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# Biochemical Composition in Relation to the Energetics of Growth and Sexual Maturation in the Ommastrephid Squid Illex argentinus

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*Phil. Trans. R. Soc. Lond. B* 1994 **344**, 201-212
doi: 10.1098/rstb.1994.0061

# Biochemical composition in relation to the energetics of growth and sexual maturation in the ommastrephid squid *Illex argentinus*

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## SUMMARY

The proximate (protein, lipid, carbohydrate and ash) and elemental (C, H, N and P) composition of the major tissues were measured for 18 male and 51 female *Illex argentinus* sampled from the feeding grounds over the Patagonian Shelf. In most tissues the chemical composition did not vary with sexual maturity, although the mass of the tissue increased significantly because sexual maturation and growth were proceeding simultaneously. The composition of the ovary and associated tissues (nidamental gland, oviducal gland) did change significantly during sexual maturation. Several tissues contained significant amounts of one or more unknown components. The nitrogen content of an unknown component in the testis was similar to that of DNA. In the spermatophoric complex the nitrogen content suggested the unknown fraction may be an amino acid or short peptide, whereas in the nidamental gland the nitrogen content suggested an amino-sugar or polysaccharide derivative. The digestive gland was rich in lipid and continued to accumulate substantial reserves of energy throughout the period of sexual maturation on the feeding grounds. During this period there was no evidence for the utilization of either digestive gland or mantle tissues to supply energy for gonads. Accumulation of carbon and energy (estimated stoichiometrically from carbon) during the final 50 days on the feeding grounds indicated that energy demands for tissue synthesis in females were almost twice those of the smaller males, and that a relatively small fraction of the demands were for reproductive tissues (5% in males, 15% in females). Most energy intake in this period was directed to the digestive gland (40% in males, 47% in females) and other somatic growth (54% in males, 38% in females). A preliminary power budget suggested that during the final days of feeding before migrating to the spawning grounds, energy intake of *Illex argentinus* is 4–5% body energy content per day, growth efficiencies are low (17–22%) and that energy reserves in the digestive gland would fuel migration in the absence of feeding for 14 days in males and 21 days in females.

## 1. INTRODUCTION

For many marine invertebrates reproduction is the most energy intensive period of their life, and achieving the optimum balance between competing demands such as activity, growth and reproduction is critical to fitness (Calow 1981). Elucidation of patterns of energy storage and utilization is thus important in understanding the interaction between organism and environment, and may reveal where constraints of power budgeting are most critical.

In many organisms the production of gonads is fuelled by an increased food intake as well as mobilization of previously stored reserves. In the final stages of vitellogenesis, however, body tissues may also be exhausted to provide maximum gonad output; this is particularly true of semelparous species where there is only a single bout of reproduction before death. In the benthic cephalopod *Octopus vulgaris*, for example, mantle protein is utilized to provide energy for eggs (O'Dor & Wells 1978; O'Dor *et al.* 1984). This would not seem to be a sensible strategy for a pelagic cephalopod such as a squid which, although semelparous, still requires functional muscle in order to survive in the water column (and, in species such as *Illex argentinus*, to migrate to the spawning grounds).

*Illex argentinus* is an ommastrephid squid distributed over the continental shelf and slope of the southwest Atlantic (Roper *et al.* 1984). It undergoes extensive migrations during its life cycle, moving from a presumed spawning area to the north of the Patagonian Shelf to feeding grounds over the Shelf where it grows and attains sexual maturity (Rodhouse & Hatfield 1990). Mature squid then return to the spawning grounds to reproduce and die at the end of one year (Hatanaka 1988; Hatanaka *et al.* 1985). When they are on the feeding grounds growth and sexual maturation occur simultaneously, and studies of tissue

*Phil. Trans. R. Soc. Lond.* B (1994) **344**, 201–212
*Printed in Great Britain*

production in terms of mass have shown that in both sexes the energy for maturation is provided from food intake rather than mobilization of body tissue (Rodhouse & Hatfield 1992; Hatfield *et al.* 1992). In this study we have looked in detail at the changes in composition of *Illex argentinus* tissues to refine our picture of the nutrient and energy requirements of both males and females during sexual maturation.

## 2. MATERIAL AND METHODS

### (*a*) *Sampling and dissection*

Samples of *Illex argentinus* de Castellanos, 1960 were taken from the commercial jig fishery on the Patagonian Shelf in March 1986 and returned to the U.K. frozen at −20°C. Male and female squid were carefully thawed, measured (dorsal mantle length), weighed (wet mass), and assigned a sexual maturity stage (1 to 5: Lipinski 1979). Each animal was then dissected into six component tissues: head with arms and tentacles, mantle, gonad (ovary with oviduct or testis), accessory sexual organs (nidamental and oviducal glands in females, spermatophoric complex in males), digestive gland and the remaining viscera. The length of the accessory sexual organs and the wet mass of each dissected tissue was measured. The tissue was then divided into two roughly equal portions; one was immediately refrozen for subsequent analysis of protein, lipid and carbohydrate content. The other was dried at 60°C for 24 h for subsequent determination of ash content and elemental composition. To minimize degradation of the tissues after thawing it was essential that the process of dissection was carried out quickly; in most cases the tissue had only partly thawed before it was re-frozen.

### (*b*) *Chemical analysis*

Dry mass and water content were determined following drying of the tissue. The dry tissue was ground to a fine powder with a mortar and pestle, and subsamples taken for further analysis. Ash was measured following ignition in a muffle furnace at 500°C. Phosphorus was measured according to Bartlett (1959) following digestion of a subsample of dried tissue in 72% perchloric acid at 180°C for 30 min. Samples were analysed in duplicate and the standard was $KH_2PO_4$. Carbon, hydrogen and nitrogen were measured on duplicate subsamples of dried tissue in a Carlo Erba 1106 elemental analyser. The standard was either acetanilide or 2,4-dinitrophenylhydrazone, depending on the nitrogen content of the tissue under analysis. In some cases (notably lipid-rich samples of viscera and digestive gland) the dried tissue was very oily and could neither be ground nor weighed accurately for subsequent ashing or CHN analysis. In these cases the ignition crucible or CHN sample boat was weighed empty, loaded with frozen tissue and dried. Reweighing gave the mass of dried tissue to be analysed. Although elemental analysis provides data for hydrogen in addition to carbon and nitrogen content, these data are influenced strongly by the degree of retained water in oven-dried tissue (Gnaiger & Bitterlich 1984) and hence the hydrogen data were not used further.

Protein was measured following digestion of duplicate samples of frozen tissue for 12 h in 0.1 N NaOH at 50°C. The samples were allowed to cool, and triplicate subsamples taken for assay according to Lowry *et al.* (1951) as modified by Hartree (1972); the standard was bovine serum albumin. Lipid was assayed gravimetrically after extraction into chloroform according to Bligh & Dyer (1959). Carbohydrate was assayed by reaction with phenol-sulphuric acid (Dubois *et al.* 1956); samples were digested for 30 min at 100°C in 10% trichloroacetic acid containing 10 mg silver sulphate to remove interfering halides. After cooling, $5 \times 3$ ml subsamples of the digest were cleared by centrifugation, and 100 µl subsamples taken for assay; the standard was D+glucose. These assay protocols are described more fully in Clarke (1980).

Several tissues gave analytical problems. The digestive gland was very rich in lipid (roughly 50% of the dry matter was lipid) and this interfered in the protein assay. The viscera fraction was also very oily, but more problematical was that it was not possible to divide the newly dissected material into representative replicate fractions. This tissue was therefore dried whole and analysed only for ash, C, N and P; there are consequently no data for protein, carbohydrate or lipid for either male or female viscera. There were thus eight tissue/component combinations for which some aspects of proximate composition could not be measured.

### (*c*) *Statistical analyses*

The total sample was 18 males classified into three maturity stages, and 51 females classified initially into five maturity stages. For each of these individuals, the data matrix usually consisted of 48 elements (six tissues by eight biochemical components). Data were analysed using the analysis of variance and regression (least-squares, model 1) routines in the MINITAB statistical package (version 7.1; Ryan *et al.* 1985). Because of the large number of elements being analysed, the threshold for a significant result was taken as $p = 0.01$ (the more conventional $p = 0.05$ would have resulted in too many false results).

For comparison between components and calculation of energy contents the following conversions were assumed: typical marine invertebrate protein contains 5.25% nitrogen, and the enthalpies of combustion for representative marine invertebrate protein, carbohydrate and lipids were −23.9, −17.5 and −39.5 kJ $g^{-1}$ respectively (all based on Gnaiger & Bitterlich 1984). Elemental ratios (C:N, C:P and N:P), were calculated in atomic (molar) units and not as simple mass ratios.

## 3. RESULTS

### (*a*) *Maturity Stages*

After dissection individual squid were classified into




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maturity stages as described by Lipinski (1979). This classification is based essentially on the development of the gonad and accessory sexual organs (nidamental gland in females and spermatophoric complex in males). In males only the three later stages (3–5) were found, whereas in females five stages were found. These were initially classified as Lipinski stages 1–5, but it later became clear that those individuals classified as stage 1 were better regarded as early stage 2. For the purposes of analysis of variance, however, the original classification into five levels was maintained for in many cases significant differences in composition were found between stages 2a (originally termed 1) and 2b.

The Lipinski maturity classification is valuable for statistical analysis, and the first phase of analysis was based on this classification of maturity stage. The disadvantage of this approach is that it takes no account of the fact that squid grow and mature at the same time. This simultaneous growth and sexual maturation coupled with normal individual variation means that squid of similar length may be at quite different stages of gonad development (Hatfield *et al.* 1992). The second phase of statistical analysis therefore used a continuous variable as a measure of maturity; this was the ratio of lengths of the nidamental gland and mantle. There was a highly significant ($p < 0.01$) correlation between this variable and the Lipinski classification for the 51 females analysed in this study.

### (*b*) *General features of the chemical composition*

One-way ANOVA was used to detect variation in composition with Lipinski maturity stage. With 48 data elements for each individual (six tissues by eight biochemical components) and analysing data for males and females separately, there are 96 possible analyses of variance; however eight of these tissue/component combinations have no data for technical reasons (see Methods). Of the remaining 88 analyses, 66 revealed no significant variation in composition with maturity stage ($p > 0.01$). The 22 analyses that revealed significant variation are listed in table 1.

There are two striking features of these data, namely that in most cases levels of significance are very high (in other words the changes in composition with Lipinski maturity stage are substantial), and that virtually all aspects of the chemical composition of the female gonad and nidamental gland alter change

Table 1. *Variation in chemical composition of* Illex argentinus *with sexual maturity*

(Eighteen male (maturity stages 3–5) and 51 female (maturity stages 2a–5) squid were each dissected into six tissues (head with arms and tentacles, mantle, digestive gland, ovary/testis, nidamental gland/spermatophoric complex, and remaining viscera). Each tissue was analysed for dry mass, lipid, protein, carbohydrate (all expressed as % frozen mass), C, N, P and ash content (all as % dry mass). One-way ANOVA was then used to examine variation in tissue composition between maturity stages, accepting $p < 0.01$ as indicating significant variation. *S*, number of maturity stages; *n*, number of individuals analysed; *F*, variance ratio; *p*, probability of this result arising by chance (two-tailed).

For all other 66 tissue/component ANOVAs were not significant ($p > 0.01$). Note that 8 tissue/component combinations have no data for technical reasons: see text.)

| tissue | component | *S* | *n* | *F* | *p* |
|---|---|---|---|---|---|
| *males* | | | | | |
| mantle | dry mass | 3 | 18 | 7.76 | 0.002 |
| | ash | 3 | 18 | 8.35 | 0.002 |
| | protein | 3 | 18 | 8.83 | 0.001 |
| head | C | 3 | 18 | 7.80 | 0.002 |
| viscera | C | 3 | 18 | 10.31 | 0.0006 |
| *females* | | | | | |
| ovary | dry mass | 5 | 51 | 63.2 | $< 10^{-6}$ |
| | ash | 5 | 51 | 29.9 | $< 10^{-6}$ |
| | N | 5 | 51 | 93.3 | $< 10^{-6}$ |
| | C | 5 | 51 | 58.7 | $< 10^{-6}$ |
| | P | 5 | 25 | 12.7 | $< 10^{-4}$ |
| | lipid | 5 | 25 | 18.0 | $< 10^{-5}$ |
| | protein | 5 | 51 | 29.0 | $< 10^{-6}$ |
| nidamental gland | dry mass | 5 | 51 | 7.95 | $< 10^{-4}$ |
| | ash | 5 | 51 | 45.7 | $< 10^{-6}$ |
| | N | 5 | 51 | 46.4 | $< 10^{-6}$ |
| | C | 5 | 51 | 5.1 | 0.002 |
| | P | 5 | 25 | 33.8 | $< 10^{-6}$ |
| | lipid | 5 | 22 | 6.71 | 0.0014 |
| | protein | 5 | 49 | 7.11 | 0.0002 |
| | carbohydrate | 5 | 19 | 17.5 | $< 10^{-4}$ |
| mantle | ash | 5 | 51 | 4.26 | 0.007 |
| viscera | N | 5 | 51 | 7.17 | $< 10^{-4}$ |




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Table 2. *Proximate composition of* Illex argentinus

(Eighteen male (maturity stages 3–5) and 51 female (maturity stages 2a–5) squid were each dissected into six tissues. Each tissue was then analysed for total dry mass, protein, carbohydrate and lipid. Data are presented as mean ± standard error. *n*, number of individuals analysed; ND, no data (see Methods); * indicates that significant variation in composition with maturity stage was detected by one-way ANOVA (see table 1), and a mean was therefore not calculated.

| | % frozen mass | | | |
|---|---|---|---|---|
| tissue | dry mass | protein | lipid | carbohydrate |
| *males* | | | | |
| *n* | 18 | 18 | 13 | 13 |
| testis | 21.1 ± 0.23 | 10.6 ± 0.40 | 1.17 ± 0.03 | 1.02 ± 0.02 |
| spermatophoric complex | 22.6 ± 0.54 | 9.5 ± 0.61 | 1.58 ± 0.15 | 1.68 ± 0.21 |
| digestive gland | 55.0 ± 1.26 | ND | 27.6 ± 1.51 | 1.32 ± 0.07 |
| mantle | * | * | 1.70 ± 0.09 | 0.35 ± 0.02 |
| head | 20.9 ± 0.22 | 17.2 ± 0.47 | 1.63 ± 0.07 | 0.32 ± 0.02 |
| viscera | 22.7 ± 0.75 | ND | ND | ND |
| *females* | | | | |
| *n* | 51 | 51 | 25 | 25 |
| ovary | * | * | * | 0.84 ± 0.03 |
| nidamental gland | * | * | * | * |
| digestive gland | 57.9 ± 1.11 | ND | 31.3 ± 2.03 | 1.16 ± 0.04 |
| mantle | 21.9 ± 0.23 | 14.8 ± 0.24 | 1.85 ± 0.06 | 0.03 ± 0.01 |
| head | 21.0 ± 0.19 | 16.0 ± 0.23 | 1.76 ± 0.04 | 0.30 ± 0.01 |
| viscera | 21.8 ± 0.34 | ND | ND | ND |

during sexual maturation. Because these data are expressed as a percentage of the mass (dry or frozen, depending on the component measured) they are measures of concentration, not overall content. These changes indicate that the nature of the tissue is changing as sexual maturation proceeds; the overall amount of any given component in a particular tissue will obviously depend on both the composition and tissue mass.

In all other cases the composition of the tissue remains much the same throughout sexual maturity, although changes in total amount consequent upon changes in overall mass would not be revealed by this type of analysis. The mean proximate and elemental composition are summarized in tables 2 and 3 for those tissues where there was no significant variation in composition with Lipinski maturity stage.

Most tissues exhibited proximate compositions of 78–80% water, 10–17% protein, 12% lipid and small amounts of carbohydrate (table 2, fresh mass basis), and elemental compositions of 43–45% carbon, 12–13% nitrogen and 0.5–2% phosphorus (table 3, dry

Table 3. *Elemental composition of* Illex argentinus

(Eighteen male (maturity stages 3–5) and 51 female (maturity stages 2a–5) squid were each dissected into six tissues. Each tissue was then analysed for ash content and elemental composition. Presentation as for table 2.)

| | (% dry mass) | | | |
|---|---|---|---|---|
| tissue | carbon | nitrogen | phosphorus | ash |
| *males* | | | | |
| *n* | 18 | 18 | 13 | 18 |
| testis | 42.4 ± 0.17 | 13.3 ± 0.05 | 2.12 ± 0.04 | 9.83 ± 0.13 |
| spermatophoric complex | 42.1 ± 0.32 | 13.1 ± 0.13 | 2.01 ± 0.05 | 10.3 ± 0.16 |
| digestive gland | 54.9 ± 0.87 | 5.9 ± 0.35 | 0.62 ± 0.04 | 3.43 ± 0.47 |
| mantle | 43.8 ± 0.93 | 12.3 ± 0.06 | 1.26 ± 0.02 | * |
| head | * | 12.9 ± 0.07 | 1.06 ± 0.01 | 7.96 ± 0.12 |
| viscera | * | 9.1 ± 0.36 | 0.96 ± 0.05 | 7.88 ± 0.34 |
| *females* | | | | |
| *n* | 51 | 51 | 25 | 51 |
| ovary | * | * | * | * |
| nidamental gland | * | * | * | * |
| digestive gland | 58.6 ± 0.87 | 5.3 ± 0.26 | 0.57 ± 0.04 | 2.55 ± 0.29 |
| mantle | 43.3 ± 0.28 | 12.2 ± 0.07 | 1.21 ± 0.01 | * |
| head | 45.8 ± 0.16 | 12.6 ± 0.04 | 0.98 ± 0.01 | 7.85 ± 0.13 |
| viscera | 43.9 ± 0.67 | * | 0.98 ± 0.02 | 7.67 ± 0.19 |

mass basis). These are typical compositions for general marine invertebrate tissue and in most of these cases the measured components explained over 90% of the dry matter (table 4). It is rare for a measured proximate composition to explain all of the dry mass. The unexplained matter represents in part material not measured by any of the assays used (for example nucleic acids, a variety of small intracellular molecules such as intermediary metabolites, and mucus). Typically these amount to about 5% of the dry mass of an organism, although in specific tissues they may comprise much more. However, the errors involved in the assays for protein, carbohydrate and lipid also accumulate in the unexplained fraction; thus if carbohydrate or protein are underestimated (for example by the use of an inappropriate calibration standard) then the unexplained matter will increase correspondingly.

Those tissues where the sum of protein, lipid, carbohydrate and ash indicated that a significant component was missing were the mantle in females, and the gonads and accessory tissues in both sexes. In males the missing components comprised about 30% of the dry mass, whereas in females the amount of the missing component varied with maturity stage (table 4; figure 1).

### (*c*) *Mantle*

In males the composition of the mantle tissue varied with Lipinski maturity stage; the most significant differences were in protein, ash and total dry matter content (table 1). These changes were small and can best be explained on the basis of minor variations in protein and water content (table 2). The nitrogen content did not vary significantly with maturity stage, confirming that the variations in protein were small (table 3). The measured composition explained 92% of the dry matter and despite variations in some individual components there was no variation in the total fraction explained through sexual development (table 4). Carbon:nitrogen and nitrogen:phosphorus ratios were typical of protein-rich tissues (mean values 4.2 and 21.7 respectively).

In female mantle tissue there was a slight variation in percentage ash content, but otherwise the composition remained similar throughout sexual maturation (tables 1–3). However, the proximate composition only explained 86% of the dry matter, which is rather low (table 4). The measured protein and nitrogen contents are fully comparable (14.8% protein on a frozen mass basis equates to 12.9% nitrogen on a dry mass basis, compared with a measured overall nitrogen content of 12.2%) and so it is unlikely that the missing component contains nitrogen. The carbon:nitrogen and nitrogen:phosphorus ratios were similar to those of males (mean values 4.2 and 22.5 respectively). The reason for the low percentage of total dry matter explained in female mantle tissue is unclear.

In neither males nor females was there a significant variation in percentage nitrogen with maturity stage. Although there was a small variation in protein content in males, the biochemical composition data confirm the findings from tissue mass measurements and histology (Rodhouse & Hatfield 1990, 1992; Hatfield *et al.* 1992) that *Illex argentinus* does not utilize mantle tissue to fuel gonad development, at least during the period of the life-cycle covered by these samples.

### (*d*) *Digestive gland*

In both males and females the digestive gland was very rich in lipid (31% and 28% of the frozen mass respectively). This high lipid content is reflected in the very high carbon content and low nitrogen content, the low ash content and the very low water content of the digestive gland. Carbon:nitrogen ratios were high (mean values 11.5 in males, 14.4 in females), and carbon:phosphorus ratios higher than in any other tissue (mean values 237 in males, 295 in females). Furthermore, as the composition did not vary with maturity stage in either males or females, there was no evidence of significant selective utilization of lipid (or

Table 4. *Percentage of total dry matter in* Illex argentinus *explained by summing protein, lipid, carbohydrate and mineral ash*
(Data were calculated for each squid individually, and means calculated separately for each tissue where all data were available. Accessory gland: nidamental gland (females) or spermatophoric complex (males). Data are presented as mean and standard error (s.e.). *n*, number of individuals for which a full data set was available.)

| | % total dry matter explained | | | | | |
|---|---|---|---|---|---|---|
| | males | | | females | | |
| tissue | *n* | mean | s.e. | *n* | mean | s.e. |
| gonad | 13 | 71.5 | 1.92 | | * | |
| accessory gland | 12 | 67.1 | 3.47 | | * | |
| digestive gland | 13 | 93.7 | 2.86 | 25 | 95.4 | 3.71 |
| mantle | 13 | 92.3 | 1.48 | 25 | 85.7 | 1.91 |
| head | 13 | 97.6 | 3.14 | 25 | 93.4 | 1.64 |

* ANOVA showed significant variation with maturity stage:
ovary: $F = 3.84$, $p = 0.019$
nidamental gland: $F = 6.04$, $p = 0.007$

any other component) during sexual maturation. The digestive gland grows at a faster rate than the rest of the body during maturation (Rodhouse & Hatfield 1990) and so it is clear that up to the moment the population moves from the feeding grounds, the substantial energy reserves held in the digestive gland have not been utilized to fuel gonad growth.

### (*e*) *Female reproductive tissues*

The mean dry mass of the ovary (including oviduct) varied from 0.79 g in stage 2a squid, to 23.7 g at stage 5. During development the various biochemical components accumulated at different rates, and so the percentage composition of these tissues changed (tables 2 to 3). As would be expected for ovarian tissue, the major organic constituents were protein and lipid. Protein increased in concentration from 13.2% (frozen mass) at stage 2a, to 41.8% at stage 5, and lipid increased from 1.5% to 8.2% over the same period. These were accompanied by a decrease in percentage ash from 9.2% to 4.9% (dry mass).

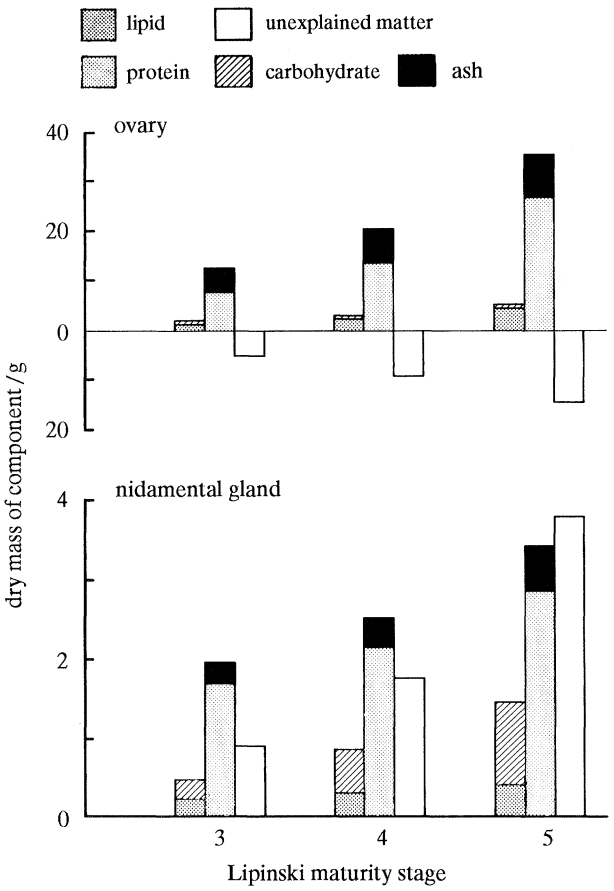


Figure 1. Proximate composition of the ovary and nidamental gland of female *Illex argentinus* as a function of Lipinski maturity stage. All data are presented as absolute amount (g) of component, and the clear box shows the amount of unexplained matter. In the ovary the summation of protein, lipid, carbohydrate and ash always exceeded the measured dry matter and so the unexplained material is negative (see text). Because the size of the reproductive tissues is small in the early stages, only data for Lipinski stages 3–5 are shown.

Changes in percentage composition such as these indicate that the nature of the ovarian tissue is changing during sexual development. This, however, is of less ecological interest than the overall accumulation of material and in *Illex argentinus* the maturation of the ovary involves a substantial accumulation of protein and lipid, particularly in the later stages (figure 1).

Summing all of the measured components in the ovary explains more than 100% of the measured dry matter, and the discrepancy increases with Lipinski maturity stage (figure 1). This indicates that there is unlikely to be any significant component that has been missed in the analysis, but also that there are errors in the analytical protocols used. A possible source of error is that the standard protein and carbohydrate used in these assays were not representative of those found in the reproductive tissues, and hence the assays have overestimated the true mass of protein and/or carbohydrate present. The error is most likely to be in the protein component, as the measured protein equates to more than 100% of the measured nitrogen at all stages; because carbohydrate is only a minor component and lipid was measured gravimetrically. Because of this discrepancy the energy content of gonad tissues was estimated from carbon content rather than proximate composition (see below).

Further difficulties were found in interpreting the biochemical composition of the nidamental gland. As with the ovary there were significant changes in percentage composition with maturity stage (tables 1 to 3), and there were substantial accumulations of organic components with development. In the nidamental gland, however, the major components were protein and carbohydrate (rather than lipid), and there was a substantial unidentified fraction (figure 1). This unknown component reached an average of 38% of the total dry matter by stage 5, and comparison of protein and nitrogen data suggested that this component contained about 6.6% nitrogen (s.d. 2.7, $n = 18$). The nitrogen content of the unexplained fraction did not vary with maturity stage (oneway ANOVA, $p > 0.01$) which would suggest that the amount, rather than the nature, of the unknown material increased during development of the nidamental gland.

### (*f*) *Male reproductive tissues*

The major component of both the testis and the spermatophoric complex was protein, which accounted for roughly half the organic matter (table 2). There were smaller amounts of lipid and carbohydrate but these components together with ash explained only 70% of the dry matter in both testis and spermatophoric complex (table 4). The unknown component in the testis appears to be different from that detected in the female nidamental gland, for estimation of the nitrogen content (by subtracting of protein nitrogen from total nitrogen) gave a value of 15.1% (s.e. 0.30, 13 estimates). The nitrogen content of DNA depends on the base composition but values of 15–18% are typical. This suggests that the unex-




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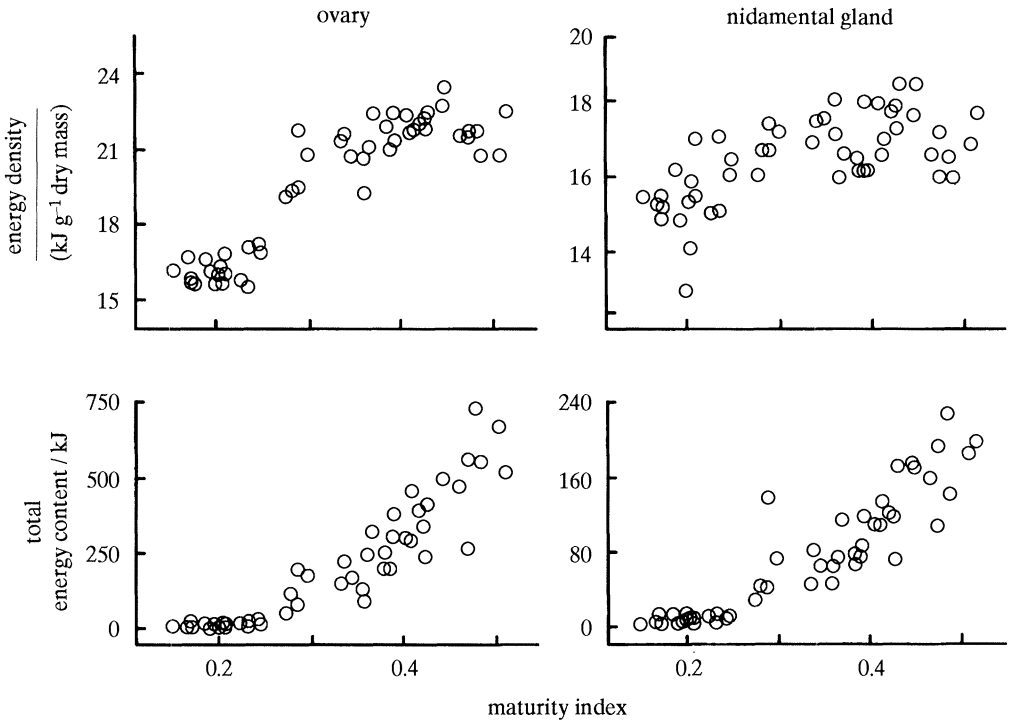


Figure 2. Changes in the energy density (kJ g$^{-1}$, dry mass) and total energy content (kJ) in reproductive tissues of female *Illex argentinus* in relation to maturity index.

plained fraction in the testis may be predominantly nucleic acid. In the spermatophoric complex the mean nitrogen content of the unexplained fraction was 25.7% (s.e. 4.82, 12 estimates).

### (*g*) *Energy content of tissues*

Energy content may be estimated from chemical composition data in two principal ways. The first is to use the proximate composition and use the standard enthalpies of combustion to estimate the energy content due to protein, lipid and carbohydrate. This technique can only be used where complete and reliable proximate composition data are available (table 4).

We have therefore used the alternative approach, which is to estimate energy content from carbon content. The underlying rationale here is that, to a first approximation, the energy content of a tissue is dependent on the lipid content, and lipid is richer in carbon than either carbohydrate or protein. Rough as these assumptions are, a number of studies have demonstrated reasonable correlations between energy estimated from carbon content and energy content measured directly by calorimetry (see, for example, Salonen *et al.* 1976). A firmer basis is provided by the stoichiometric approach of Gnaiger & Bitterlich (1984) although this too is based on the assumption of average compositions for representative marine invertebrate protein, carbohydrate and lipid (and hence may be less applicable to specific tissues than to whole organisms). We have employed the Gnaiger/Bitterlich algorithm to estimate energy content in the tissues of *Illex argentinus*, and the results are shown in table 5.

In those tissues where complete proximate composition data exist comparison of energy content estimated from carbon and from proximate composition shows that estimates from carbon are systematically, and significantly, lower (table 5). Taking the data set as a whole, estimates from proximate composition are greater by an average factor of 1.24. The largest discrepancy is for ovarian tissue at Lipinski stage 5; part of the explanation for this, however, must be that the proximate composition is itself in error for the sum total of individual estimates of protein, lipid, carbohydrate and ash for this tissue exceeds 100% (figure 1).

The general pattern that emerges from the energy contents of individual tissues, as estimated from carbon, is that most tissues have energy contents typical of non-fatty marine invertebrates ($-15$ to $-18$ kJ g$^{-1}$ dry mass) but digestive gland (both sexes) and ovary are energy rich due to their high lipid contents. There is no indication that the energy reserves located in the digestive gland are used up to the time that the squid leave the feeding grounds (analysis of variance against Lipinksi maturity stage, and against maturity index: $p > 0.01$ for both sexes). This matches the conclusions reached by Hatfield *et al.* (1992) and Rodhouse & Hatfield (1992) from analysis of organ growth patterns and the histology of the mantle.

## 4. DISCUSSION

Detailed data on proximate or elemental composition are of relatively little interest in themselves; their value lies in what they can tell us about the ecology or




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Table 5. *Energy content of* Illex argentinus *tissues*

(Energy content was estimated from carbon content according to Gnaiger & Bitterlich (1984), and from proximate composition assuming enthalpies of combustion of −17.5, −23.9 and −39.5 kJ $g^{-1}$ for carbohydrate, protein and lipid respectively. Data presented as mean ± s.e., with number of individuals in parentheses. For tissues labelled (S5) chemical composition varied with maturity stage and energy contents were calculated only for individuals at Lipinski stage 5. ND, no data (incomplete proximate analysis); *, proximate composition in error. In all cases estimates from proximate analysis were significantly greater than those from carbon content (all $p < 0.01$).)

| | energy content (kJ $g^{-1}$ dry mass) | |
|---|---|---|
| tissue | carbon | proximate |
| *males* | | |
| testis | −15.4 ± 0.46 (18) | ND |
| spermatophoric complex | −15.1 ± 0.93 (18) | ND |
| digestive gland | −24.6 ± 2.51 (18) | ND |
| mantle (S5) | −16.9 ± 0.65 (10) | −20.8 ± 1.25 (5) |
| head | −18.4 ± 0.60 (18) | −22.5 ± 2.78 (13) |
| viscera (S5) | −19.9 ± 2.24 (10) | ND |
| *females* | | |
| ovary | −21.7 ± 0.85 (10) | −33.7 ± 5.06 (5)* |
| nidamental gland (S5) | −17.1 ± 0.78 (10) | ND |
| digestive gland | −27.1 ± 4.10 (51) | ND |
| mantle | −16.5 ± 0.96 (50) | −20.0 ± 2.48 (25) |
| head | −17.9 ± 0.81 (51) | −21.8 ± 1.97 (25) |
| viscera (S5) | −15.1 ± 3.19 (10) | ND |

physiology of the organism in question. Two aspects that are of interest in the detailed data for *Illex argentinus* presented here are comparison with data available for other species, and an estimate of the energy intake required to fuel sexual maturation. Unfortunately there are few other data on tissue composition in cephalopods with which to compare our data, but there are useful metabolic measurements that can be used to infer aspects of the energetics.

### (*a*) *Tissue composition*

Of the six tissues into which each individual squid was dissected, data for the head (including the arms and tentacles) and viscera reveal compositions typical of marine invertebrate tissue. With the exception of two components in the viscera (carbon in males, nitrogen in females) there was no variation with maturity.

The mantle also showed little variation with maturity, and overall it is clear that, at least during the stage of the life cycle for which samples are available, *Illex argentinus* does not utilize mantle tissue to provide energy. This contrasts sharply with the situation in *Octopus vulgaris* (O'Dor & Wells 1978; O'Dor *et al.* 1984), and the difference is clearly allied to ecology. *Octopus vulgaris* guards its brood of eggs in a secluded place, and after spawning has little further use for locomotion (being semelparous the adult dies some time after the eggs have hatched). Males, although less sedentary, undergo a similar degeneration in muscle tissue. In *Illex argentinus*, and by inference other pelagic cephalopods, there is a continuing need for the mantle tissue to be fully functional for locomotion.

Neither is there any indication that the substantial energy reserves held in the digestive gland are utilized up to the time the *Illex argentinus* move northwards to the spawning grounds (and out of the sampling area). The digestive gland is rich in lipid (table 2), and has the highest energy content of any of the tissues analysed (table 5). The mass of the digestive gland increases during sexual maturation (which proceeds in parallel with somatic growth), and there are no significant changes in percentage composition throughout the sampling period. Whilst *Illex argentinus* will undoubtedly gain a small energetic advantage from the buoyancy of the lipid stored in its digestive gland (28–31% dry mass, roughly half the organic matter), it is likely that the primary role of this lipid is as an energy reserve. Probable functions of this energy reserve are to fuel the migration to the spawning ground and to fuel the final stages of vitellogenesis.

The gonads and accessory reproductive tissues of both males and females all contain significant organic components either missed or poorly estimated by the standard analytical procedures employed in this study. In males, the testis and spermatophoric complex both contain an unknown component which comprises 30% of the dry mass. The unknown component in the testis has a nitrogen content of about 15% and this corresponds well to the nitrogen content of DNA. Estimates of the nitrogen content of the unknown component of the spermatophoric complex were more variable (mean 25.7, s.e. ± 4.8). It is possible that this unknown component may be a simple compound of low molecular mass. An amino acid is a possibility and the usual amino acids found in marine invertebrates vary in nitrogen content from 8 to 32% (median 15%), a range consistent with the values estimated here.

Whereas the percentage composition of the male reproductive tissues remained constant through sexual

development, the composition of both the ovary and the nidamental gland in females varied during sexual maturation (figure 2). In the nidamental gland the unexplained material reached almost 40% dry mass at maturity. The nitrogen content of this unknown component was 6.6% dry mass, and its elemental composition did not appear to vary with maturity stage. The low nitrogen content suggests that the unknown component is not an amino acid, and is more likely an amino-sugar or perhaps a small glycopeptide not measured by the conventional assays for carbohydrate or protein.

The ovary also changed in composition throughout maturation, although here the unexplained material was always negative (that is the sum of protein, lipid, carbohydrate and ash always exceeded the measured total dry matter). Components such as nucleic acids and small molecular mass metabolites will not have been measured, and so these data indicate that at least one of the major components has been overestimated significantly. Because lipid and ash were both estimated gravimetrically, the errors must presumably lie in the estimation of protein or carbohydrate. The 'unexplained' material is an order of magnitude greater than the measured carbohydrate content, and hence the error most likely lies predominantly with the protein determination. Although bovine serum albumin is clearly not a typical marine invertebrate protein, there have been few indications from other studies that the error introduced by its use as a standard is ever severe.

### (*b*) *The energetics of sexual maturation in* Illex

The detailed chemical data we have acquired for *Illex argentinus* allow us to make some estimates of the energy invested in the various body components during sexual maturation. When making such estimates, ecologists traditionally use units of energy (joules) or energy per unit time (power, watts), whereas oceanographers interested in patterns of material and energy flux have tended to use carbon or nitrogen as the unit. In this study we have measured carbon content directly; because of the difficulties with proximate composition discussed above we have confined our discussion of energy flux (power) to energy content estimated stoichiometrically from carbon content.

We have confined calculations to the final 50 days of growth and maturation on the feeding grounds; from age 300 to 350 days the increase in the mass of individual organs of *Illex argentinus* is rapid and approximately linear. The increase in tissue mass was estimated by solving the individual allometric equations for each tissue (from Rodhouse & Hatfield 1990) for age 300 and 350 days and calculating the difference. The increase in mass was then converted to an increment in absolute carbon and energy content, using the composition data obtained in this study. Where carbon or energy content had been found to vary significantly with maturity stage, only data from Lipinski maturity stage 5 were used. The resulting pattern of distribution of carbon and energy between tissues is shown in table 6.

Table 6. *Fate of ingested carbon and energy in male and female* Illex argentinus *during the final 50 days of sexual maturation on the feeding grounds before migrating to the spawning grounds*

(Data calculated from allometric relationships in Rodhouse & Hatfield (1990), and carbon and energy content data from this study. Data calculated as total carbon (g) or energy (kJ) increment for each tissue separately, and summed to give total body increments; percentage data are the percentage of the total increment sequestered in a given tissue. Note that the carbon and energy data are not directly equivalent, for the calculation of energy content takes into account the ash content of the tissue (Gnaiger & Bitterlich 1984).)

| | increment in 50 days | | | |
|---|---|---|---|---|
| | tissue carbon/g | % | energy/kJ | % |
| *males* | | | | |
| total body increment in 50 days | 29.7 | | 1214 | |
| mantle | 8.0 | 26.8 | 306 | 25.2 |
| head | 8.2 | 27.6 | 321 | 25.2 |
| digestive gland | 11.0 | 36.9 | 490 | 40.4 |
| viscera | 0.8 | 2.5 | 32 | 2.6 |
| testis | 0.9 | 3.0 | 32 | 2.6 |
| spermatophoric complex | 1.0 | 3.2 | 34 | 2.8 |
| *females* | | | | |
| total body increment in 50 days | 52.2 | | 2200 | |
| mantle | 12.6 | 24.1 | 479 | 21.8 |
| head | 8.2 | 15.7 | 321 | 14.6 |
| digestive gland | 22.4 | 43.0 | 1038 | 47.2 |
| viscera | 1.2 | 2.3 | 41 | 1.8 |
| ovary | 5.4 | 10.4 | 230 | 10.5 |
| nidamental gland | 2.2 | 4.3 | 87 | 4.0 |
| oviducal gland | 0.1 | 0.2 | 5 | 0.2 |




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Males and females differ both in the absolute intake of carbon and energy, and also how this intake is used within the body. In males the average increase in body carbon from day 300 to 350 is just over 6 g, equivalent to 250 kJ. Most of this carbon is incorporated into the digestive gland (36%), although there is substantial growth in other somatic tissue, with 28% of the carbon being invested in the mantle, and 30% in the remaining somatic tissues. Reproductive tissues together contain only 5.7% of the carbon incorporated into tissue during this period.

By the age of 300 days, female *Illex argentinus* are larger than males, and growing faster (Rodhouse & Hatfield 1990). In the period from day 300 to 350, females incorporate an average of 22 g carbon (or 935 kJ) into new tissue. Of this the majority again goes to somatic tissue (23% to the mantle, 17% to the head, arms, tentacles and viscera) with the digestive gland being the major recipient (44% of the carbon, 48% of the energy). Reproductive tissues, although growing faster than in males, accumulate only 16% in total (table 6).

These data indicate that not only are *Illex argentinus* on the feeding ground not utilizing somatic tissue for reproduction, they are actively increasing the amount of non-reproductive tissue through rapid growth. At present we do not know the function of the large energy reserves which are located in the digestive gland, and which are still being accumulated as the squid leave the feeding grounds to migrate to the spawning grounds. Likely possibilities are to fuel the energy costs of migration and to provide energy for the final phase of sexual maturation. It is also possible that once the spawning grounds have been reached the somatic tissues are also utilized to provide energy for reproduction; unfortunately we have no samples from the spawning grounds to examine this possibility.

A rough estimate of the energy content of whole squid can be obtained by comparing the increase in total body mass during the latter stages of maturation on the feeding grounds (data for *Illex argentinus* of age 300 and 350 from Rodhouse & Hatfield 1990) and the total energy incorporated into different tissues during the same period (table 6). The mean energy contents calculated in this way are 5.49 kJ $g^{-1}$ fresh mass (1.31 kcal $g^{-1}$) in males, and 6.57 kJ $g^{-1}$ fresh mass (1.57 kcal $g^{-1}$) in females.

### (*c*) *A preliminary power budget for maturing* Illex argentinus

The data on carbon intake during the final 50 days on the feeding grounds may be used to construct a preliminary power budget for this period. Such a budget must perforce be very approximate, for data in several areas are missing and must be estimated from elsewhere. We have good data for the amount of energy incorporated into new tissue during this period (table 6), but we must also allow for the metabolic costs of tissue synthesis. These costs are unknown for squid, but Parry (1983) has argued that in marine invertebrates in general, costs of synthesis average 20–30% of the energy incorporated into new tissue; we have used a value of 20%. The level of excretory losses is unknown, but data from fish suggest that these are generally small and hence we have ignored them in this preliminary budget.

The oxygen consumption of *Illex argentinus* has not been measured, but there are literature data for *Illex illecebrosus* (DeMont & O'Dor 1984) a species which is very similar both morphologically and ecologically. In this study 95 measurements of respiration rate were made of squid over a period during which they grew from 43 to 443 g, and the ambient temperature increased from 8.3 to 18.2°C. These data were fitted by a multiple regression relating oxygen consumption, body mass, temperature and activity level. They represent the most complete data currently available for incorporating into an energy budget, and the regression equation was solved for a squid of body mass equivalent to male and female *Illex argentinus* at day 325 (469 and 800 g fresh mass respectively), a temperature of 10°C (representative of the water temperature on the feeding grounds) and activity levels of 0 and 100%. An activity level of 0% will approximate basal metabolism, whereas that at 100% activity should indicate the incremental cost of continuous swimming activity.

It is unlikely, however, that squid on the feeding grounds will be fully active all the time and so the power required for locomotor activity will be less than the 100% activity figure predicted from the equation of DeMont & O'Dor (1984). How much less is very difficult to decide in the absence of field activity data, although O'Dor (1988) found a good fit to migration data based on tagged squid on the assumption of an overall activity level of 50%. We have therefore used a figure of 50% in the preliminary budget.

These data have been compiled into a speculative power budget in table 7. To convert all estimates to the same units, oxygen consumption data have been converted to energy utilization rates using the standard conversion factor of 4.63 cal per millilitre of oxygen (Elliot & Davison 1975). An oxygen consumption of 1 ml $h^{-1}$ is thus equivalent to a rate of energy utilization of 5.38 mW.

The tissue energy content and oxygen consumption data in table 7 can be used to estimate daily energy intake (% total body kJ $d^{-1}$) and growth efficiency. The calculated daily ingestion rates (4.3 to 4.8% per day) are close to those observed for *Illex illecebrosus* (3.5–6.7, mean 4.6%: Hirtle *et al.* 1981), and within the overall range of 1.3 to 14.9% for cephalopods in data collated by O'Dor & Wells (1987). The gross growth efficiencies calculated for *Illex argentinus* (17–22%) are, however, generally lower than the range of mean values collated by O'Dor & Wells (1987) for cephalopods (27–69%).

These calculations are very preliminary but they do point to some general features. These are that locomotory costs in squid are high, that relatively low proportions of ingested energy are diverted to tissue synthesis, and that during the period on the feeding grounds when growth and sexual maturity are proceeding simultaneously, more energy is diverted to new somatic tissue than to reproductive tissue. As a




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Table 7. *Preliminary power budget for male and female* Illex argentinus *during the final 50 days of growth and sexual maturation on the feeding grounds prior to migration to the spawning grounds*

(All data averaged over the period 300 to 350 days since hatching; mass of male: 547 g (energy content 3002 kJ), mean fresh mass of female: 694 g (energy content 4555 kJ). 1 kJ $d^{-1}$ = 11.57 mW.)

| | power/mW | |
|---|---|---|
| | males | females |
| tissue synthesis | | |
| gonad | 7.3 | 53.5 |
| accessory tissues | 7.9 | 21.4 |
| digestive gland | 113.5 | 240.3 |
| mantle | 70.8 | 111.0 |
| other somatic tissues | 81.4 | 82.9 |
| cost of synthesis | 56.2 | 101.8 |
| excretory losses | unknown | unknown |
| basal metabolism | 465.1 | 560.0 |
| cost of activity (50% active) | 786.3 | 997.6 |
| total power requirement | 1518 | 2294 |
| total power (corrected for 90% absorbtion efficiency) | 1670 | 2086 |
| ingestion (% body kJ $d^{-1}$) | 4.8 | 4.3 |
| growth efficiency (%) | 16.8 | 22.3 |

result when mature *Illex argentinus* leave the feeding grounds to migrate northwards to their spawning area, there are substantial energy reserves located in the digestive gland. We can use the estimated power requirements for active swimming derived from *Illex illecebrosus*, and the measured energy reserves in the digestive gland in *Illex argentinus* at day 350 to estimate the migratory endurance of mature squid of both sexes. Assuming that migration involves an average of 50% maximum locomotor activity (as estimated by O'Dor 1988) and is fuelled solely by the complete depletion of the digestive gland reserves, the migratory endurance of male and female *Illex argentinus* is estimated at 7.6 and 10.4 days respectively.

An alternative estimate of endurance can be obtained by using the equation given by O'Dor (1982) which gives oxygen consumption as a function of body mass and swimming speed. The swimming speed of *Illex argentinus* during migration can be estimated if we assume that they leave the feeding grounds on 15 May and that the mean spawning date is 30 June (see Rodhouse & Hatfield 1990), giving an estimated migration time of 46 days. The shortest distance from the feeding grounds to the closest area where rhynchoteuthions (newly hatched paralarvae) have been collected is 1850 km along the 200 m isobath. Allowing for the known current speed of 35–40 cm $s^{-1}$ in the direction of migration gives an overall migration swimming speed of 7–12 cm $s^{-1}$. Solving the equation for a swimming speed of 0.1 m $s^{-1}$ and representative body masses of 547 g (male) and 694 g (female) fresh mass suggests migratory endurances of 14 and 21 days respectively.

Neither calculation suggests an overall endurance that matches the estimated migration time, even when allowance is made for the contribution from a favourable current. This suggests that *Illex argentinus* may need to feed on migration to the spawning grounds. The difference in endurance between the sexes may be explained partly by females using their reserves both for migration and as a final input of energy into the maturing ovary.

The material for this study was collected as part of a study of the biology of *Illex argentinus* funded by the Falkland Islands Government Fisheries Department by a grant to P.G.R. at the British Antarctic Survey. We thank Ken Patterson (FIDC), Myriam Booth (BAS) and the Fishing Master, Officers and crew of the vessel *Showa Maru 23* for help with collection of specimens. We also thank Bruce Lamden for converting a handwritten algorithm derived from Gnaiger & Bitterlich (1984) into a working ANSI C programme for desktop computer.

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*Received 11 October 1993; accepted 17 November 1993*





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