have extended spermatophore production as in other squids.

Introduction

Antarctic squid is a major component in the diet of numerous predators of the Southern Ocean all through the year, ranging from small penguins to large toothed whales (Xavier and Cherel 2009). Little is known about reproduction of the Antarctic squid (Nesis et al. 1998; Laptikhovsky and Arkhipkin 2003; Collins and Rodhouse 2006). Indeed, there is still considerable debate to how Antarctic squid (particularly those living and feeding in deep waters) become available to surface/near-surface feeders, such as albatrosses (Xavier et al. 2013). One of them, the giant warty squid, *Kondakovia longimana* Filippova, 1972, inhabits Antarctic epi- and mesopelagic waters and also occurs near the sea floor (Nesis 1987; Collins and Rodhouse 2006). Adult *K. longimana* are an important prey species for sperm whales *Physeter macrocephalus* and elephant seals *Mirounga leonina*, whereas sub-adults and juveniles are preyed upon by albatrosses and penguins (Cherel and Weimerskirch 1999; Xavier and Cherel 2009). Little is known about reproduction of *K. longimana* species, but its life span may take several years and it might be a polycyclic spawner (Bizikov 1991; Jarre et al. 1991; Laptikhovsky et al. 2013). Females of *K. longimana* of < 670 mm mantle length (ML) and up to 7 kg body weight (BW) have been reported as immature, with gonad weights of about 1–2% BW (Clarke 1980; Bizikov 1991). A few, large females of this species, which were found occasionally at the surface, and one captured by a longline, were 670–1080 mm ML (7–30 kg BW) (Clark 1980; Vacchi et al. 1994; Lu and Williams 1994; Lynnes and Rodhouse 2002; Laptikhovsky et al. 2013). There has only been one report of a mature male (Clarke 1980), which was 350 mm ML and ca. 1 kg BW and was found in a sperm whale stomach.

The original description of the species was based on three small immature females (Filippova 1972). A schematic drawing of the spermatophore of *K. longimana* was provided by Clarke (1980), who stated that this one mature male specimen known to science had spermatophores in two narrow-sized groups: 51.3–55.5 mm and 81.5–82.6 mm (23.3–23.6% ML). Until now, no further mature male specimens have been reported (Bolstad 2008).

Spermatophores and other parts of soft bodies are known to be found in stomach contents and boluses of adult albatrosses (Cherel and Weimerskirch 1999; Xavier et al. 2003 a,b; Xavier et al. 2004), that together with fresh beaks, allow identification of these body parts (Clarke 1962; Clarke 1980; Xavier et al. 2005). This also provides an opportunity for a taxonomically important description of the spermatophores of *K. longimana*, as the earlier description of a single spermatophore (Clarke 1980) did not provide key information and has been viewed as “difficult to interpret” (Hess 1987).

Materials and methods

Reproductive systems of two male *K. longimana* (identified by fresh beaks attached directly to body tissues) were found in the food of two albatross species at Bird Island, South Georgia (54°01S, 38°03W) in September 2009:

Sample A - stomach contents of a chick of Wandering Albatross *Diomedea exulans* L. were collected following Xavier et al. (2003a). After a parent fed its chick, the chick was inverted over a bucket and its stomach contents collected. Nests were labelled to avoid pseudo-replication and to avoid resampling from chicks. The animal procedures used in this study were reviewed and approved by the Joint British Antarctic Survey - Cambridge University Animal Welfare and Ethical Review Committee. Permits to operate were issued by the Government of South Georgia and the South Sandwich Islands.

Sample B- bolus from an adult Grey-headed Albatross *Thalassarche chrysostoma* (Forster) collected following Alvito et al. (2015). Bolus, regurgitated by an adult grey headed albatross that had recently arrived at the colony was collected from the ground during a daily visit to the nesting site.

The lower rostral length of the squid beaks was 13.4 mm (sample A) corresponding to 478 mm ML and 2545 g BW, and 9.2 mm (sample B) corresponding to 320 mm ML and 778 g BW according to the allometric equations of Xavier and Cherel (2009).

Both bunches of spermatophores and spermatangia (ejaculated spermatophores) with associated empty tunics were preserved first in 4% formalin and then transferred into 70% ethanol. Total length was measured to the nearest 1 mm in all undamaged spermatophores and empty envelopes. More detailed morphometric features were measured (to 0.1 mm) for a subsample of 27 spermatophores (sample A) and 25 spermatophores (sample B) under a Leica MZ 125 binocular microscope.

Results

The reproductive system of the first *K. longimana* (sample A) consisted of remnants of the spermatophoric sac that contained a total of 27 intact spermatophores, and 88 empty tunics (56 of which were undamaged) left after spermatophore ejaculation. These envelopes were accompanied by 30 spermatangia and some amorphous pieces of those without a normal spermatangial structure. In this sample, the spermatophore length (SL) varied from 63–90 mm (Fig. 1, 2), mostly being 65–70 mm. Empty envelopes were 57–79 mm long. The seminal reservoir (SR) occupied more than a half of the SL (Table 1), with a very small rear empty part behind it. SR was tight and occupied all available

space inside the spermatophore envelope behind the cement body. The cement body was homogenous, closely attached to the seminal reservoir, without a distinctive separation from the spiral filament of the ejaculatory tube. The head was distinctive, with about two loops of the spiral filament inside and a strong thick thread. Spermatangium length varied from 26–60 mm, of which 17–38 mm were represented by a bulky, stick-like body, and the rest consisted of a long trailing tail of inner envelopes.

The reproductive system from sample B contained an intertwined pack of spermatophores (N=62, of which 54 were measurable) and empty envelopes (N=50, of which 37 were measurable). Spermatophores were smaller, as expected for a smaller male (Fig. 2). Their morphology was identical to that described above, and the morphometry was very similar (Table 1): SL ranged from 42–69 mm (mostly 55–65 mm), and the empty envelopes were 38–68 mm long.

Discussion

Seabirds often forage on spent Antarctic deep-sea cephalopods that float up after spawning is well known (Lininski and Jackson 1989; Croxall and Prince 1994; Cherel and Weimerskirch 1999; Xavier et al. 2013). Indeed, rising to the surface after spawning is a common fate of females of many meso- and bathypelagic squid (e.g. Onychoteuthidae, Gonatidae, Histioteuthidae and Cranchiidae) that undergo gelatinous degeneration during maturation, and may explain the common occurrence of large deep-water squids in the stomachs of seabirds, including those incapable of diving (Nesis et al. 1998; Xavier et al. 2013). To our best knowledge, maturing and mature squid of the family Onychoteuthidae were never reported from surface layers, where they could be available to seabirds, as during maturation they descend into deep-sea well before become able to reproduce (Arkhipkin and Nigmatullin 1997, Laptikhovsky et al. 2007). In contrast to this, spent male and female onychoteuthids were regularly found floating at the surface (e.g. Clarke 1966; Arkhipkin and Nigmatullin 1997; Bolstad and Hoving 2011 and references within). There is no trawl fishery around South Georgia to suggest that these two males of *K. longimana* might be captured from deep-seas and then discarded by fishermen though we cannot exclude a sheer theoretical possibility of capturing these squid by two longliners operating in the area. However, throughout the entire longlining history, which is closely monitored by observers onboard it occurred just once (Laptikhovsky et al. 2013). Therefore we think that the possibility of these *K. longimana* being brought up to the surface otherwise but by post-spawning body degeneration is highly unlikely.

The spermatophores of *K. longimana* in our study looked normal and were functional, as they had ejaculated following the albatrosses' predation. They were very dissimilar to those of other spent squid, in which the spermatophores have short, thin, semi-transparent, seminal reservoirs and a progressively increasing rear empty part, as reported in senescent male oceanic squid *Illex argentinus* (Laptikhovsky and Nigmatullin 1992) and *Gonatus antarcticus* (V. Laptikhovsky, unpublished), following a gradual decline in testis activity. Rigid and dense seminal reservoirs occupied 42–72% SL (mostly ca. 55–60% SL) in *K. longimana* and looked as those in other large cold- and temperate water onychoteuthids, including *Onykia robsoni* (~ 70% SL), *O. robusta* (62–78% SL), *O. ingens* (38–51% SL) and *Onychoteuthis borelijaponica* (66–70% SL), though they were longer than reported in tropical and subtropical representatives of the family: *Onychoteuthis banksi* and *Ancistroteuthis lichtensteini* (9–33%) (Hess 1987; Laptikhovsky 1990; Hoving et al. 2016). The SR of *Kondakovia* had similar proportions to other Antarctic squids from other families, such as *Galiteuthis glacialis* (42.4% SL) and *Mesonychoteuthis hamiltoni* (72.6–85.4% SL) (Hess 1987). Numerous empty envelopes and spermatangia indicated that the spermatophores of male *Kondakovia* foraged by albatrosses may have been ejaculated.

Our results show that in moribund male *K. longimana* the spermatophoric sacs contained at least a hundred normal spermatophores and there was no evidence of malfunctioning of the reproductive system. We might suppose that senescence processes in both sexes of *K. longimana* occurs together with morphological and physiological changes in the mantle leading to spent and partially spent individuals rising to the surface, as has been recorded in large polynyas in the southern Indian Ocean, Ross Sea and Scotia Sea (Lu and Williams 1994; Vacchi et al. 1994; Lynne and Rodhouse 2002). If such a process would occur faster than full degeneration of the reproductive system, a male might die or lose neutral buoyancy before being fully spent. It is noticeable that the beaks of senescent *K. longimana* (darkened wings, n=72) sampled from the diet of wandering albatross belonged to animals with mode of 435 mm ML (Cherel and Weimerskirch 1999), which is much smaller than the maximum reported size of immature females. Possibly, the buccal masses of adult females are too big for albatrosses to swallow, and this part of the body may be more prone to seabirds foraging.

Analysis of existing information allows to conclude that senescent male *K. longimana* are 320–480 mm ML (0.8 – 2.5 kg BW) mm and are thus about 1/10 the weight of senescent females (7–29 kg or more), emphasising the sexual dimorphism of this species. Generally, males of medium-sized and large nectonic oceanic oegopsid squid are either of the same size as females as noted in the Thysanoteuthidae (Nigmatullin et al. 1995), or are slightly smaller than females, whereby the maximum reported ML for males is about 70–85% of the maximum reported female ML (and

approximately half the body mass), as reported in Ommastrephidae, Gonatidae, Architeuthidae and Onychoteuthidae (Filippova et al. 1997; Jereb and Roper 2010; Hoving et al. 2004, 2015). Only in *Sthenoteuthis* and *Ommastrephes* (Ommastrephidae) are females 1.5-2 times larger and 2-5 times heavier than males (Zuev et al. 2002; Jereb and Roper 2010).

The possible reason of extreme sexual dimorphism in *K. longimana* (~10 times difference in weight between males and females) is the need to maximise reproductive output by channelling most of the available resources into female somatic and generative growth in food – restricting environment. On the other hand, a broad spectrum of adult size allows for a wider size spectrum of prey (as do smaller squid hunt smaller fish), so potentially reducing intraspecific competition and enhancing available trophic resources.

Our data also show that male *K. longimana* have extended spermatophore production, as other squids and in contrast to that supposed by Clarke (1980). Spermatophore production in this species likely lasts to the very last moment of life, and the bimodal spermatophore size structure noticed by Clarke (1980) could be the result of males producing them seasonally and living for several years, as female do.

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References

- Arkhipkin AI, Nigmatullin CM (1997) Ecology of the oceanic squid *Onychoteuthis banksi* and the relationship between the genera *Onychoteuthis* and *Chaunoteuthis* (Cephalopoda: Onychoteuthidae). J Mar Biol Ass UK 77: 839-869
- Bizikov VA (1991) A new method of squid age determination using the gladius. In: Jereb P, Ragonese S, Boletzky S (eds) Squid age determination using statoliths. N.T.R.-I.T.T.P. Special publications no. 1. Mazara del Vallo, Italy, pp. 39-51

196 Bolstad KS (2008) Systematics of the Onychoteuthidae Gray, 1847 (Cephalopoda: Oegopsida). PhD
 197 thesis, Earth & Oceanic Sciences Research Institute Auckland University of Technology
 198 Bolstad KS, Hoving HJT (2011) Spermatangium structure and implantation sites in onychoteuthid
 199 squid (Cephalopoda: Oegopsida). *Mar Biodivers Rec* 4; e5: 1-6
 200 Cherel Y, Weimerskirch H (1999) Spawning cycle of onychoteuthid squids in the southern Indian
 201 Ocean: new information from seabird predators. *Mar Ecol Prog Ser* 188:93-104
 202 Clarke MR (1962) The identification of cephalopod "beaks" and the relationship between beak size
 203 and total body weight. *Bull Brit Mus Nat Hist (Zool)* 8:1-480
 204 Clarke MR (1966) A review of the systematics and ecology of oceanic squids. *Adv Mar Biol* 4: 91-300.
 205 Clarke MR (1980) Cephalopoda in the diet of sperm whales of the southern hemisphere and their
 206 bearing on sperm whale biology. *Discovery Repts* 37:1-324
 207 Collins MA, Rodhouse PGK (2006) Southern ocean cephalopods. *Adv Mar Biol* 50:191-265
 208 Croxall JP, Prince PA (1994) Dead or alive, night or day: how do albatrosses catch squid? *Antarct Sci*
 209 6:155-162
 210 Filippova JA (1972) New data on the squids (Cephalopoda: Oegopsida) from the Scotia Sea
 211 (Antarctic). *Malacologia* 11:391-406
 212 Filippova JA, Alexeev DO, Bizikov VA, Khromov DN (1997) Commercial and mass cephalopods of the
 213 World Ocean: a manual for identification. VNIRO Publ. Moscow (In Russian).
 214 Hess SC (1987) Comparative morphology, variability, and systematic applications of cephalopod
 215 spermatophores (Teuthoidea and Vampyromorpha). PhD thesis, University of Miami, USA
 216 Hoving HJT, Roeleveld MAC, Lipinski MR, Melo Y (2004) Reproductive system of the giant squid
 217 *Architeuthis* in South African waters. *J Zool Lond* 264:153-169
 218 Hoving, H.J.T., Arkhipkin, A.I., Laptikhovsky, V.V., Marian, E.A.R. 2015. Mating tactics in the sub-
 219 Antarctic deepsea squid *Onykia ingens* (Cephalopoda: Onychoteuthidae). *Polar Biol* 39:
 220 1319-1328
 221 Jarre A, Clarke M, Pauly D (1991) Re-examination of growth estimates in oceanic squids: the case of
 222 *Kondakovia longimana* (Onychoteuthidae). *ICES J Mar Sci* 48:195-200
 223 Jereb P, Roper CFE (2010) (eds) Cephalopods of the world. An annotated and illustrated catalogue of
 224 cephalopod species known to date. 2. Myopsid and Oegopsid Squids. FAO Species catalogue
 225 for fishery purposes 4, FAO, Rome
 226 Laptikhovsky VV (1990) Spermatozoid morphology of the oceanic cephalopoda, their concentration
 227 in spermatophores and activity. *Zoologicheskij Zh* 69:21-28 (In Russian with English
 228 summary)
 229 Laptikhovsky V, Arkhipkin A (2003) The reproductive features of a mature female of the deep-sea
 230 planktonic squid *Galiteuthis glacialis* (Cephalopoda: Cranchiidae) from the Southern Ocean.
 231 *Polar Res* 22: 395-397
 232 Laptikhovsky VV, Nigmatullin ChM (1992). Caracteristicas reproductivas de machos y hembras del
 233 calamar (*Illex argentinus*). *Frente Maritimo* 12:23-38
 234 Laptikhovsky VV, Arkhipkin AI, Hoving HJT (2007) Reproductive biology in two species of deep-sea
 235 squid. *Mar Biol* 152:981-990
 236 Laptikhovsky V, Collins MA, Arkhipkin A. (2013) First case of possible iteroparity among coleoid
 237 cephalopods: the giant warty squid *Kondakovia longimana*. *J Moll Stud* 79:270-272
 238 Lipinski MR, Jackson S (1989) Surface-feeding on cephalopods by procellariiform seabirds in the
 239 southern Benguela region, South Africa. *J Zool Lond* 218:549-563
 240 Lu CC, Williams R (1994) *Kondakovia longimana* Filippova, 1972 (Cephalopoda: Onychoteuthidae)
 241 from the Indian Ocean sector of the Southern Ocean. *Antarct Sci* 6:231-234
 242 Lynnes AS, Rodhouse PG (2002) A big mouthfull for predators: the largest recorded specimen of
 243 *Kondakovia longimana* (Cephalopoda: Onychoteuthidae). *Bull Mar Sci* 7:1087-1090
 244 Nesis K (1987) Cephalopods of the world. T. F. H. Publications, Neptune City, New Jersey
 245 Nesis KN, Nigmatullin CM, Nikitina IV (1998) Spent females of deepwater squid *Galiteuthis glacialis*
 246 under the ice at the surface of the Weddell Sea (Antarctic). *J Zool Lond* 244:185-200

247 Nigmatullin CM, Arkhipkin AI, Sabirov RM (1995) Age, growth and reproductive biology of diamond-
 248 shaped squid *Thysanoteuthis rhombus* (Oegopsida: Thysanoteuthidae). Mae Ecol Prog Ser
 249 124:73-87
 250 Sabirov RM (1995) Spermatophorogenesis and reproductive strategy in males of ommastrephid
 251 squids (Oegopsida: Ommastrephidae). PhD thesis, Institute of Evolutionary Morphology,
 252 Moscow, Russia (in Russian),
 253 Vacchi M, Greco S, La Mesa M (1994) *Kondakovia longimana* Filippova 1972 (Onychotheuthidae) from
 254 Terra Nova Bay, Ross Sea. Antarct Sci 6:283-283
 255 Xavier JC, Cherel Y (2009) Cephalopod beak guide for the Southern Ocean. British Antarctic Survey,
 256 Cambridge
 257 Xavier JC, Cherel Y, Roberts J, Piatkowski U (2013) How do cephalopods become available to
 258 seabirds: can fish gut contents from tuna fishing vessels be a major food source of deep-
 259 dwelling cephalopods? ICES J Mar Sci 70:46-49
 260 Xavier JC, Croxall JP, Cresswell KA (2005) Boluses: an effective method to assess the proportions of
 261 cephalopods in the diet of albatrosses. Auk 122:1182-1190
 262 Xavier JC, Croxall JP, Reid K (2003a) Inter-annual variation in the diet of two albatross species
 263 breeding at South Georgia: implications for breeding performance. Ibis 145:593-610
 264 Xavier JC, Croxall JP, Trathan PN, Rodhouse PG (2003b) Inter-annual variation in the cephalopod
 265 component of the diet of wandering albatrosses *Diomedea exulans* breeding at Bird Island,
 266 South Georgia. Mar Biol 142:611-622
 267 Xavier JC, Trathan PN, Croxall JP, Wood AG, Podestá GP, Rodhouse PG (2004) Foraging ecology and
 268 interactions with fisheries of wandering albatrosses at South Georgia. Fisheries Oceanogr
 269 13:324-344
 270 Zuev G, Nigmatullin C, Chesalin, M, Nesis K (2002) Main results of long-term worldwide studies on
 271 tropical nectonic oceanic squid genus *Sthenoteuthis*: an overview of the Soviet
 272 investigations. Bull Mar Sci 71:1019-1060
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276 Figure captions:

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278 Figure 1. Detailed illustration of the spermatophore of *Kondakovia longimana*. A – the
279 spermatophore, B – spermatophore head, C – end of the cement body (an expansion of inner tunics
280 around the beginning of ejaculatory tube is not always present). Scale bars 1 mm.

281 Figure 2. Length-frequency distributions of spermatophores both intact and empty envelopes: A-
282 sample A, B – sample B.

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