

‘Superbull’ males: what role do they play and what drives their appearance within the *Doryteuthis gahi* Patagonian Shelf population?

Jessica B. Jones^{id} ^{A,B,C,G}, Graham J. Pierce^{A,D,E,F}, Paul Brickle^{A,C},
Zhanna N. Shcherbich^B and Alexander I. Arkhipkin^{A,B}

^ASchool of Biological Sciences, University of Aberdeen, Zoology Building, Tillydrone Avenue, Aberdeen, AB24 2TZ, Scotland, UK.

^BFisheries Department, Falkland Islands Government, Stanley, PO Box 598, FIQQ 1ZZ, Falkland Islands.

^CSouth Atlantic Environmental Research Institute, PO Box 609, Stanley Cottage, Stanley, FIQQ 1ZZ, Falkland Islands.

^DCentro de Estudos do Ambiente e do Mar, Universidade de Aveiro, PT-3810-193 Aveiro, Portugal.

^EDepartamento de Biologia, Universidade de Aveiro, PT-3810-193 Aveiro, Portugal.

^FInstituto de Investigaciones Mariñas (Consejo Superior de Investigaciones Científicas), Eduardo Cabello 6, E-36208 Vigo, Spain.

^GCorresponding author. Email: jjones@fisheries.gov.fk

Abstract. Cephalopod populations exhibit high variability in life history characteristics, such as longevity and size-at-age. The aim of this study was to understand how characteristics of a newly described ‘superbull’ male morph in *Doryteuthis gahi* populations (Patagonian Shelf) arise and whether there is a selective advantage. At the population level, it is speculated that superbulls provide temporal and spatial connectivity, but individual benefit is less obvious. Age structure and reproductive potential of males was investigated to determine whether superbulls could provide connectivity. Environmental variables affecting size-at-age were explored to ascertain whether morphological differences were primarily phenotypically driven. Superbulls from the autumn spawning cohort were significantly older than the residual population, with added longevity potentially leading to spawning with the following cohort. A reduction in relative testis weight was apparent in superbulls, but spermatophore production remained high. Generalised additive mixed models indicated temperature, location and hatch year had significant effects on size-at-age. Weak correlations between warm El Niño–Southern Oscillation phases and superbull abundance were found. The results suggest that superbulls provide temporal connectivity and arise through phenotypic plasticity, likely providing connectivity as a side effect of body shape and size rather than a genetically selected advantage.

Additional keywords: cephalopods, plasticity, population structure, reproduction, southwest Atlantic, statolith.

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Introduction

Many fish populations have a multigenerational structure that provides a reserve of reproductive potential and genetic variability. With several year classes of reproductively viable adults available, this risk-spreading strategy reduces the potential for population collapse (O’Dor 1998). Conversely, most cephalopods are characterised by an annual life cycle. With annual recruitment depending solely on the success of a single generation, cephalopods are thought to compensate for a lack of temporal risk spreading by producing genetically and phenotypically diverse offspring that are able to survive in a heterogeneous marine environment (Pierce and Guerra 1994; O’Dor 1998).

Although this is a group selectionist argument for phenotypic plasticity, it is plausible if many populations exist and population persistence depends on plasticity.

The Patagonian long-finned squid *Doryteuthis gahi* is abundant around the Falkland Islands, where it is targeted by a commercial trawl fishery (Arkhipkin *et al.* 2007). These squid undergo spatial ontogenetic migrations from their shallow inshore spawning and nursery grounds (depth ~5–50 m) to offshore feeding grounds on the Falklands shelf (depth ~200–350 m; Arkhipkin *et al.* 2004). *D. gahi* is a small-sized squid typically attaining 13–16-cm dorsal mantle length (DML; Arkhipkin *et al.* 2007). Squid are targeted during two

commercial fishing seasons, which correspond to the appearance of the two spawning cohorts, namely the spring spawning cohort (SSC) and the autumn spawning cohort (ASC), on their shared feeding grounds (Patterson 1988; Hatfield *et al.* 1990; Arkhipkin and Middleton 2003; Arkhipkin *et al.* 2007).

Most myopsid squid populations are characterised by the presence of two size-dependent behavioural morphotypes of mature males on spawning grounds (Hall and Hanlon 2002; Hanlon *et al.* 2002; Wada *et al.* 2005; Shashar and Hanlon 2013): (1) large males, referred to as ‘bull’ males, have dominant behaviour, forming mating pairs with females; and (2) smaller subordinate individuals or ‘sneaker’ males that are of similar size or smaller than females. The small size of ‘sneaker’ males facilitates a close approach to mating pairs, where the ‘sneaker’ males then attempt to elicit extrapair copulations (EPCs; Hanlon and Messenger 1998). Some authors have pointed to evidence for the existence of more than two size groups in males, for example Collins *et al.* (1999) for *Loligo forbesii*, implying that plasticity in growth form extends beyond the existence of two reproductive strategies in males. Anomalous large males, much larger than the typical mature males, have been found to occur in populations of several loliginid squid (Mangold 1987; Pierce *et al.* 2013; Jones *et al.* 2019). A recent study investigating the intrapopulation body shape variation of *D. gahi* found that males exceeding 20–25 cm DML had a significantly different body shape than the rest of the population and were suggested to be a third ‘superbull’ morph (Jones *et al.* 2019). These superbull males had a substantially more elongated body, a heavier fin and a larger fin area than the rest of the population.

Owing to the streamlined shape and large fins characteristic of this morph, it is likely that these superbull males have enhanced gliding abilities and are able to undertake longer migrations than the rest of the population (Arkhipkin *et al.* 2015a). It was therefore hypothesised that these superbull males extend their migratory routes, providing geographic connectivity by migrating between remote spawning areas (Jones *et al.* 2019). In other species, anomalously large individuals have delayed maturation and an extended life cycle. For example, in the eastern Pacific, temperature determines whether jumbo squid *Dosidicus gigas* have a 1-year life cycle (early maturation and a small size) or a 1.5- to 2-year life cycle (delayed maturation and a large size; Arkhipkin *et al.* 2015b). If this is the case for *D. gahi*, these individuals may also potentially maintain temporal connectivity by reproducing with the following spawning cohort.

The intrinsic or extrinsic mechanisms driving the existence of these superbull males are presently unknown and the hypothesis that they provide geographical or temporal connectivity is yet to be confirmed. Given that (spatial or temporal) connectivity is a population-level phenomenon, the existence of superbull males might be explained by either a selective advantage at an individual level (e.g. enhanced reproductive output relative to smaller males, either through increased gamete holding capacity or through increased success in conflicts with other males) or selective pressure favouring phenotypic variability, the expression of which may be environmentally driven (O’Dor 1998). It is plausible that populations with higher individual variability or plasticity are more persistent. Several sources of evidence are

relevant to the existence of superbulls and the selective pressures at work:

- evidence of enhanced swimming ability or longer life would indicate the potential for providing spatial or temporal connectivity
- evidence of superbulls mating with squid in distant locations or with squid from the next generation would confirm that connectivity is achieved
- evidence of enhanced reproductive output would confirm the possibility of a direct selective advantage for superbulls, if this characteristic is selected
- evidence of consistent genetic differences at one or more locus, between superbulls and other males, would suggest the superbull characteristics are heritable
- evidence that the occurrence of superbulls is environmentally driven would provide circumstantial evidence against heritability.

Considering that the superbulls exhibit a high degree of interannual variation in abundance, it is likely that their occurrence is driven by extrinsic phenotypic variation rather than any genetic influence. Small changes in environmental conditions (most often temperature) and food availability are known to have significant effects on life history characteristics such as longevity and size-at-age in squid (Pecl *et al.* 2004). Variability of growth in *D. gahi* has been largely attributed to variation in temperature during early life stages, with squid hatched in summer (at higher temperatures) being significantly larger than squid of the same age hatched during the winter months (Hatfield 2000). Clearly environmental influences have a marked effect on the population characteristics of this species. Indeed, large-scale events such as the propagation of the El Niño–Southern Oscillation (ENSO) from the Pacific Ocean have been correlated with population abundance in *D. gahi* (Waluda *et al.* 2004). The ENSO is recognised as an important driver of sea surface temperature (SST) variability in south-west Atlantic and Antarctic waters (Trathan and Murphy 2003; Meredith *et al.* 2008; Baylis *et al.* 2012). However, the effects of large-scale events on the size-at-age relationship have not yet been investigated.

The aim of this study was to quantify the reproductive output and compare squid age between spawning cohorts and between superbulls and smaller males to investigate the potential that superbulls provide temporal or geographic connectivity. In addition, the effect of extrinsic variables, predominantly temperature and food availability, on the size-at-age relationship was investigated to determine whether the appearance of superbull males is phenotypically driven, the hypothesis being that these males have experienced similar environmental conditions that have resulted in their large size. We examined this in two ways: (1) comparing size-at-age relationships across years to see whether differences are environmentally driven; and (2) relating the annual abundance of superbulls to large-scale temperature anomalies.

Materials and methods

Biological sampling

An existing dataset representative of the squid population within the Falkland Islands interim conservation and management zone

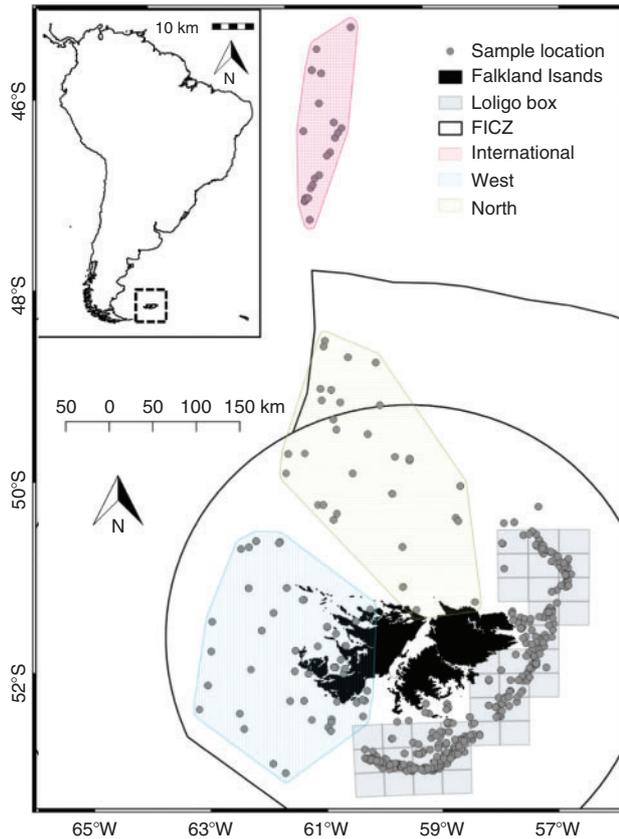


Fig. 1. Sample locations within the Falkland Interim Management and Conservation Zone (FICZ) and international waters. Samples were divided into four areas (samples in close proximity to the 'Loligo box', the commercial fishing region, are included in the area 'Loligo') for the area variable in the generalised additive mixed models. The box delineated by the dashed line in the inset indicates the study location relative to mainland South America.

(FICZ) and international waters north of the zone was extended during this analysis to incorporate additional superbull males (defined as those exceeding 25 cm DML; Fig. 1), which are a rare component of the fishery catch. Data were sourced from three different datasets from the Falkland Islands Fisheries Department, as detailed below:

- Data source 1: random samples of aged male specimens ($n = 7505$) collected and frozen by scientific observers on board commercial fishing vessels during the two commercial fishing seasons (Season 1: March–May; Season 2: July–October) and from two research cruises (the *RV Dorada* and *RV Castelo*) between fishing seasons (between 1998 and 2007); measurements and statoliths were taken from fortnightly samples of 'mixed' squid (unsorted by size; Table 1)
- Data source 2: non-random aged samples including rare superbull males exceeding 25 cm DML ($n = 162$), collected and frozen during 2014 and 2015, to ensure squid of a large size were represented within the sample (Table 1)
- Data source 3: random length–frequency data ($n = 921\,742$) from males collected daily by scientific observers during the

Table 1. Summary of samples collected by cohort from Data sources 1 and 2

There were two spawning cohorts: autumn (ASC) and spring (SSC). Data show the mean (\pm s.e.m.) dorsal mantle length (DML) and the number of samples collected (n) by year of collection

Year of collection	ASC		SSC	
	n	DML (cm)	n	DML (cm)
1998	61	9.6 \pm 0.4	4	14.0 \pm 1.0
1999	893	13.9 \pm 0.2	416	14.9 \pm 0.2
2000	968	12.3 \pm 0.1	901	12.4 \pm 0.1
2001	388	13.3 \pm 0.3	635	14.8 \pm 0.2
2002	172	9.7 \pm 0.2	480	13.3 \pm 0.2
2003	239	16.5 \pm 0.4	501	16.8 \pm 0.2
2004	182	15.7 \pm 0.5	338	16.4 \pm 0.2
2005	415	16.1 \pm 0.4	226	15.9 \pm 0.3
2007	460	14.3 \pm 0.3	226	14.3 \pm 0.4
2014	41	25.0 \pm 1.7	60	30.3 \pm 1.1
2015	44	17.4 \pm 0.8	17	18.8 \pm 1.6

two commercial fishing seasons within the designated fishing zone (the 'Loligo box'; Fig. 1) between 1998 and 2017 for construction of length–frequency graphs and distributional maps to aid in data interpretation.

Frozen samples from Data sources 1 and 2 were thawed overnight and processed in the Falkland Islands Fisheries Department laboratory. Squid were measured (DML; ± 1 mm), weighed (total bodyweight (BW); ± 1 g) and visually assessed for sex and maturity stage according to a modified version of Lipinski (1979), using a maturity scale ranging from 1 (immature) to 6 (spent). Statoliths were dissected from the cephalic cartilage and stored in 96% ethanol. A subsample of aged males ($n = 386$) collected between 1999 and 2007 had additional data collected including mantle weight (MW), digestive gland weight (DGW) and reproductive organ weights (i.e. testis weight (TW), Needham's complex weight (NCW) and the reproductive system weight, $RW = TW + NCW$). To determine how the proportion of somatic weight allocated to different organs changes relative to size, four indices were computed, defined as:

$$i = \frac{a}{BW - a} \times 100$$

where I is the gonadosomatic index (GSI), testis index (TWI), Needham's complex index (NCI) or digestive gland index (DGI) and a is the corresponding organ weight (RW, TW, NCW or DGW). Samples from Data source 3 were processed at sea and were measured for DML (± 5 mm) and visually assessed for maturity stage.

Age estimation

One statolith per specimen was mounted concave side up for age analysis, then ground and polished on both sides using wet waterproof grinding paper to expose the nucleus. The thin transverse section produced was embedded in mounting medium (Canada balsam, Merck KGaA, Darmstadt, Germany)

and covered with a cover glass for observation. Statoliths were read under the transmitted light of an Olympus BX51 compound microscope (Olympus Corporation, Tokyo, Japan) at 400× magnification, with a phase-contrast Nomarski effect used to improve readability (Arkhipkin and Shcherbich 2012).

Statoliths were first read in the lateral dome from the natal ring to the first prominent check. This check was followed into the rostrum and read in the rostrum to the tip. Because the ‘one ring = one day’ hypothesis has been validated in several loliginid species, including *Sepioteuthis lessoniana* (Jackson *et al.* 1993), *Lolliguncula brevis* (Jackson *et al.* 1997), *Loligo reynaudii* (Lipinski *et al.* 1998), *Loligo vulgaris* (Villanueva 2000) and *Loligo plei* (Jackson and Forsythe 2002), total increment number was assumed to represent postembryonic age in days (from here onwards referred to as age). To minimise counting errors, the total number of growth increments for each specimen was taken as the mean of at least two counts. If the difference between these counts exceeded 10%, a third count was made. If the difference between the second and third readings still exceeded 10%, the statolith was rejected from further age analysis. For Data source 1, statolith ages were intermittently verified by a second reader. For Data source 2, 6% of age counts were verified by a second reader.

Environmental variables

SST data were extracted from the Integrated Global Ocean Services System database (IGOSS nmc Reyn_SmithOiv2 monthly; http://iridl.ldeo.columbia.edu/SOURCES/IGOSS/nmc/Reyn_SmithOiv2/monthly/, accessed 20 February 2018) to determine how average monthly SST affected the size-at-age relationship (Reynolds *et al.* 2002). SST data were collected between 53.5 and 45.5°S and 63.5 and 56.5°W to obtain average monthly SST values for the whole sampling area. Mean monthly chlorophyll-*a* concentration (mg m⁻³) was extracted for the same area from the European Union Copernicus marine service information system and was used as a proxy for productivity at month of hatching and the month following month of hatching (to account for a lag between primary production and zooplankton production). Data were derived from global ocean colour satellite observations (L4_NRT_OBSERVATIONS_009_033 and L4_REP_OBSERVATIONS_009_082) at a resolution of 4 × 4 km using SeaWiFS, MODIS-Aqua, MERIS, VIIRS and OLCI-S3A sensors (<http://marine.copernicus.eu>, accessed 20 February 2018).

To assess whether the number of superbull males appearing in the fishery was affected by broad-scale climate variability in temperature, the southern oscillation index (SOI) was used. The SOI is the standardised difference in surface pressure anomalies between Tahiti in the Central Pacific (17°33'S, 149°37'W) and Darwin in Australia (12°28'S, 130°51'E), with negative SOI values indicating a warm phase of the ENSO. Yearly SOI values were accessed on the Australian government Bureau of Meteorology website (<http://www.bom.gov.au/climate/current/soihtm1.shtml>, accessed 15 February 2018).

Statistical analysis

Reproductive indices were plotted against squid size (DML) and fitted with local regression smoothers (locally estimated scatterplot smoothing (LOESS)) to visually assess reproductive

output, with DGI also plotted with LOESS to investigate whether energy for gonad development was derived from stored sources (most often sourced from the lipid-rich digestive gland) or diet. Hatch date was determined by back-calculation of the total number of increments from the date of capture. Based on hatch date, each male was assigned to a spawning cohort. Squid hatched between April and November were considered to belong to the ASC and those hatching between December and March were assigned to the SSC (Arkhipkin *et al.* 2013). Data exploration was undertaken for aged samples following procedures described by Zuur *et al.* (2010). Extreme outliers identified in bivariate scatterplots and Cleveland dotplots were removed. Nine recorded values were considered implausible (0.001% of the dataset) and were discarded before analysis. To explore the potential that superbulls provide temporal connectivity between cohorts, age was modelled by means of a generalised linear model (GLM) with a Gaussian distribution and an identity link:

$$A_i = \beta_0 + \beta_1(\text{cohort}_{1i}) + \beta_2(\text{type}_{2i}) + \beta_3(\text{cohort}_{1i}, \text{type}_{2i}) + \varepsilon_i$$

where $\varepsilon_i \sim N(0, \sigma^2)$, A_i is the post-hatching age, in days, of individual squid i , cohort is fitted as a categorical variable with two levels (ASC and SSC) and type is fitted as a categorical variable with two levels (superbull (≥ 25 cm DML) and normal (< 25 cm DML)). The significance of an interaction term between cohort and type was evaluated by comparing GLM models with and without an interaction, using a Chi-squared test. Only mature male squid (Maturity stages 5 and 6) were used in this analysis ($n = 3883$). Effects from the GLM model were exhibited using the package ‘effects’ (Fox 2003) in the R environment (ver. 3.3.0, R Foundation for Statistical Computing, Vienna, Austria, see <https://www.R-project.org/>, accessed 25 July 2018).

Generalised additive models (GAMs) and generalised additive mixed models (GAMMs) with Gaussian distributions and identity links were used to describe variation in squid size (DML) in relation to environmental variables, using data from all aged males that had complete sets of environmental data ($n = 7308$). Several models were fitted, with Akaike information criterion (AIC; Akaike 1973) and Akaike weights used in conjunction with a forwards model selection procedure (based on biological knowledge of the species and the objectives of the study) to identify the optimal model (Wagenmakers and Farrell 2004). Examination of the residuals of the simplest model, using DML as the response variable and age as the smoothed explanatory variable, suggested a transformation was necessary to avoid violating the assumptions of normality and to reduce heterogeneity. A Box–Cox transformation (Box and Cox 1964) was applied to DML in all models, which improved residual fit, defined as:

$$y_i^\lambda \begin{cases} \frac{y_i^\lambda - 1}{\lambda}, \lambda \neq 0 \\ \ln y, \lambda = 0 \end{cases}$$

where y_i is the DML of an individual squid i and λ is an exponent between -5 and 5 that transforms the data to the best approximation of a normal distribution curve. To incorporate

dependency among observations found in the same level of maturity, the addition of a random intercept for each maturity stage (categorical; six levels) was investigated (i.e. a_j , if squid i is from maturity level j , where a_j are assumed to be independent and identically distributed (i.i.d.) random effects) (Wood 2008). Putative explanatory variables were as follows: hatch_yr (year of hatch, categorical, 13 levels), col_year (year of sample collection, categorical, 11 levels) age (days, continuous), lon,lat (of collection, continuous), area (of collection, spatial categorical data with four levels, see Fig. 1), SST (SST during month of hatching ($^{\circ}\text{C}$), continuous), CHL (chlorophyll- a during month of hatching (mg g^{-1}), continuous) and CHL+1 (chlorophyll- a during month after hatching (mg g^{-1}), continuous). Either hatch_yr or col_year was incorporated into the model to account for interannual variation in size. Multipanel scatter plots and variance inflation factors (VIF) indicated strong collinearity between SST and CHL (Zuur *et al.* 2010). Therefore, the covariates SST and CHL were included in separate models. Smoothers for fixed variables were fitted using thin plate regression splines (Wood 2003). To avoid over-smoothing and to make the relationship between the response and explanatory variable clearer and more interpretable, the number of degrees of freedom for the environmental variables SST, CHL and CHL+1 was restricted to 6. Model assumptions were verified by plotting residuals against fitted values and against all potential covariates (Zuur and Ieno 2016). The additions of certain fixed effects were verified by applying F -tests. All statistical modelling was performed in the package 'mgcv' (Wood 2011) in R (R Foundation for Statistical Computing).

Cross-correlation analysis was used to determine the correlation between SOI anomalies and absolute abundance of superbull males. Absolute abundance was calculated by multiplying the number of superbulls found in Data source 3 in each year by a catch factor. The catch factor divided the total annual catch (obtained from fishery statistics reports; Falkland Islands Government 2017) by the minimum total catch found for the period 1998–2015 to improve the accuracy of interannual comparisons.

Results

Reproductive v. somatic growth

BW, MW and DGW showed a consistent increase with increasing DML (Fig. 2a–c). DGW showed more variability than the other two weight variables. However, RW, which is the sum of TW and NCW, reached an asymptote at 25–30 cm DML and showed a high degree of variation (Fig. 2d). When the constituents of RW were considered separately, TW started to decline after 25–30 cm DML (Fig. 2e), whereas NCW consistently increased with body size (Fig. 2f).

Reproductive and digestive gland indices were calculated for each individual (Fig. 3). Above 25 cm DML, the GSI dropped markedly along with the TWI. There was a negative linear relationship between DGI and squid size. The NCI increased from ~15 cm DML, reaching an asymptote at 23–25 cm DML. This suggests that although the RW is increasing (Fig. 2d), less energy is being directed into reproductive growth for individuals exceeding 25 cm DML.

Age structure

The modal size of mature males was 14 cm DML, with a mean size of 17.4 cm DML and a maximum size of 44.5 cm DML. Of the 3883 mature-aged specimens, 427 individuals were superbull males exceeding 25 cm DML. Of these, there were 234 superbull males assigned to the ASC and 193 assigned to the SSC, showing a similar distribution of month of hatching (Fig. 4a) to the rest of the male population (Fig. 4b). Peak hatching for both superbulls and the main population was found during December–January, with a second smaller hatching peak during July–September in the main population and August–October in the superbulls (despite peaks in hatch date, superbulls were still a small proportion of the entire population during these months). Hatching occurred almost all year round. Superbulls of the SSC were only collected in 4 months, peaking from September–October and those of the ASC were found in 8 months, with two main peaks in May and August. Maximum post-hatching age in male *D. gahi* was estimated at 368 days (a 41 cm DML superbull individual), the only individual exceeding 360 days. The GLM applied to the mature aged males indicated that the SSC was significantly younger than the ASC ($t = -7.65$, $P < 0.001$) and that the superbull males were significantly older than normal-sized males in both cohorts ($t = 22.87$, $P < 0.001$; Fig. 5). A likelihood ratio test indicated that the inclusion of an interaction term significantly improved model fit ($\chi^2 = 10541$, $P < 0.001$). Superbull males found within the ASC were the oldest with a predicted mean age of 286 days. Superbull males had a larger variation in age than the normal male morph (Fig. 5).

Environmental effects

The candidate models that explored the effects of environmental variables on the size-at-age relationship are given in Table 2. The inclusion of year of hatch (Model 2) rather than year of collection (Model 1) lowered the AIC and significantly improved model fit (d.f. = 2.43, $F = 126.2$, $P < 0.001$). To remove the effect of maturity stage, a random intercept was applied for each level of maturity, which also had a significant positive effect on model fit (d.f. = 3.82, $F = 65.1$, $P < 0.001$ using the restricted maximum likelihood (REML) estimation; see Fig. S1 of the Supplementary material). Although the inclusion of all three environmental variables (SST in month-of-hatch, chlorophyll- a in month of hatch and chlorophyll- a in month after hatch) lowered the AIC, SST improved model fit the most, followed by chlorophyll- a in month of hatch (see Fig. S2 of the Supplementary material). We assessed the residuals of Model 4 for spatial dependency and could not find any obvious patterns in the residuals (see Fig. S3 of the Supplementary material). However, we included spatial components at different spatial scales, with AIC favouring the simpler spatial model, separating the population into four broad regions ('Loligo box', $n = 5521$; west, $n = 844$; north, $n = 578$; international, $n = 365$; Fig. 1). Partial effects of the smooth from the more complex Model 7, using longitude and latitude, suggested the largest individuals were located in the north-west coastal waters of west Falklands and, more generally, all inshore coastal regions around the Falkland Islands (Fig. 6). Large individuals were also located in western international waters in the partial effects, but this could be an artefact resulting from the small sample size in

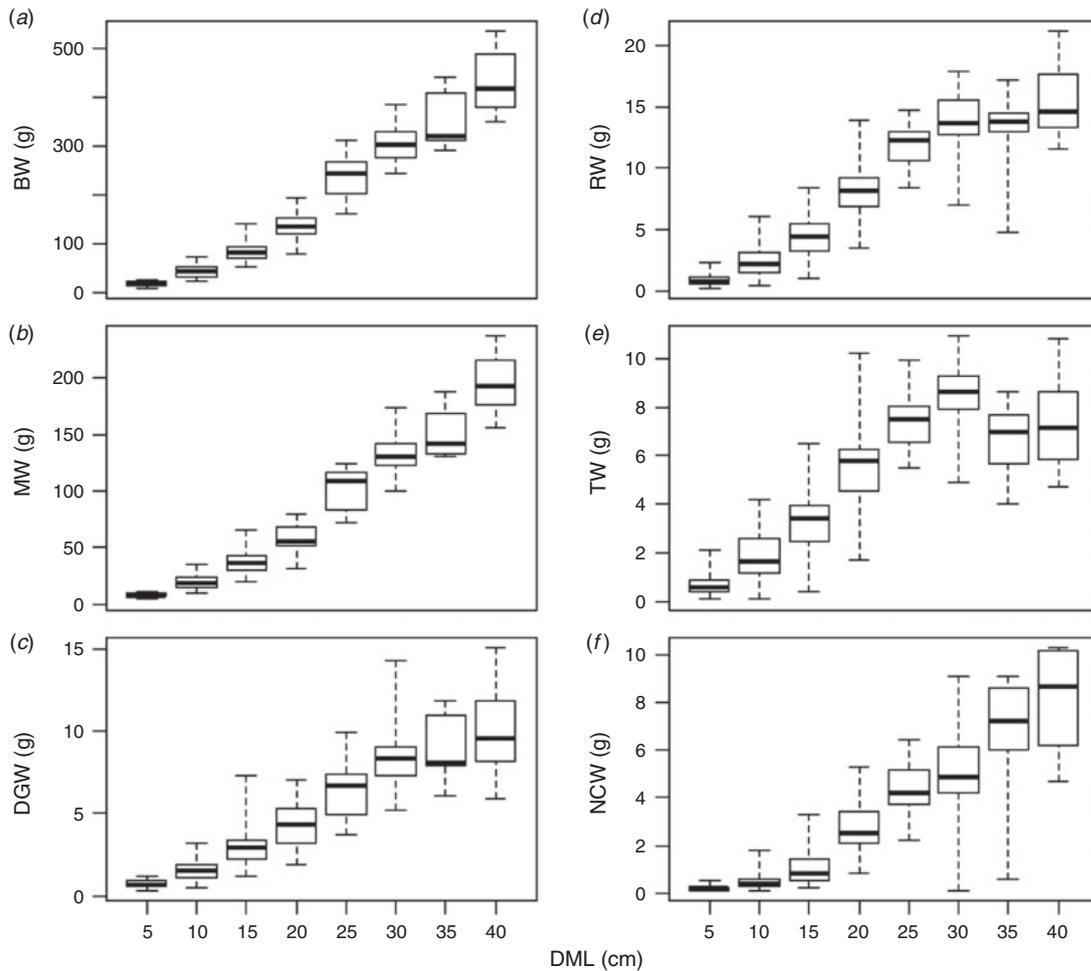


Fig. 2. Boxplots showing how mean (a) bodyweight (BW), (b) mantle weight (MW), (c) digestive gland weight (DGW), (d) reproductive weight (RW), (e) testis weight (TW) and (f) Needham's complex weight (NCW) change with dorsal mantle length (DML; grouped every 5 cm). The boxes show the interquartile range, with the median value indicated by the horizontal line; whiskers show the range.

this region. Based on raw distributional plots of superbull males collected during the first and second fishing seasons, first-season superbulls were often caught to the west of the Falkland Islands (as suggested by the partial effects of Model 7), contrary to normal-sized males of the first and second seasons, which were caught within the 'Loligo box' region. Second-season superbulls occurred more often to the north of the 'Loligo box' (for distributional plots, see Fig. S4–S7 of the Supplementary material).

The most parsimonious model in Table 2 was Model 9, in which the age effect had a smooth term that differed by area (Fig. 7). Comparison of models with and without separate smooths for the effect of age indicated that the inclusion of separate smooths significantly improved the model (d.f. = 7.87, $F = 13.5$, $P < 0.001$). The shape of all the smooths for the effect of age demonstrated that squid of a larger size were older. There appears to be a discontinuity in the age–length relationship in the 'Loligo box' area at ~200 and 300 days (Fig. 7b). To the north of the zone, the shape of the smoother for the effect of age was almost linear (Fig. 7c). As expected, error increased at

model extremes. Partial effects of area suggest that the 'Loligo box' area has the largest individuals, whereas areas in the rest of the conservation zone (north and west) have the smallest individuals (Fig. 8a). The optimal fitted GAMM demonstrated that SST ($F = 145.18$, $P < 0.001$) had a significant negative linear relationship with squid size, with error increasing with SST (Fig. 8b). Within this model, the effect of SST became a negative linear effect. Model validation indicated that no strong residual patterns were evident (see Fig. S8 of the Supplementary material).

Between-year differences

Annual SOI (Fig. 9a) was compared to the absolute abundance of large males exceeding 25 cm DML to determine how broad-scale environmental variation affects their presence in the fishery, given that there is a high degree of interannual variability in population size distribution (Fig. 9b). It seems that the appearance of superbull males coincides with negative SOI anomalies (i.e. warm ENSO phases), contrary to the results of the GAMM analysis, which found that squid achieve a larger

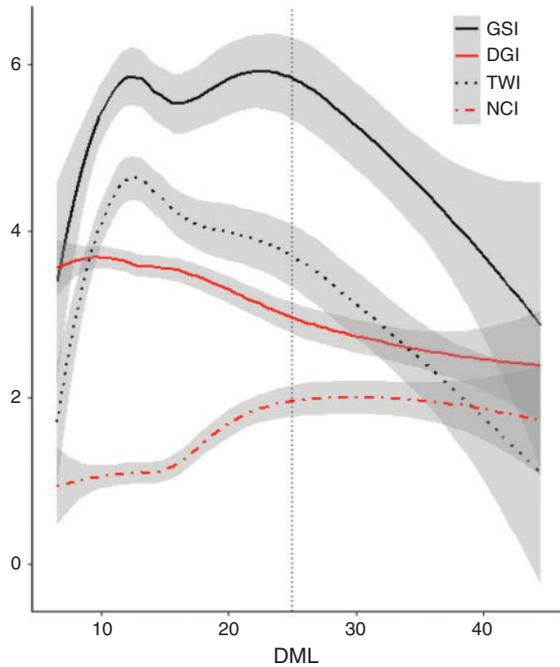


Fig. 3. Locally estimated scatterplot smoothing (LOESS) smoothers for all four indices calculated: gonadosomatic index (GSI), digestive gland index (DGI), testis weight index (TWI) and Needham's complex index (NCI). Shaded areas indicate 95% confidence intervals. The dotted vertical line indicates dorsal mantle length (DML) of 25 cm. Span = 0.75.

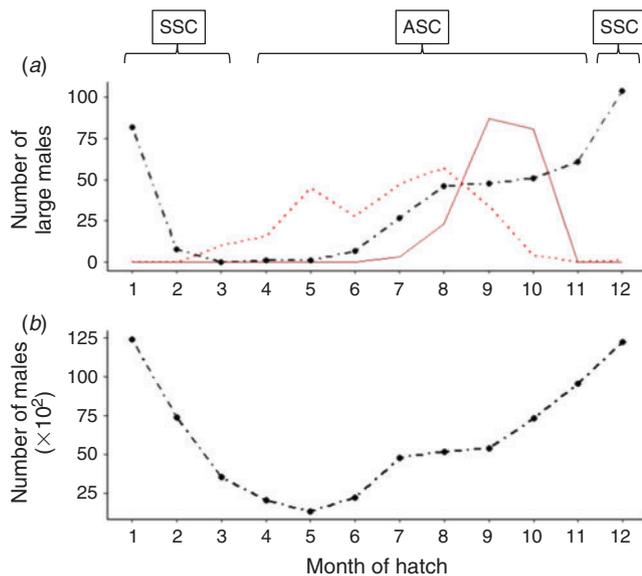


Fig. 4. Number of (a) males exceeding 25-cm dorsal mantle length (DML) and (b) all aged males hatched (dot-dashed lines) or collected (dotted line is the autumn spawning cohort (ASC); the solid line is the spring spawning cohort (SSC)) in each month with the cohort (ASC or SSC) that squid hatching in each month are purported to belong to indicated at the top.

size-at-age at higher temperatures during month of hatching. However, cross-correlation analysis indicates that the correlation between SOI and superbull absolute abundance is weak

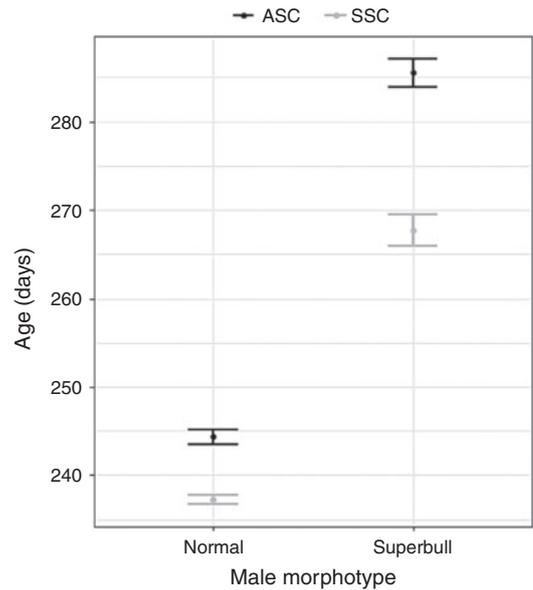


Fig. 5. Effect display for the interaction of cohort and male morphotype in the generalised linear model fit to age (days) with 95% confidence limits included. ASC, autumn spawning cohort; SSC, spring spawning cohort.

(-0.37 with a lag of 1 year) and non-significant at the 5% level (critical level = -0.47).

Discussion

To fully understand the ecology of a species, many factors must be taken into consideration. Population connectivity is usually determined by methods that can track an individual's life history throughout ontogeny, such as tagging (Gilly *et al.* 2006). However, these techniques are difficult to implement on loliginid squid, which are often too fragile for an external tag, have a short post-capture survival time and are lacking a suitable attachment site that does not inhibit their behaviour (Arkhipkin 2005). In this case, in order to obtain a complete picture of ontogenetic movements, a toolbox approach is required, where a range of independent techniques provides information at different temporal scales that can be used to understand connectivity (Sturrock *et al.* 2012). This can be achieved using methods such as elemental analysis of statoliths (Jones *et al.* 2018) or genetic methods (Shaw *et al.* 2004), but can also be achieved by analysing more basic information, such as size-at-age data. This study has made several steps in advancing our understanding of why superbull males exist within the *D. gahi* population using a toolbox approach.

The streamlined shape and large fins characteristic of the superbull morph are hypothesised to result in an extension of their migratory routes, providing a population-level benefit by connecting geographically separate subpopulations within Falkland Islands waters (Arkhipkin *et al.* 2015a; Jones *et al.* 2019). In addition to this connectivity between remote spawning areas, it is also possible that these squid provide temporal connectivity between spawning cohorts. At present, the mechanisms connecting the two cohorts are unresolved (Arkhipkin *et al.* 2013). Genetic studies using allozyme markers

Table 2. Models applied to *Doryteuthis gahi* size-at-age data for all males with complete sets of environmental data ($n = 7308$), numbered for easy reference and with expressions included

The notation $f_1(\text{age}_i) : \text{area}_i$ means that one smoother is used for each geographical area and the area is fitted as a mean term. $\text{DML}_{ij}^{\lambda}$, Box-Cox-transformed dorsal mantle length (DML) of squid i ; DML_{ij} , squid i within maturity stage j (random effect); a_j , random intercept for each maturity stage j and $\varepsilon_i \sim N(0, \sigma^2)$; col_yr , sample collection year; hatch_yr , hatch year; SST, sea surface temperature during month of hatching; lon, lat , longitude/latitude; CHL, chlorophyll- a during month of hatching; CHL+1, chlorophyll- a during month after hatching (CHL+1); %DE, percentage deviance explained; AIC, Akaike's information criterion; AIC wt, Akaike weights

Model	Expression	d.f.	%DE	AIC	ΔAIC	AIC wt.
1	$g\{\text{DML}_{ij}^{\lambda}\} = f_1(\text{age}_i) + \text{col_yr}_i + \varepsilon_i$	16.3	68.2	-9601.5	11 588.9	<0.001
2	$g\{\text{DML}_{ij}^{\lambda}\} = f_1(\text{age}_i) + \text{hatch_yr}_i + \varepsilon_i$	18.7	68.2	-9898.2	11 292.3	<0.001
3	$g\{\text{DML}_{ij}^{\lambda}\} = f_1(\text{age}_i) + \text{hatch_yr}_i + a_j + \varepsilon_{ij}$	23.4	76.4	-20 062.6	1127.8	<0.001
4	$g\{\text{DML}_{ij}^{\lambda}\} = f_1(\text{age}_i) + f_2(\text{SST}_i) + \text{hatch_yr}_i + a_j + \varepsilon_{ij}$	26	77.0	-20 301.6	888.9	<0.001
5	$g\{\text{DML}_{ij}^{\lambda}\} = f_1(\text{age}_i) + f_2(\text{CHL}_i) + \text{hatch_yr}_i + a_j + \varepsilon_{ij}$	28.1	76.9	-20 144.5	1046	<0.001
6	$g\{\text{DML}_{ij}^{\lambda}\} = f_1(\text{age}_i) + f_2(\text{CHL}+1_i) + \text{hatch_yr}_i + a_j + \varepsilon_{ij}$	28	76.6	-20 122.5	1068	<0.001
7	$g\{\text{DML}_{ij}^{\lambda}\} = f_1(\text{age}_i) + f_2(\text{SST}_i) + f_3(\text{lon}_i, \text{lat}_i) + \text{hatch_yr}_i + a_j + \varepsilon_{ij}$	50.6	78.4	-20 944.1	246.4	<0.001
8	$g\{\text{DML}_{ij}^{\lambda}\} = f_1(\text{age}_i) + f_2(\text{SST}_i) + \text{area}_i + \text{hatch_yr}_i + a_j + \varepsilon_{ij}$	29	77.3	-21 098.3	92.2	<0.001
9	$g\{\text{DML}_{ij}^{\lambda}\} = f_1(\text{age}_i) : \text{area}_i + \text{SST}_i + \text{hatch_yr}_i + a_j + \varepsilon_{ij}$	35.3	77.6	-21 190.5	0	0.999

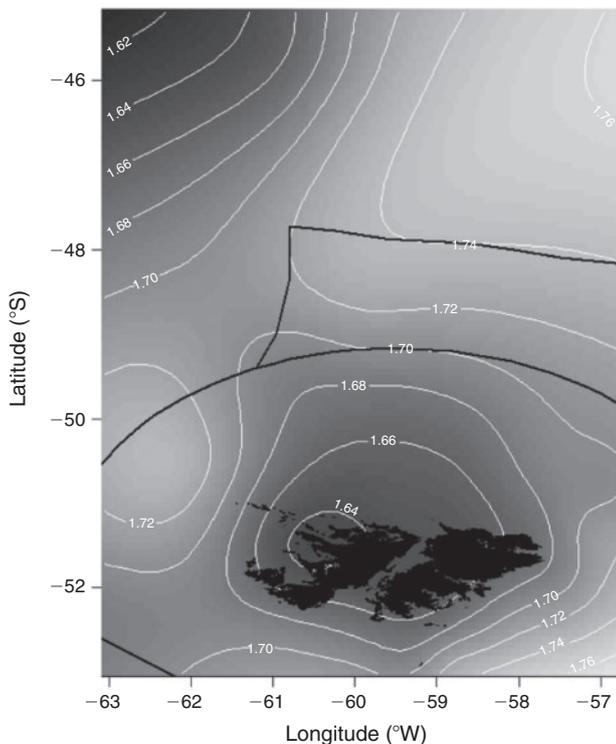


Fig. 6. Contour plot of the partial effects from the Generalised additive mixed Model 7 (Table 2) of longitude/latitude. Higher values indicate larger individuals. The black shaded region indicates the position of the Falkland Islands; black lines indicate the position of the Falkland Interim Management and Conservation Zone.

found no evidence of genetic differentiation among temporally distinct samples, suggesting that squid from both cohorts belong to a single interbreeding population (Carvalho and Pitcher 1989; Shaw *et al.* 2004). Several mechanisms have been proposed to allow for interbreeding between different seasonal cohorts, including early maturation and mating of males with mature

females from the previous cohort, a wide variation in spawning time resulting in overlap or an extended life cycle of more than 1 year for some individuals (Mesnil 1977; Boyle *et al.* 1995; Arkhipkin *et al.* 2013). Therefore, superbulls were hypothesised to have a life cycle exceeding 1 year (including the period of embryogenesis), providing the population level benefit of temporal connectivity (Jones *et al.* 2019). In order for a population-level benefit to occur, these squid would have to be reproductively viable. On an individual level, an enhanced reproductive output compared with normal-sized individuals may be a selective advantage. Alternatively, the existence of superbulls may be a by-product of phenotypic plasticity, with no specific individual selective advantage. The selective advantage of plasticity would, of course, also occur at the population level, but selection is also arguably plausible if populations with (heritable) higher plasticity are more persistent over evolutionary time. This latter scenario is consistent with the fact that the occurrence of superbulls is rare (Jones *et al.* 2019).

In the present study there was no evidence of enhanced reproductive output in superbulls, but the data do suggest that superbull males are reproductively able to maintain connectivity, whether it is geographical or temporal. Results indicate some trade-off between somatic and reproductive growth in those exceeding 25 cm DML, with a reduction in TW relative to BW (TWI) and with the NCW increasing synonymously with BW. Although there is a trade-off, this should not inhibit the ability of these superbull males to reproduce with females on spawning grounds because NCW (responsible for spermatophore production and accumulation) is maintained. Most likely, the relative reduction in TW is due to increased weight of muscles in the mantle and fins to maintain a streamlined shape. Energy for production of reproductive organs appears to be derived from diet rather than a stored energy source, given the gradual decline of the DGI with size. An obvious drop would suggest that lipid deposits within the digestive gland are being used as an energy source at the onset of reproductive maturation (Guerra and Castro 1994; Moltschaniewskyj and Semmens 2000).

As shown in previous growth papers (Arkhipkin and Roa-Ureta 2005) and from the significant relationship between

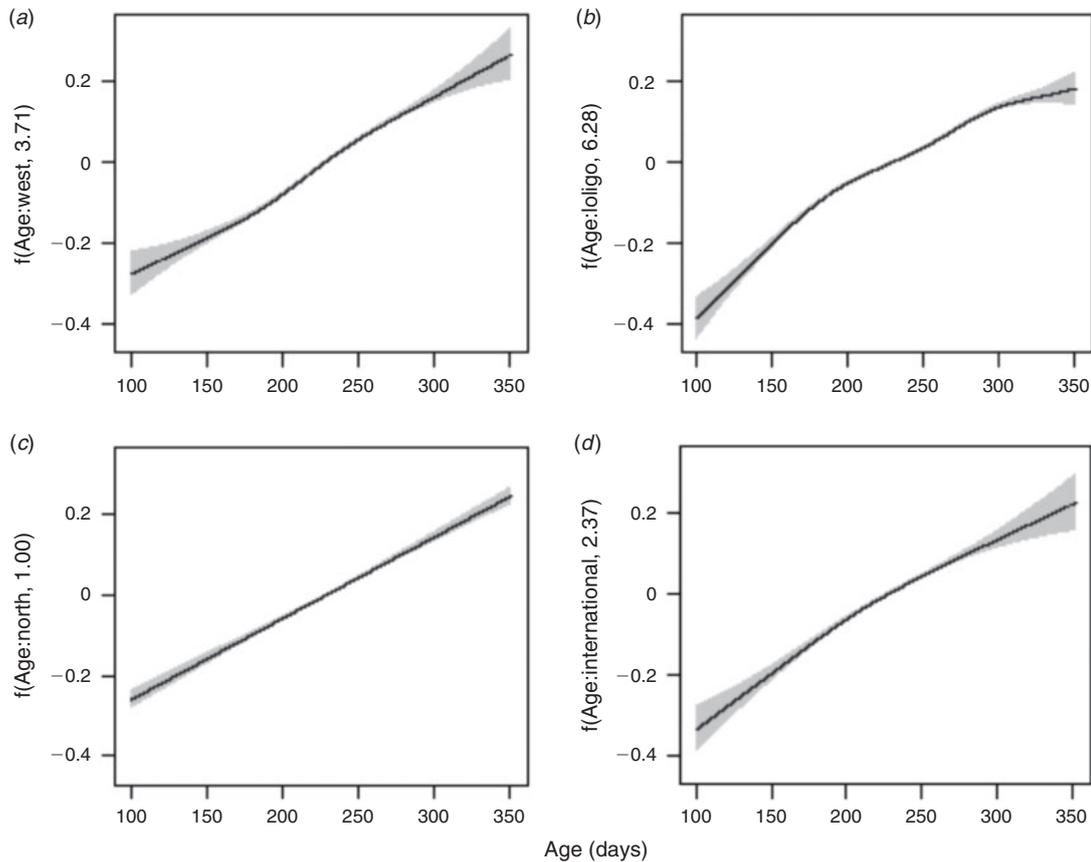


Fig. 7. Estimated smoothing curves from the optimal model (Model 9; Table 2) for age (days) and shaded point-wise 95% confidence intervals by area: (a) west, (b) 'Loligo box', (c) north and (d) international. Expected degrees of freedom are given in smoothing terms.

age and DML in the GAMM results in this study, there is a positive relationship between age and DML. Therefore, if a proportion of the population had an extended lifespan, it would be expected to be the largest animals, including superbull males, which are at the extreme positive end of the size distribution. In the present study, even after the inclusion of non-random samples of the largest individuals, maximum post-hatching age was estimated at 368 days, comparable to previous ageing studies (Hatfield 1991; Arkhipkin 1993). Results from the GLM suggest that the oldest individuals within the population are superbull males from the ASC, with a mean post-hatching age of 286 days. There is a large contrast in mean age between superbull males from the SSC and ASC and a larger variation in age for superbulls compared with the rest of the mature male population. Before hatching, embryo development is estimated at 51–65 days in austral summer (SSC) and 125 days in the winter (ASC; Arkhipkin and Middleton 2003). Inclusion of the long embryo development period suggests that superbulls from this particular cohort may have a life cycle exceeding 1 year. Mesnil (1977) proposed that squid hatched from eggs laid early in the breeding season are capable of reproduction and completion of their life cycle within 1 year. Those arising from the latter part of one season may not be reproductively competent in the next year, but live to breed at

the start of the following breeding season (i.e. delayed maturation and an extended life cycle), and thus contribute towards connectivity between spawning groups. This hypothesis is consistent with the higher mean post-hatching ages of the superbull males from the ASC, which are likely providing temporal connectivity between spawning cohorts. Peak spawning for the ASC occurs from May to June, whereas for the SSC peak spawning occurs from October to November (Arkhipkin *et al.* 2007). The ASC extending their life cycle to breed with individuals from the SSC is far more likely because the difference in spawning time is smaller than if the SSC extended their life cycle to breed with individuals from the ASC (a 2- to 4-month gap rather than a 4- to 6-month gap). Sample collection dates of superbulls from each cohort further confirm this hypothesis. Peak collection for the SSC superbull samples was September–October. The SSC remain on their offshore feeding grounds until the end of October, when they rapidly migrate inshore to spawn. The ASC move inshore to spawn during March–May, with peak spawning during May–June (Arkhipkin *et al.* 2013). Collection of superbulls from the ASC peaked during both May and August, with superbull males from this cohort present in samples for several months of the year. The peak in collection during May coincides with the migration of the ASC to their spawning grounds, whereas

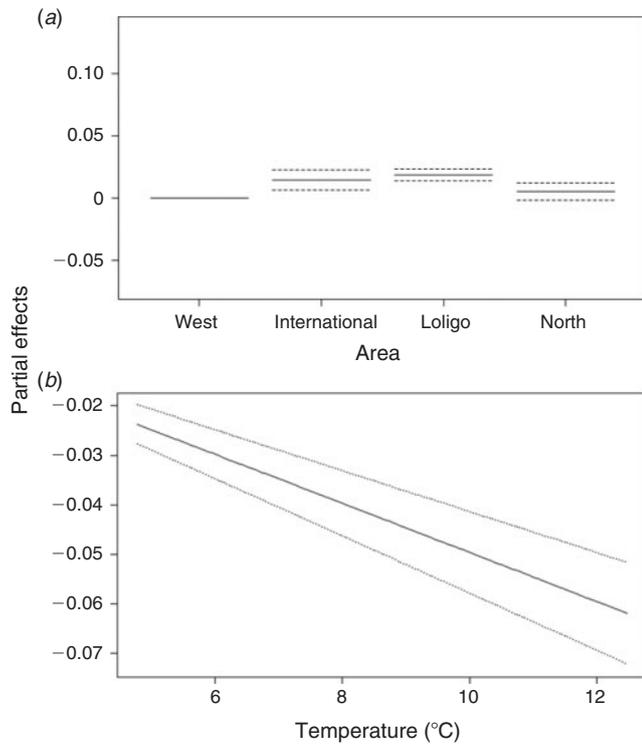


Fig. 8. Partial effects from the generalised additive mixed Model 9 (Table 2) for (a) area and (b) sea surface temperature during month of hatching (solid lines). Dashed lines indicate point-wise 95% confidence intervals.

the peak during August, well beyond the migration and spawning period for this cohort, provides further evidence that some individuals may have an extended life cycle. The wide variation in ages, wide distribution in hatching dates (also found in Shaw *et al.* 2004) and the availability of superbulls in samples during different times of the year suggest that there are several pathways to achieving this superbull morph, either through slow continuous growth and an extended life cycle or fast growth and a normal life-span, which provides evidence against heritability and instead favours the hypothesis that this morph arises through phenotypic variation.

Variability in size at maturity and adult size in cephalopods has largely been attributed to two main factors: temperature and food availability (Pecl *et al.* 2004). The response to extrinsic factors can be sex specific; a 5-year study on *Sepio-teuthis australis* found that interannual differences in squid size, condition, reproductive investment and possibly growth rate were sex specific (Pecl *et al.* 2004). This is evident in the *D. gahi* population, which shows the most pronounced size and shape variability in the male population (Jones *et al.* 2019). The partial effects of temperature in Model 9 suggested that there was a significant linear negative relationship between SST during month of hatching and DML. This is in contrast with the findings of Hatfield (2000), who found that *D. gahi* hatched in months with high mean monthly SST were significantly larger than those hatched in winter months. A larger size-at-age for individuals hatched in warmer conditions is evident in field

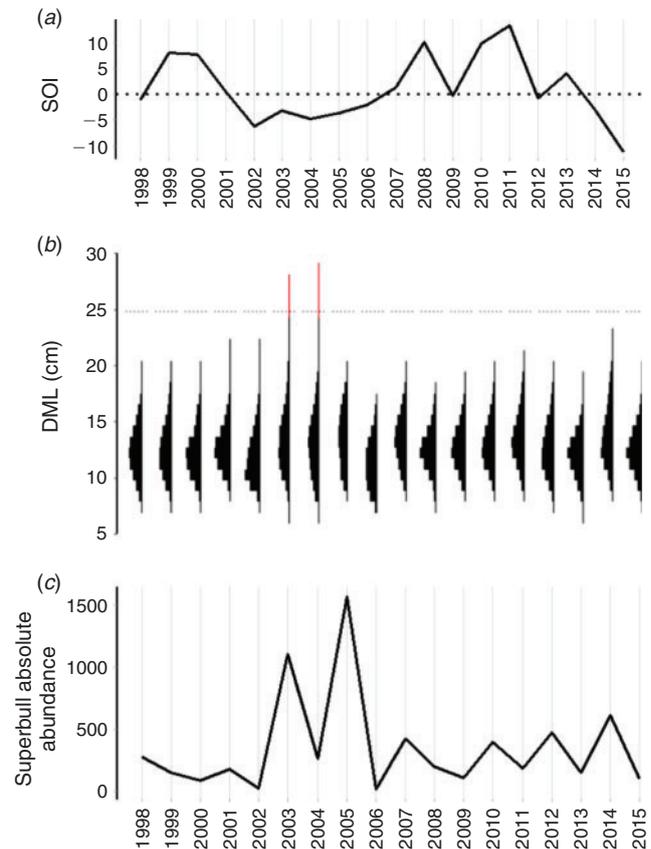


Fig. 9. (a) Southern oscillation index (SOI) and sea surface temperature (SST) anomalies generated by the El Niño–Southern Oscillation (ENSO), (b) percentage frequency graphs of the entire annual sample with the dotted line indicating the cut-off point between superbull and normal males and (c) absolute abundance (number of superbulls in each annual length–frequency sample multiplied by catch factor; see Materials and methods).

studies of other loliginid species (Moreno *et al.* 2007) and is consistent with the Forsythe hypothesis, which suggests that increased temperature during a squid's early growth phase may result in accelerated growth and potentially a larger maximum size (Forsythe 2004). However, a negative relationship was found in the jumbo squid *D. gigas* (Arkhipkin *et al.* 2015b). Low temperatures during early ontogeny are associated with slow growth, delayed maturation and an extended life cycle, resulting in a large size. This pattern was also found when comparing broad spatial regions (equatorial, tropical and subtropical) for the loliginid squid *S. lessoniana* (Jackson and Moltchanivskyj 2002). For *D. gigas* in Peruvian waters, low temperatures resulted in a large size and a life cycle that extended from 1 to 2 years (Arkhipkin *et al.* 2015b). In the present study, large-scale temperature variability found weak non-significant correlations between warm ENSO phases and the abundance of superbulls when lagged by 1 year. Although lower temperatures during month of hatching resulted in larger individuals, higher temperatures during other periods of ontogeny (i.e. juvenile and adult stages) could result in accelerated growth and perhaps a larger size.

The inclusion of CHL as a smoother improved model fit during the GAMM analysis, yet the effect of CHL was not as significant as the inclusion of SST, especially with a 1-month lag. The 'Loligo box', the fishing zone for the *D. gahi* trawl fishery, is occupied primarily by waters associated with the northward-flowing Falkland Current, consisting of sub-Antarctic superficial water in the upper layer and Antarctic intermediate water in the lower layer (Arkhipkin *et al.* 2004). Mixing of these waters with inshore waters results in high productivity in the summer and winter (Agnew 2002). The persistent macrozooplankton densities mean that *D. gahi*, which predominantly feeds on *Euphausia* spp. and the pelagic amphipod *Themisto gaudichaudii* (excluding self-cannibalism, which is most likely net feeding), is rarely food limited (Guerra *et al.* 1991; Brickle *et al.* 2001).

There appeared to be a significant spatial effect, with the largest individuals found in the 'Loligo box' for the optimal model, which included broad-scale spatial variability. However, for the model including a smoother of longitude and latitude, partial effects indicated that large individuals were located to the west of the Falkland Islands, which is more aligned with the raw data. Uneven sample distribution is a limitation in this study, with the majority of samples originating from the commercial fishing area ('Loligo box'). Despite this discrepancy, a spatial effect is evident within both models, which are likely capturing residual unexplained environmental variation (such as upwelling effects or differences in temperature based on depth). The age-length relationship also differed significantly between areas. The 'Loligo box' in particular had discontinuity in the age-length relationship, which means either that growth accelerates after a certain size or that two different growth trajectories are present within the 'Loligo box' (potentially superbulls and normal individuals). Different growth trajectories for different morphs would be logical if observed differences were phenotypically driven.

In conclusion, the ecological role of superbulls within the *D. gahi* population is still not fully understood; however, superbulls from the ASC are likely to provide temporal connectivity between cohorts. Without evidence of enhanced swimming ability there is no direct way to confirm that these individuals provide geographic connectivity. There was no evidence of enhanced reproductive output in superbull males (which would confer a selective advantage), but they are still reproductively viable. Recently, spermatozoa from spermatozoophores of the loliginid squid *L. reynaudii* (Iwata *et al.* 2018) and *Heterololigo bleekeri* (Iwata *et al.* 2015) have been found to be dimorphic, associated with the two alternative 'sneaker' and 'bull' mating tactics. A useful future step to aid in understanding whether and how the reproductive status of superbull males differs relative to the rest of the male population would be to analyse the morphology of spermatozoa from mature males of different sizes. Although there may not be a reproductive advantage in terms of the relative weight of reproductive organs, sperm shape may be advantageous. Because temperature, area and, to a lesser extent, food availability have significant effects on the age-length relationship, it appears that the existence of these large superbulls can be attributed to phenotypic variability. Understanding why these superbull males exist may help us to discern how this commercially important species is connected on the Patagonian Shelf.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Declaration of funding

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