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ON THE USE OF AMMONIUM FOR BUOYANCY IN SQUIDS

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(Figs. 1–8)

Squids (teuthoids) fall into two distinct groups according to their density in sea water. Squids of one group are considerably denser than sea water and must swim to stop sinking; squids in the other group are nearly neutrally buoyant. Analyses show that in almost all the neutrally buoyant squids large amounts of ammonium are present. This ammonium is not uniformly distributed throughout the body but is mostly confined to special tissues where its concentration can approach half molar. The locations of such tissues differ according to the species and developmental stage of the squid. It is clear that the ammonium-rich solution are almost isosmotic with sea water but of lower density and they are present in sufficient volume to provide the main buoyancy mechanism of these squids. A variety of evidence is given which suggests that squids in no less than 12 of the 26 families achieve near-neutral buoyancy in this way and that 14 families contain squids appreciably denser than sea water [at least one family contains both types of squid]. Some of the ammonium-rich squids are extremely abundant in the oceans.

INTRODUCTION

Earlier work has shown that squid of the family Cranchiidae achieve neutral buoyancy by accumulating high concentrations of ammonium ions in acidified coelomic liquids which comprise almost two-thirds of the weight of the animals in air (Denton, Gilpin-Brown & Shaw, 1969). The present paper gives an account of the use of ammonium ions for buoyancy in other families of oceanic squids and surveys what may be inferred regarding buoyancy from the structure and appearance of species whose chemistry has not yet been studied. It is shown that in several families the major part of the buoyant ammoniacal liquid lies not in the coelomic cavity but in special tissues in other parts of the body. Brief accounts of some of this work have already been published (Clarke, Denton & Gilpin-Brown, 1969; Denton & Gilpin-Brown, 1973). Small muscles and nerves are found in the ammoniacal tissues but their relationships with the compartments containing strong ammoniacal solutions have yet to be studied in detail.

MATERIALS AND METHODS

A number of species of pelagic oceanic squid were caught close to the Canary Islands during cruises of R.R.S. 'Discovery' in 1967, 1969 and 1976. The majority of specimens were captured using the mid-water trawls, RMT 8 and RMT 90 (Clarke, 1969; Baker, Clarke & Harris, 1973). While fishing, the net was monitored in the ship and was opened and closed on demand so that the depth of capture was known. Of the specimens used 70 % were obtained from depths between 250 and 600 m where the salinities lie between 35·7 ‰ and 35·9 ‰ and temperatures between 15 °C and 11 °C (Fuglister, 1960).

A sheltered collecting area in the lee of Fuertaventura and the stability of R.R.S. 'Discovery' made observations and experiments on living or fresh animals very much easier than is usually

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the case on board ship. The effect of the ship's motion on weight measurements, carried out with a beam balance and two torsion balances of 10 g and 1 g capacities, was minimized by using a gimbals table. Mechanical vibration was damped by placing the table itself on anti-vibration mounts secured to the deck. All specimens were weighed in filtered sea water obtained from 300 to 400 m depth and kept in polythene bottles in a cold room at about 10 °C. The distribution of buoyancy between different parts of an animal (Table 2) was found by cutting the specimen into parts and weighing each part both in air and in sea water. Liquid losses, which occurred from the cut surfaces and fragile tissues, varied between 1 % and 7 % of the animal's weight in air. Under shipboard conditions, small errors were introduced during weighing because of differences between the temperatures of the animals and the sea water in which weighings were made. For small animals, and small parts of animals, losses of water by evaporation in the hot dry laboratory could also give small errors. To calculate the buoyancy of positively buoyant animals, pieces of very thin lead wire were added until the animals became slightly denser than sea water. The lead was later weighed ashore.

When buoyancy measurements had been made, specimens (including whole animals, residual carcasses and samples obtained from them) were either analysed at sea (pH, osmolarity, freezing point determinations and some ammonia determinations) or stored for later analysis ashore. Samples were stored in previously prepared dry glass tubes or screw-topped bottles which were placed in polythene bags before deep freezing. When thawing samples for analysis, these were allowed to reach room temperature before removing the outer polythene bags: this avoided condensation on the inner container and possible contamination of contents when the tubes were opened. Depressions of freezing point were determined either with a Fiske Osmometer or a Ramsay–Brown apparatus. pH was measured by glass electrode. Ammonia was measured by the indo-phenol colorimetric method of Crowther & Large (1956). Sodium, potassium, magnesium and calcium were determined by flame photometry and atomic absorption spectrometry. Chloride was determined by the Conway micro-diffusion method (Conway, 1940). Sulphate was either estimated accurately by the barium iodate method or estimated approximately by comparing the precipitates produced by adding an excess of barium chloride solution to the unknown solution and to standards prepared by diluting sea water with known amounts of distilled water. Amino acids plus ammonia were measured by the ninhydrin method, and total nitrogen by Kjeldahl's method.

RESULTS

Non-buoyant and buoyant squids

Fig. 1 and Table 1 show how clearly squid fall into two groups with respect to buoyancy. In species of the Loliginidae, Ommastrephidae, Enoploteuthidae (except for *Ancistrocheirus*), Brachioteuthidae and Ctenopterygidae, the ratio of weight in sea water over weight in air is greater than 2 %: such squids must swim actively to prevent themselves from sinking. On the other hand, as previously shown for the Cranchiidae, species of the Gonatidae, Octopoteuthidae, Mastigoteuthidae, Chiroteuthidae, Histioteuthidae and the gelatinous enoploteuthid *Ancistrocheirus lesueuri* (D'Orbigny) weigh very little in sea water. Some liquids in the latter families were probably even closer to neutral buoyancy when alive than is suggested by Fig. 1 and Table 1 since they are fragile and some were possibly slightly damaged during capture.

Body liquids

When the nearly neutrally buoyant squids were cut into pieces considerable quantities of liquid could usually be expressed from the cut surfaces of the buoyant parts and this loss of liquid was accompanied by loss of buoyancy. Thus, for example, the two large ventral arms of a specimen of *Mastigoteuthis* were buoyant, weighing 1·98 g in air and −13 mg in sea water (Table 2). After expressing about 1 ml of liquid from the cut

Table 1. *Buoyancy of representatives of various squid families*

Family	Species	*a*-weight in air (g)	*b*-weight in water (g)	*b*/*a* × 100
Loliginidae	*Loligo forbesi*	68·3	+2·5	+3·66
	Alloteuthis subulata	7·2	+0·225	+3·13
Ommastrephidae	*Todarodes sagittatus*	—	—	+2·86
	Todaropsis eblanae	390	+10·0	+2·56
Enoploteuthidae	*Pyroteuthis margaritifera*	4·93	+0·14	+2·84
	Abraliopsis pfefferi	1·06	+0·047	+4·43
	Ancistrocheirus lesueuri	4·37	+0·015	+0·34
Brachioteuthidae	*Brachioteuthis* sp.	3·85	+0·09	+2·34
Gonatidae	*Gonatus fabricii*	80·0	+0·04	+0·05
Ctenopterygidae	*Ctenopteryx siculus*	23·0	+0·50	+2·17
Octopoteuthidae	*Octopoteuthis danae*	120	+0·52	+0·43
Mastigoteuthidae	*Mastigoteuthis* sp.	*17·0	+0·01	+0·05
Chiroteuthidae	*Chiroteuthis* sp.	5·9	+0·01	+0·16
	Chiroteuthis veranyi (larva)	*3·0	−0·002	−0·006
	C. veranyi (adult)	90	+0·09	+0·103
Histioteuthidae	*Histioteuthis bonnellii*	7·5	0·0	0·0
	H. reversa	39·5	+0·01	+0·025
		*25·4	+0·05	+0·197
	H. meleagroteuthis	*49·0	+0·057	+0·116
	Histioteuthis sp.	0·84	+0·005	+0·59
	Histioteuthis sp.	22·0	0·0	0·0
	Histioteuthis sp.	35·0	+0·021	+0·06

* Variations of buoyancy within some of these animals are shown in Table 2.

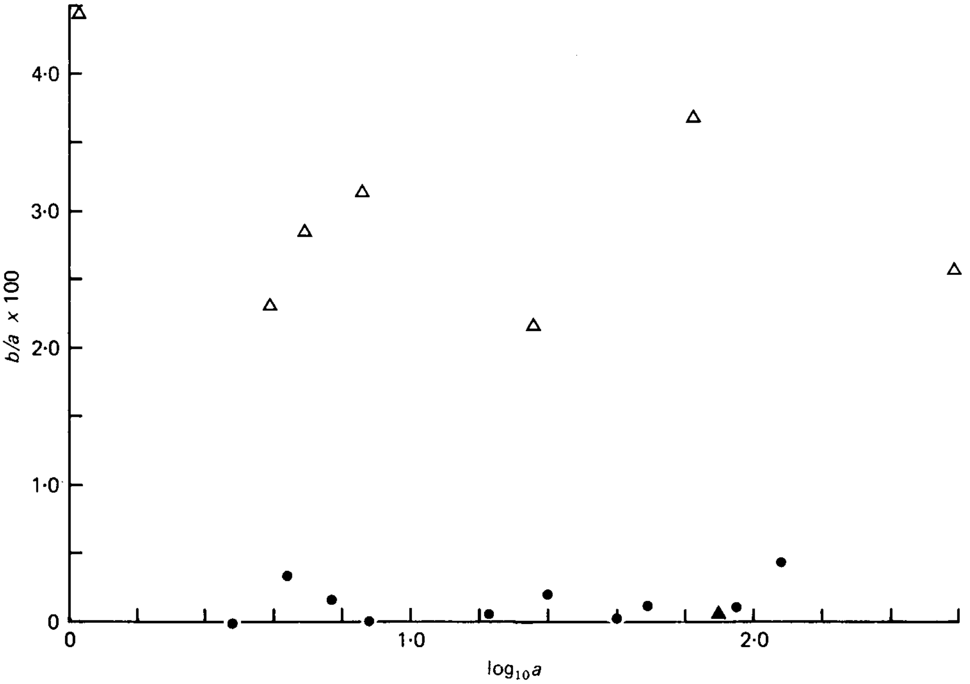

Fig. 1. The weight of squids in sea water (*b*) expressed as a percentage of the wet weight in air (*a*) plotted against $\log_{10}a$. Values are those given in Table 1, omitting the three *Histioteuthis* specimens not identified to species. (●), Squid containing large amounts of ammonium; (▲), *Gonatus fabricii* which accumulates large volumes of fat in its liver; (△), other squid.

surfaces, the arms weighed 0·94 g in air and +2·5 mg in sea water. Thus 1·04 g of the liquid within these arms had given a lift of 15·5 mg in sea water. The density of this liquid was therefore 1·011 and very close to that of the ammoniacal body fluids of the Cranchiidae (Denton *et al.* 1969).

Table 2. *The buoyancy of different parts of buoyant squid*

Species	*a*-weight in air (g)	*b*-weight in sea water (g)	*b*/*a* × 100
O. danae			
Mantle	20·5	−0·119	−0·58
Arms	24·2	−0·036	−0·15
Remainder (except fin)	*43·5	*+0·308	*+0·71
Fin	31·8	+0·367	+1·16
Mastigoteuthis sp.			
Two large arms	1·98	−0·013	−0·66
Remainder	*15·02	*+0·023	*+0·15
C. veranyi (Larva)			
Middle 'neck' region	*0·56	*−0·015	−2·68
Head and arms	0·88	+0·004	+0·46
Mantle and viscera	0·73	+0·009	+1·23
H. meleagroteuthis			
Arms	13	−0·052	−0·40
Mantle	9	−0·017	−0·19
Remainder	*27	*−0·126	+0·47
H. reversa			
Four arms	4·3	*−0·030	−0·70
Viscera and pen	2·63	*−0·005	−0·19
Mantle	5·4	+0·015	+0·28
Remainder of head	12·8	+0·060	+0·47

* By difference.

Samples of liquid were obtained from the buoyant regions of a number of species of squid either by squeezing the fresh tissues or by draining the liquids from them after deep freezing. For some specimens of *Histioteuthis* blood was drawn from the afferent branchial vessels and liquid taken from behind the lens of the eye. Results of analyses made on the liquids are given in Table 3.

The ammonium concentrations of the liquids obtained from buoyant regions were always extremely high and, where comparisons were made, very many times higher than those found in the blood or eye fluids of the same animal. The freezing point determinations show that the total osmotic concentrations of all these liquids were close to sea water (Δ_t close to −1·9 °C, osmolarity close to 1050 m-osmol). The small differences from sea water would not have given major changes in buoyancy and we feel certain, moreover, that some of these differences arose from the fact that the liquids had to be sampled, manipulated and measured either in the ship's very dry laboratories or after storing for considerable periods in a deep freeze. Packard (1972) calculated from the results of Amoore, Rodgers & Young (1959), that in *Octopus*, the liquids in the posterior chamber of the eye were slightly hyperosmotic to the blood and surrounding sea water. Our results do not indicate such a difference for the Histioteuthidae.

Table 3. *Properties of liquids obtained from buoyant and other parts of various squid compared with those of sea water*

	pH	Na^+ (mM)	K^+ (mM)	NH_4^+ (mM)	Sum of cations measured	Cl^- (mM)	Freezing point depression (°C)
Sea water							
Salinity 35‰	—	481	10	—	491	546·7	1·85
Oceanic 400 m	—	—	—	$\leqslant 10^{-2}$	—	—	1·91
			Mastigoteuthidae				
307/A *Mastigoteuthis* sp.							
Arms	5·9	—	—	—	—	—	1·93
Eyes	6·4	—	—	—	—	—	—
			Chiroteuthidae				
19/D *Chiroteuthis*							
Head and arms	—	—	—	160	—	—	2·08
'Neck' region	6·3	—	—	276	—	—	2·07
Mantle and viscera	6·2	—	—	125	—	—	2·28
			Octopoteuthidae				
6/C *Octopoteuthis danae*							
Mantle fluid	—	216	14	279	509	561	2·14
			Histioteuthidae				
16/B *Histioteuthis reversa*							
Arms	—	154	22	435	611	547	2·44
19/B *H. reversa*							
Arms	5·1	95	16	503	614	480	1·95
Mantle	5·1	192	29	394	615	546	—
Eye	6·6	336	29	75	440	596	1·84
27/B *H. meleagroteuthis*							
Arms	—	165	16	482	663	564	—
Mantle	—	119	23	427	569	541	—
Eye	—	530	17	21	568	555	—
324/B *Histioteuthis* sp.							
Arms	6·3	—	—	391	—	—	2·20
Blood	—	—	—	10	—	—	—
Eye	6·5	—	—	3	—	—	—
325/D *Histioteuthis* sp.							
Arms	6·3	—	—	345	—	—	1·92
Blood	7·2	—	—	5	—	—	2·20
Eye	7·1	—	—	4	—	—	1·87
326/B *Histioteuthis* sp.							
Arms	—	—	—	380	—	—	1·82
Blood	7·1	—	—	5	—	—	1·93
Eye	6·7	—	—	3	—	—	1·77
Histioteuthis (averages of values given above)							
Arms	5·9	138	18	423	629	530	2·07
Blood	7·2	—	—	7	—	—	2·07
Eye	6·7	433	23	21	504	576	1·83
Mantle	—	156	26	410	592	544	—
Architeuthis sp.							
Arm	—	116	34	228	378	337	—
Mantle	5·8	88	41	338	467	417	1080 [m-osmol]

Table 3 gives results of analyses made on several species of squid. These show that some body liquids contained a great deal of ammonium and much less sodium than sea water and that chloride was the principal anion. Sulphate was always very low in concentration relative to sea water. This composition adequately explains the low densities of the buoyant liquid and, since tissues rich in ammonium are very extensive, ammonium must play a major role in making these squid nearly neutrally buoyant.

The quantitative contribution to lift of ammoniacal liquids will depend on the total amount of ammonium which an animal contains in relation to the weight in sea water of its 'sinking' components such as protein, amino acids and chitin. If we assume, as a first approximation, that the only 'sinking' components of an animal are protein and amino acids of density 1·33 and the only component giving lift is a solution rich in ammonium chloride like the coelomic liquid of a cranchiid squid (approximately 475 mM ammonium and 1·010 density), then it is very easy to show that in sea water neutral buoyancy will be given when the ammonium nitrogen equals about two-thirds of the combined protein nitrogen and amino acid nitrogen. Analyses of whole animals were, therefore, made on specimens of two species of squid known to have tissues rich in ammonium, i.e. *Histioteuthis reversa* (Verrill) and *Octopoteuthis danae* Joubin and on a specimen of *Sepia officinalis* L., an animal which is known to be very muscular and to have a different buoyancy mechanism. The general analytical procedure was: (1) to add acidified water to the minced animal, centrifuge and analyse the supernatant for various components; (2) to repeat (1) until the supernatant contained negligible quantities of the substances being studied and (3) to dry the residue at 110 °C to constant weight (*W*) and then digest it with concentrated sulphuric acid for Kjeldahl determinations of total nitrogen. This nitrogen was assumed to be protein nitrogen. The total amounts of the substances present in solution were calculated from their concentrations in the supernatants and the volumes of these supernatants.

The quantity most difficult to calculate satisfactorily was the weight of water in the animal. An estimate of water was made by subtracting, from the initial wet weight, the final dry weight (*W*) and estimated weights of soluble components. The weight of soluble amino acids was taken as 6·25 times the alpha-amino nitrogen they contained, and the total weight of other soluble components as three-hundredths of the wet weight of the animal. The errors in estimates of water made in this way cannot be greater than a few percent and cannot affect any of the conclusions reached.

The results of these analyses show (Table 4, line *h*): (i) that in the *Sepia* the ammonium could only have counterbalanced a very small fraction of the weight in sea water of its proteins and amino acids and (ii) that in the *Histioteuthis* and *Octopoteuthis* the ammonium was more than sufficient in quantity to counterbalance their proteins and amino acids and could indeed have counterbalanced other sinking components as well. In the latter two animals the accumulation of ammonium must, therefore, have been the major buoyancy mechanism. There are striking differences between the animals of Table 4 in the concentrations of ammonium averaged over their whole body liquids (line *i*). The *Histioteuthis* and *Octopoteuthis*, with average concentrations of 361 mM and 229 mM respectively, resembled closely the cranchiid squid [we calculate that for the *Helicocranchia* studied by Denton *et al.* (1969) the average concentrations of ammonium were around 300 mM]. The average concentration of ammonium in the *Sepia* was only about 1/900th of that found in the *Histioteuthis*.

Table 4. *Results of analyses of three species of cephalopod*

Component	Derivation	*Histioteuthis reversa*	*Octopoteuthis danae*	*Sepia officinalis* without cuttlebone
(*a*) Wet weight in air (g)	Measured	36·6	39·7	3·36
(*b*) Weight of dry residue (*W*) (g)	Measured	0·77	0·82	0·25
(*c*) Weight of water (g)	$a-b-\left(\frac{6{\cdot}25e+30a}{1000}\right)$	34·4	37·1	2·9
(*d*) Total NH_4 nitrogen (mg)	Measured	174	119	0·015
(*e*) Total amino-acid nitrogen (mg)	Measured	50	98	14
(*f*) Total protein nitrogen (mg)	Measured	78	75	34
(*g*) Total sinking nitrogen (mg)	$e+f$	128	173	48
(*h*) Ratio $\frac{\text{floating nitrogen}}{\text{sinking}}$	$\frac{d}{e+f}$	1·36	0·69	0·0003
(*i*) Average NH_4^+ concentration (mM)	$\frac{1000d}{14c}$	361	229	0·4
(*j*) Average Na^+ concentation (mM)	Measured	116	92	321
(*k*) Average K^+ concentration (mM)	Measured	23	35	54
(*l*) Average Mg^{2+} concentration (mM)	Measured	14	1·8	39
(*m*) Average Ca^{2+} concentration (mM)	Measured	0·4	0·5	7·6
(*n*) Total cations measured (mM)	$(i+j+k+l+m)$	514	358	422
(*o*) Total Cl^- concentration (mM)	Measured	—	398	289

Variation of density within the body

That different regions of the body had different densities was immediately apparent when specimens were cut up and the parts placed in sea water. In one specimen of *Histioteuthis reversa* the arms floated but, when these arms were cut into two, the bases floated whereas the tips sank. Table 2 gives the weights in air and sea water of different parts of five species from four families. It shows, for example, that in *Octopoteuthis danae* about 75 % of the lift is derived from the mantle and 25 % from the arms while the reverse situation applies in *Histioteuthis meleagroteuthis* (Chun) since the arms supply 75 % of the lift and the mantle the remainder. In both animals the muscular fins are the densest regions. In *H. reversa* it is again the arms which provide the greater part of the lift and in *Mastigoteuthis* this function is performed above all by the two enlarged ventral arms. In the doratopsis larva of *Chiroteuthis veranyi* (Férussac) the head is separated from the mantle by a long 'neck' or 'collar' region, which is lost in the adult (Fig. 2). It is this region which provides the lift for the whole animal (Table 2).

Further dissection of buoyant squid into smaller parts showed that, within the buoyant regions, buoyancy was often localized in particular tissues. For instance closer study of the mantle of *Histioteuthis* showed that the middle and inner layers floated, but the outer layer was denser than sea water and sank.

Although the concentrations of ammonium shown in Table 4 are very high they are, nevertheless, average figures for whole specimens. Since some tissues (e.g. blood and

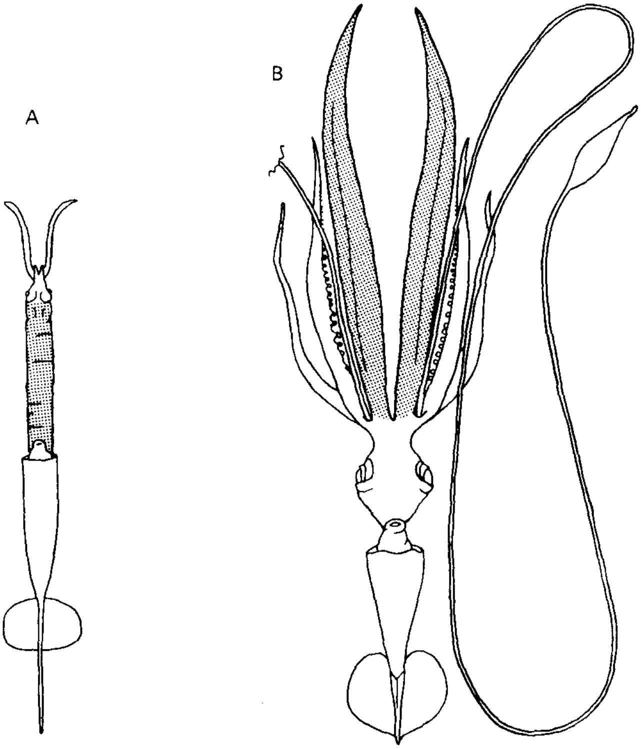

Fig. 2. *Chiroteuthis veranyi*. (A) Doratopsis larva with a mantle length of 1 cm (after Naef, 1923). (B) Adult with a mantle length of 6·8 cm (after Pfeffer, 1912). The most buoyant regions are shaded.

liquid from the eyes, Table 3) contained very little ammonium, the buoyant parts of the animals must have contained concentrations even higher than the average.

High ammonia concentrations in other squid families

Many accounts in the literature show that *Architeuthis* species are regularly found floating dead or dying at the sea surface (Clarke, 1966) and that it has proved useless for bait because pieces float (Denton & Gilpin-Brown, 1973). When pieces of arm and mantle removed from a deep-frozen *Architeuthis* (taken alive off Newfoundland) were made available, analysis showed that the colourless liquids, which exuded from the samples when they thawed, were very similar in composition to those obtained from squids previously analysed and known to be made nearly neutrally buoyant by accumulation of ammonium (Table 3). For the *Architeuthis* tissues, osmolarity was determined by measuring vapour pressures with a Hewlett-Packard osmometer.

There is a very much greater quantity of ammonium in squids using it for buoyancy than in the dense, non-buoyant squids and, if there is sufficient ammonium to impart positive buoyancy to a tissue, a strong smell of ammonia can be detected when this tissue is placed in a strong sodium hydroxide solution and warmed. The smell is not detected when the tissues are non-buoyant and contain only low concentrations of ammonium as with arms or mantle of *Loligo* and *Ommastrephes*. This simple test clearly demonstrated high concentrations of ammonium in intact mantles of the octopoteuthid squid, *Taningia danae* Joubin (Fig. 3A), and the enoploteuthid squid, *Ancistrocheirus lesueuri*, collected from sperm whale stomachs. The latter was subsequently found to be nearly neutrally buoyant (Table 1).

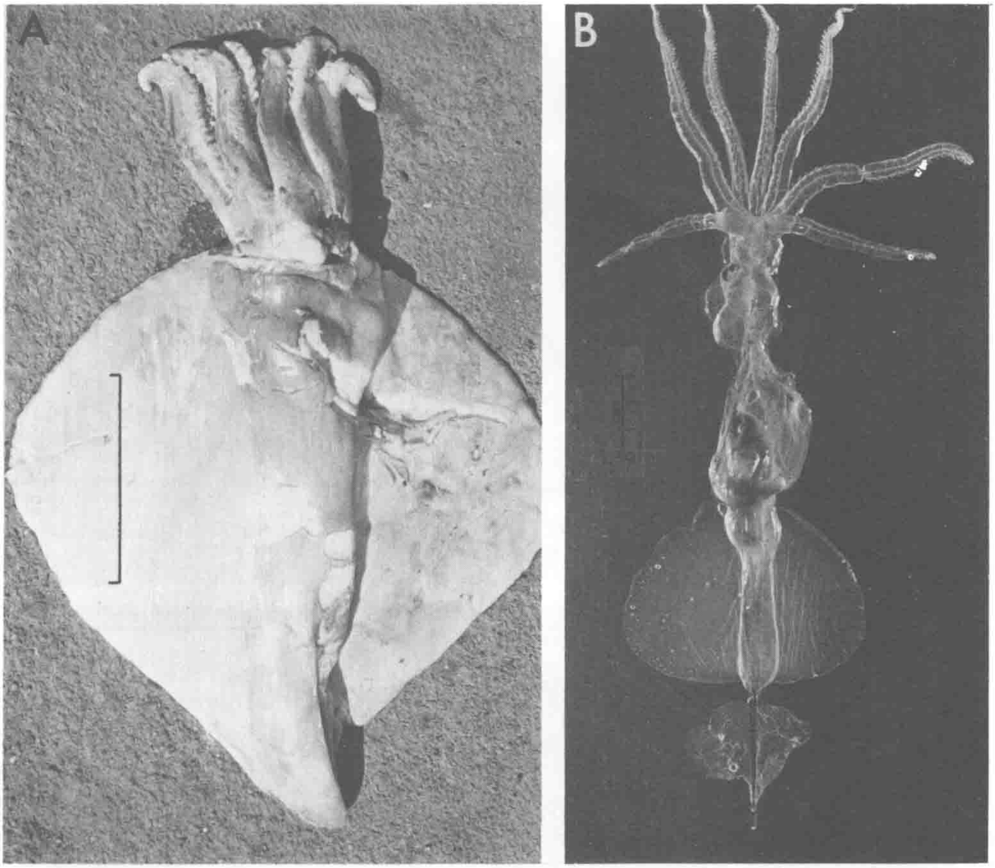

Fig. 3. Ventral views of (A) *Taningia danae* from a sperm whale's stomach and (B) *Grimalditeuthis bonplandi*: a rare squid with the unusual feature of a second fin and probably an ammoniacal species. Scale bars: A, 20 cm; B, 3 cm.

Histology of buoyant tissues

In sections of the arms and mantle of non-buoyant squids the muscular layers occupy a large proportion of the total area. For example, the mantle of the onychoteuthid *Onychoteuthis banksi* (Leach) or the arm of the ommastrephid *Todarodes sagittatus* (Lamarck) (Fig. 4B) are composed almost entirely of densely packed muscles. Squids in the Cranchiidae that are neutrally buoyant by retention of ammonium in the coelom (Denton *et al.* 1969) also have muscular mantles and arms (Fig. 5). On the other hand, in squids such as *Octopoteuthis*, *Histioteuthis*, *Chiroteuthis* and *Mastigoteuthis*, known to be neutrally buoyant by storage of ammonium within the body tissues, muscles occupy

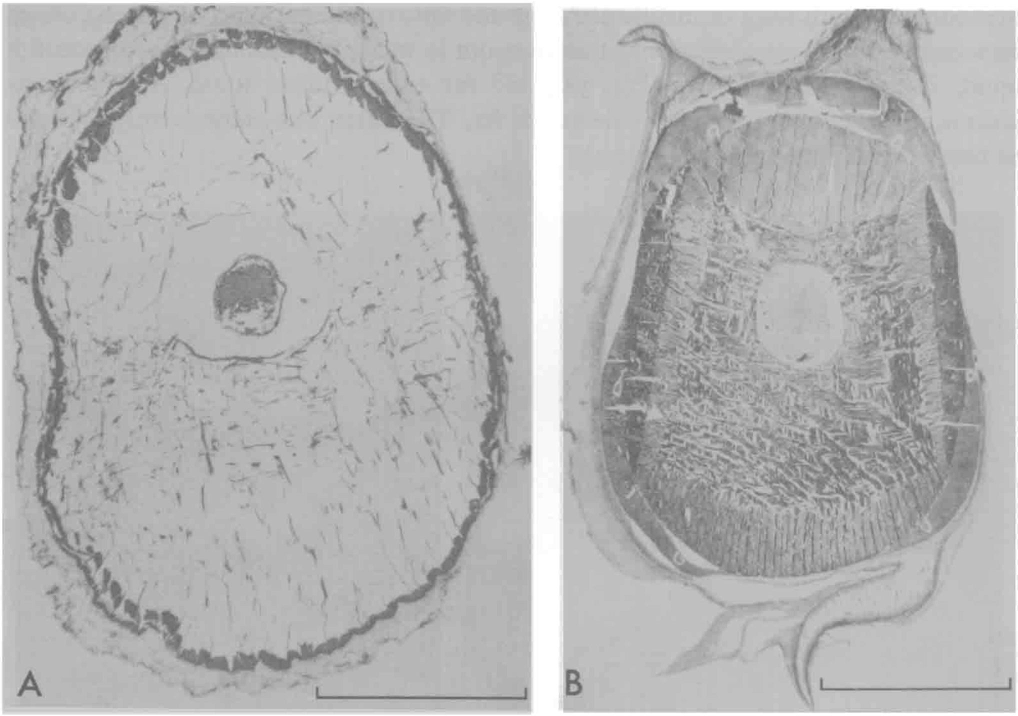

Fig. 4. Transverse sections of the arms of (A) *Mastigoteuthis* sp. and (B) *Todarodes sagittatus*. Scale bars: A, 1 mm; B, 5 mm.

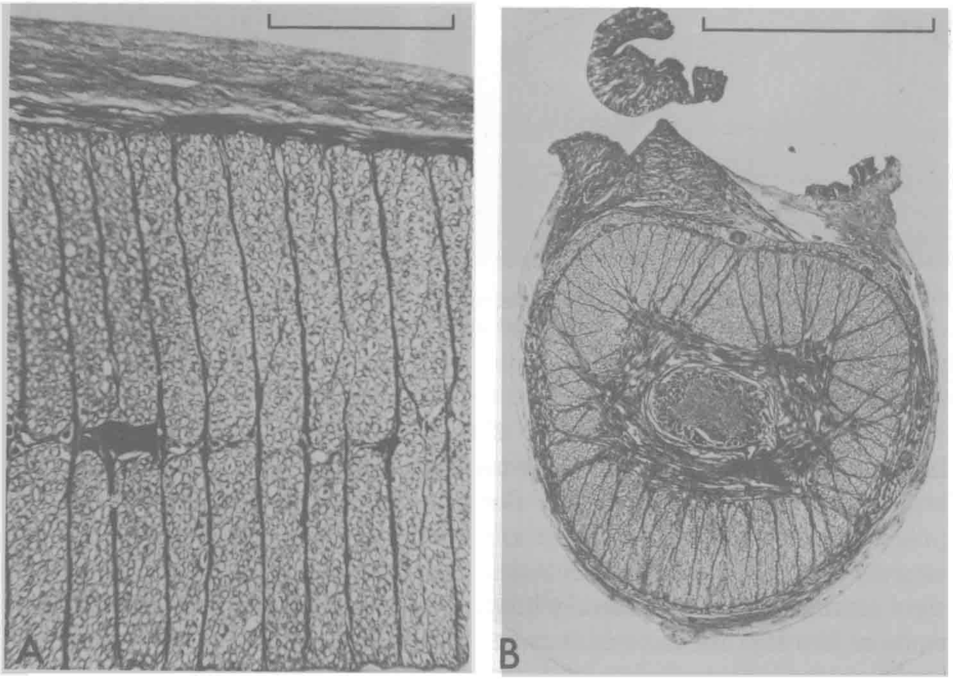

Fig. 5. *Helicocranchia pfefferi*. Sections of (A) mantle and (B) arm. Scale bars: A, 0·2 mm; B, 0·5 mm.

very much less of the total area of sections of the arms and mantle (Figs. 4A, 6A). Between rather widely scattered muscles the tissue often consists of a thin-walled reticulum with a 'honeycomb' appearance (Fig. 6B).

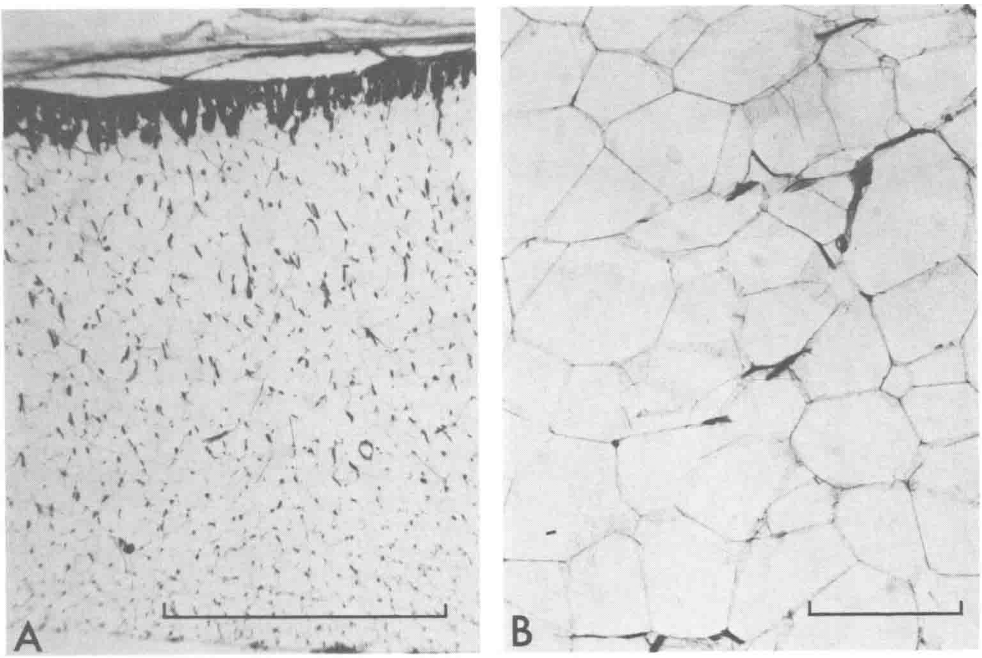

Fig. 6. Sections of the mantles of (A) *Octopoteuthis* sp. and (B) *Histioteuthis bonnellii* showing reticulate tissues. Scale bars: A, 0·5 mm; B, 0·2 mm.

Since this reticulum accounts for most of the volume of the arms and mantle it is certain that most of the ammonium within the arms and mantle must be enclosed in the reticulum. If the tissues other than the reticular ones contain little ammonium then the relative volumes of reticulum and other tissues within an organ can be related to their respective densities and the density of the organ. For example, we know from our earlier calculations (p. 262) that the liquid expressed from an arm of *Mastigoteuthis* had a lift of approximately 15 mg per g. We also know that 1 g of arm had a lift in sea water of about 6·6 mg (Table 2). In addition, we can assume from weighings made on muscles of non-buoyant squid that dense tissues, consisting mainly of muscle, will probably have a weight in sea water of about 4·5 % of their weight in air, i.e. 1 g will weigh 45 mg in sea water. Then, if x is the fractional weight of the dense tissue and $(1-x)$ that of the buoyant liquid, the relative amounts of dense tissue and buoyant liquid will be given by the equation

$$45x - 15(1-x) = -6{\cdot}6,$$

$$\text{so that} \quad x = 0{\cdot}14.$$

The relative areas of reticular and other tissue in transverse sections of arms of *Histioteuthis* were measured from camera lucida drawings made on graph paper. From these measurements we estimate that the combined volume of the central core, consisting

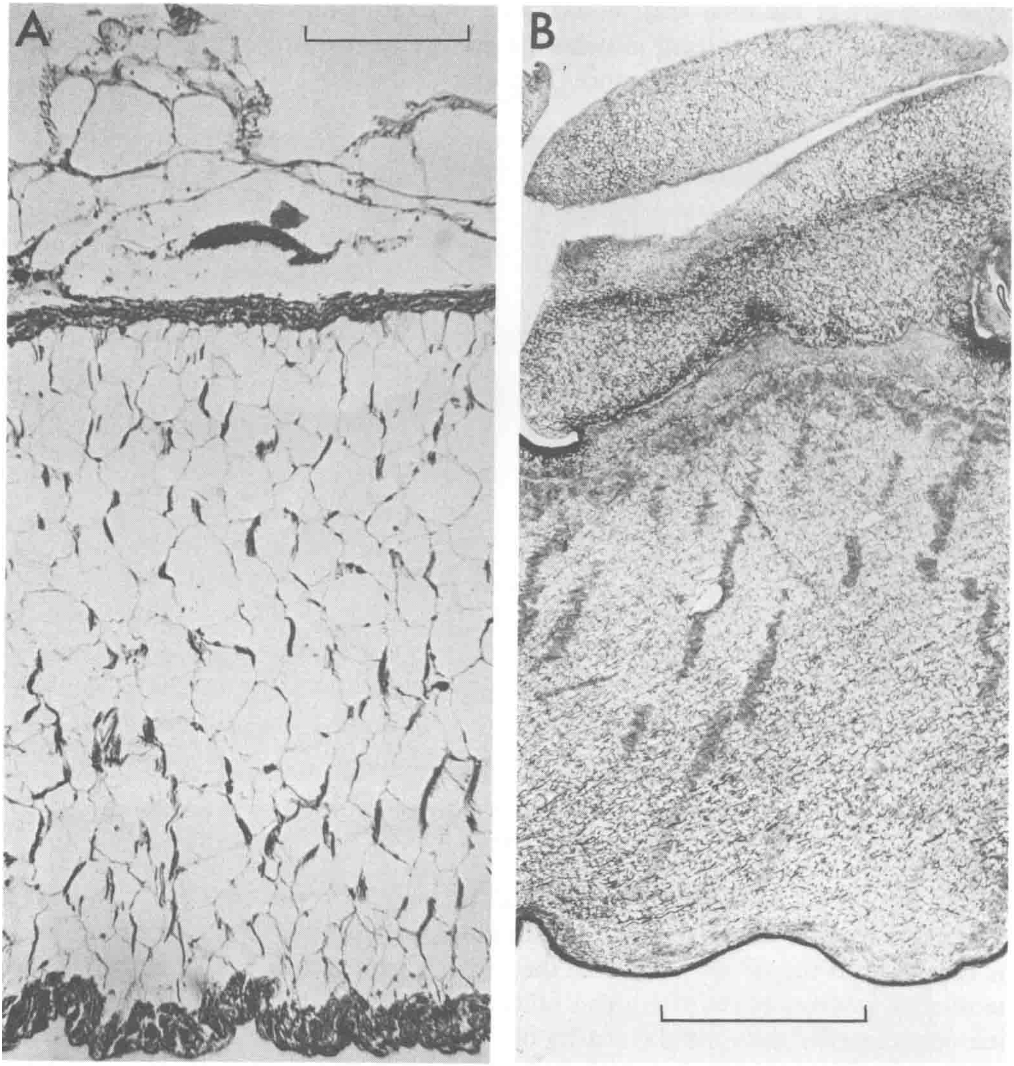

Fig. 7. *Lepidoteuthis grimaldii.* Sections of the mantles of (A) larva and (B) adult. Scale bars: A, 0·2 mm; B, 1 mm.

mainly of the nerve and the sheath of longitudinal muscles, amounted to about 9% of the total volume. This figure, which should be increased by some small amount to include the scattered dense tissues whose areas were not measured, agrees reasonably well with that obtained by the above calculation (14%) which was based on the assumption that *all* the reticular tissue contained an ammonium-rich solution like that obtained by expressing liquid from the arms.

Generally, if gelatinous and reticular tissues comprise a large proportion of a pelagic marine animal their function is to impart lift. Two buoyancy mechanisms have been described which use such tissues. The first involves the substitution, within the body liquids, of the sulphate ions of sea water by chloride ions. This substitution can only

Table 5. *Conclusions concerning the buoyancy of living teuthoid families and the evidence upon which they are based*

The species list includes most of those examined by the authors. Four families (*) have not been examined and tentative conclusions are drawn from descriptions and published drawings. X shows where evidence from two sources conflicts. D = dense species; N = near-neutral buoyancy.

Family	Species	Evidence used to form conclusion on buoyancy: Buoyancy measured	Ammonia estimated	Histology studied	Body shape and texture	Buoyancy either measured or predicted
Loliginidae	*Loligo forbesi*	√	√	√	√	D
	Alloteuthis subulata	√	√	√	√	D
Pickfordiateuthidae	*Pickfordiateuthis pulchella*				√	D*
Ommastrephidae	*Todarodes sagittatus*	√	√	√	√	D
	Ommastrephes caroli			√	√	D
Onychoteuthidae	*Moroteuthis robusta*		√		√	D
	Onychoteuthis banksi			√	√	D
	Choanoteuthis mollis	√				D
Thysanoteuthidae	*Thysanoteuthis rhombus*			√	√	D
Brachioteuthidae	*Brachioteuthis riisei*	√	√	√	√	D
Ctenopterygidae	*Ctenopteryx siculus*	√		√	√	D
Neoteuthidae	*Neoteuthis thielei*	√			√	D
Promachoteuthidae	*Promachoteuthis megaptera*				√	D*
Lycoteuthidae	*Oregoniateuthis lorigera*			√	√	D
Psychroteuthidae	*Psychroteuthis glacialis*				√	D*
Batoteuthidae	*Batoteuthis skolops*				√	D*
Enoploteuthidae	*Pyroteuthis margaritifera*	√	√	√	√	D
	Abraliopsis pfefferi	√		√	√	D
	Ancistrocheirus lesueuri	√		√	√	N
Gonatidae	*Gonatus fabricii*	√	√	√	√	N X
Bathyteuthidae	*Bathyteuthis abyssicola*			√	√	N
Cycloteuthidae	*Cycloteuthis akimushkini*			√	√	N
	Discoteuthis laciniosa			√	√	N
Joubiniteuthidae	*Joubiniteuthis portieri*			√	√	N
Grimalditeuthidae	*Grimalditeuthis bonplandi*			√	√	N
Lepidoteuthidae	*Lepidoteuthis grimaldii*			√	√	N
Pholidoteuthidae	*Pholidoteuthis boschmai*			√	√	N ?
Architeuthidae	*Architeuthis* sp.		√		√	N
Octopoteuthidae	*Octopoteuthis danae*	√	√	√	√	N
	Taningia danae		√	√	√	N
Mastigoteuthidae	*Mastigoteuthis hjorti*	√	√	√	√	N
Chiroteuthidae	*Chiroteuthis veranyi*	√	√	√	√	N
	Valbyteuthis danae			√	√	N
Histioteuthidae	*Histioteuthis bonnellii*	√	√	√	√	N
	H. reversa	√	√	√	√	N
	H. meleagroteuthis	√	√	√	√	N
Cranchiidae	*Taonius megalops*	√			√	N
	Taonidium pfefferi	√			√	N
	Carynoteuthis sp.	√			√	N

give sufficient lift for the animal to approach neutral buoyancy if, like the pelagic medusae, ctenophores and tunicates, it contains only a very small proportion of dense tissue such as muscle (Denton & Shaw, 1961). The second mechanism involves the substitution, within body liquids, of the sodium ions of sea water by ammonium ions. This latter mechanism gives sufficient lift to counterbalance much greater quantities of dense muscular tissues. If, therefore, we find large amounts of gelatinous or reticular tissue associated with appreciable quantities of muscular tissues as in the Cranchiidae, Histioteuthidae, Octopoteuthidae, etc. we can be reasonably sure that the second method, involving substitution of sodium by ammonium ions, is the one involved. Even when density measurements have not been made we can therefore use the relative quantities of muscular and non-muscular tissues in the mantles and arms as an indication of whether the squids belong to the 'dense' group or the 'nearly neutrally buoyant' group. Based upon this criterion we can be reasonably confident that an accumulation of large amounts of ammonium takes place, not only in the families in which we have detected very large quantities of ammonium, i.e. the Cranchiidae, Octopoteuthidae, Histioteuthidae, Mastigoteuthidae, Chiroteuthidae, Architeuthidae, and one genus, *Ancistrocheirus*, of the Enoploteuthidae, but also in the Grimalditeuthidae (Fig. 3), Bathyteuthidae, Joubiniteuthidae, Lepidoteuthidae (Fig. 7) and Cycloteuthidae. Of these 12 families the Enoploteuthidae shows the greatest diversity of form and structure and is the only one which we know contains an ammoniacal, nearly neutrally buoyant, genus as well as genera belonging to the dense, non-buoyant group of squids. On the other hand, the Loliginidae, Ommastrephidae, Neoteuthidae, Ctenopterygidae, Onychoteuthidae and Brachioteuthidae are known, from density measurements, to belong to the dense group which, on the basis of their structure and appearance, probably also contains the Thysanoteuthidae and Lycoteuthidae. From comments on structure by other workers this group probably also contains the Pickfordiateuthidae (Voss, 1953), Promachoteuthidae (Roper & Young, 1968) Psychroteuthidae (Filippova, 1972) and Batoteuthidae (Young & Roper, 1968).

The evidence upon which these conclusions are based is summarized in Table 5.

The reticular tissue that contains high concentrations of ammonium may consist of thin-walled, irregularly-shaped elements as in the Histioteuthidae and the Octopoteuthidae (Fig. 6) or have much larger elements separated by thicker walls as in the Chiroteuthidae (*Chiroteuthis* (Fig. 8) and *Valbyteuthis*). Young *Lepidoteuthis* have reticulate tissue similar to *Histioteuthis* but the mantle of the adults has smaller elements that form a thick spongy layer through which pass radial muscles (Fig. 7). *Pholidoteuthis* (as described from sperm whale stomachs by Clarke, 1979) has a mantle with a similar spongy layer to that in *Lepidoteuthis* adults. It seems probable, although not proven, that this spongy tissue carries high concentrations of ammonium. In *Bathyteuthis* the reticulate layer lies completely outside well developed muscles of the mantle. The larvae of some cranchiids such as *Bathothauma* have very large, turgid-looking, transparent eye-stalks largely composed of reticulate tissue (Young, 1970). It is possible that in these young squid some ammonium is contained within the eye stalks as well as being in high concentration in the coelom.

Adults of *Gonatus fabricii* are distinct from squids of all other well studied teuthoid families by being both nearly neutrally buoyant and having relatively small quantities of ammonium. Lift in this species is given by large amounts of low density oil and this will be described elsewhere (Clarke & Denton, in preparation). While a similar mechanism is not known for other families it might be present in squids of one or two other muscular families whose density has not been measured, particularly the onychoteuthid genus *Moroteuthis*. It should also be mentioned that very young specimens may differ from the adults of the same species. This was not found in any ammoniacal families examined but young of the Gonatidae are not neutrally buoyant.

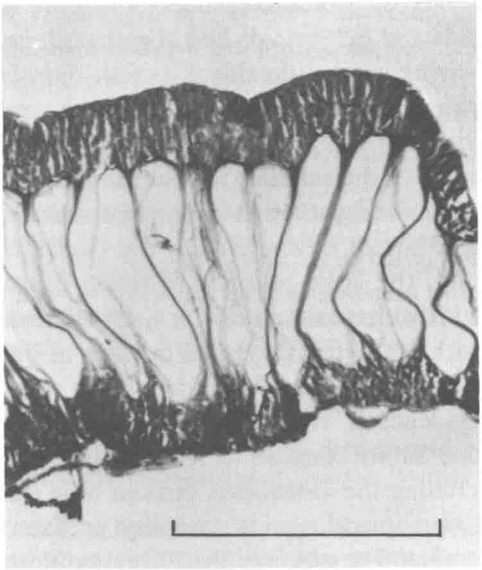

Fig. 8. *Chiroteuthis veranyi*. Section of mantle. Scale bar: 0·5 mm.

Centre of buoyancy and posture

The position a resting squid normally adopts with respect to gravity depends upon its shape and the relative density of its parts. In *Mastigoteuthis schmidti* Degner the two large ventral arms are more buoyant than the rest of the animal (Table 2) so that living animals float with these uppermost. *Chiroteuthis veranyi* adults also have very large ventral arms and normally adopt the same attitude (W. Pearcy, personal communication). However, the doratopsis larvae are very different in shape (Fig. 2A) and the more central position of the main buoyant tissue, with denser tissue in front and behind it, facilitates a horizontal attitude. *Histioteuthis* is short in relation to its width and has buoyant tissue in both arms and mantle. Its attitude is readily influenced by a very small turning couple such as that given by a small entomological pin inserted in the posterior end of the mantle.

DISCUSSION

All the evidence suggests that, since they evolved, most cephalopods have had some mechanism by which they attained near-neutral buoyancy. Mechanically, neutrally buoyant squids correspond to airships while the dense squids, which swim to stay in midwater, correspond to aeroplanes. This difference in buoyancy between species of squid must correspond to very different life styles, as do the differences in buoyancy between species of fish, e.g. between the sea horse and cod on the one hand, and the mackerel and tunny fish on the other.

We know of four methods by which cephalopods achieve neutral buoyancy. The fossil ammonoids, nautiloids and belemnoids had chambered, gas-filled shells and such shells are found in a few living genera, notably *Nautilus*, *Spirula* and *Sepia* (Denton & Gilpin-Brown, 1961, 1973). A second method of achieving neutral buoyancy, used in some oceanic octopods with extremely gelatinous bodies and very little muscle, is by substitution, within the body, of the sulphate ions of sea water by chloride ions. This is a buoyancy mechanism described for other gelatinous animals by Denton & Shaw (1961). Thirdly, the oceanic squids of one family, the Gonatidae, store large volumes of low density fats. Finally, the large majority of living cephalopods having neutral buoyancy are oceanic squids that have the coelom or special tissues filled with ammonium-rich solutions isosmotic with sea water. Of the 26 families of living teuthoids (25 listed by Roper, Young & Voss (1969) and the Pholidoteuthidae reinstated by Clarke, 1977, 1979) we consider the evidence is strong that 12 families use ammonium chloride solutions for buoyancy, one family contains dense as well as neutrally buoyant squids, and the remaining 13, including the Gonatidae, contain little ammonium.

The large number of ammoniacal squids eaten by predators shows that they are abundant in the world's oceans. For example the Histioteuthidae, the Octopoteuthidae, and the Cranchiidae are important in the diet of sperm whales and the combined weight of these three families consumed each year by these whales probably exceeds the total fish catch of all the fishing fleets of the world (Clarke, 1977). Thus, during evolution, buoyant, soft-bodied squids have largely replaced the diverse and once very prolific extinct forms with gas-filled, chambered shells. Why should such a change have taken place? At least part of the answer must be the restriction a calcareous, gas-filled chambered shell imposes upon the depth range of its possessor. All such shells contain gas at sub-atmospheric pressure and this places an absolute restriction on the depth to which an animal with such a shell can go before its shell implodes. For this reason *Sepia* is only found in the top 150 m, *Nautilus* in the top 500 m and even the tiny *Spirula* only descends to 1200 m. The use of ammonium or oil for buoyancy has allowed the spread of large, buoyant cephalopods to much greater depths than could ever have been possible for animals depending on chambered shells.

The evidence given here shows that only some of the tissues of the ammoniacal squid contain ammonium in high concentrations: blood and liquids from eyes were found to contain relatively little ammonium. We also know from work on cranchiids (Denton, Gilpin-Brown & Wright, unpublished observations) and on *Histioteuthis* (Drs Q. Bone and J. V. Howarth, personal communications) that the larger nerves from these animals will not conduct impulses if exposed to ammonium of the high concentrations known to exist in their reticular tissues.

Denton *et al.* (1969) argue that ammonium could be trapped in special tissues by simply making the liquid contained in these tissues more acid than blood. On their simple hypothesis the ratio of the concentrations of hydrogen ions between these tissues and in the blood would equal the corresponding ratio of ammonium ions. The results given on Table 3 show that their ammoniacal tissues are more acid than their blood. However, if we take the average values for *Histioteuthis* sp., the ratio of hydrogen ion concentrations between the ammoniacal liquid and blood is about 20 whilst the corresponding ratio for ammonium is over 60. This result is not compelling evidence against the simple hypothesis because the liquids expressed from the buoyant tissues must always have contained small volumes of liquids other than those of the reticular tissues. These additional liquids would almost certainly be better buffered than the strongly ammoniacal liquids and have a disproportionate effect on acidity.

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