



Ammonium content and buoyancy in midwater cephalopods

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Abstract

The majority of squid families (Teuthoidea: Cephalopoda) exchange sodium for ammonium, creating a low-density fluid that imparts lift for neutral buoyancy. However, previous methods for measuring ammonium did not distinguish between NH_4^+ and various other amine compounds. The present study, using single column ion chromatography, reassessed the cation concentrations in several midwater cephalopod species. High NH_4^+ levels were confirmed for histioteuthid, cranchiid, and chiroteuthid and related squids. A strong relationship is reported between ammonium content and body mass in *Histioteuthis heteropsis*, suggesting a gradual accumulation of ammonium coincident with an ontogenetic migration to greater depths. The bathypelagic squids *Bathyteuthis abyssicola* and *Bathyteuthis berryi*, on the other hand, contained very little ammonium but rather contained large quantities of an as yet unidentified cation. The ecological significance of this compound is not yet known. Morphology in Bathyteuthid squids suggests that the unknown cation is contained intracellularly and so, unlike sequestered ammonia, does not diminish the space available for muscle tissue. Accordingly, protein measurements in *B. berryi* mantle muscle are on par with shallower-living muscular

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squids, and in situ submersible observations reveal strong locomotory abilities relative to other deep-water squids.

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1. Introduction

Maintaining vertical position in the water column is a major energetic expense (Alexander, 1990; Webber et al., 2000). Pelagic organisms have evolved diverse strategies to reduce their densities relative to seawater in order to minimize this cost. The diversity of mechanisms used for buoyancy is exemplified by the molluscan class Cephalopoda; representatives of which employ nearly every known type of buoyancy mechanism. *Nautilus* spp., *Spirula* spp., and *Sepia* spp. all maintain gas within chambers in a hard shell (Denton et al., 1967), while females of the octopod *Ocythoe tuberculata* are among the few invertebrates known to use a gas-filled swim bladder (Packard and Wurtz, 1994). Other pelagic octopods possess extensive layers of gelatinous tissue overlying and invading the musculature that, in combination with a reduction in the concentration of heavy sulfate ions in body fluids, facilitates neutral buoyancy (Denton et al., 1967; Clarke et al., 1979). Some squids contain lipid in the digestive gland and accumulate methylamines in muscle tissue (Kelly and Yancey, 1999; Seibel et al., 2000b; Seibel and Walsh, 2002), both of which impart lift via low density and large positive partial molal volume, respectively (Sanders and Childress, 1988; Withers et al., 1994).

By far, the most prevalent method of attaining neutral buoyancy in cephalopods is the exchange of sodium for ammonium ions creating low-density fluids that impart lift (Clarke et al., 1979; Lipinsky and Turoboyski, 1983; Robison, 1989; Voight et al., 1994). While squids of the family Cranchiidae store ammonium fluid exclusively in a specialized coelomic chamber, all other ammoniacal families store it in the muscle tissue. The mantle and arms of such species are heavily invaded with specialized vacuoles that presumably contain ammonium fluid, and although nerves and muscles appear to run directly through this tissue, ammonium has not been found in the blood, suggesting that the sequestration is effective (Clarke et al., 1979).

Ammonium is a readily available by-product of the carnivorous cephalopod diet. However, because it is only slightly lighter than sodium, the ion with which it is typically exchanged, large quantities must be accumulated in order to achieve neutral buoyancy. The body mass of ammoniacal squids is comprised of roughly 50–60% ammonium fluid that reaches concentrations near 500 mM (Clarke et al., 1979, Voight et al., 1994). These massive proportions raise questions about the mechanisms and timecourse of accumulation and also pose problems for locomotion via displacement of muscle tissue. In fact, no matter what mechanism is used, neutral buoyancy and strong locomotory performance are, to some extent, incompatible goals (Alexander, 1990). For example, external shells increase drag (Jacobs and Chamberlain, 1996) and large lipid-rich digestive glands inhibit jet propulsion to a lesser extent by reducing the volume of

the mantle cavity (Hunt and Seibel, 2000). This trade-off is ecologically feasible in meso- and bathypelagic cephalopods because selection for locomotory capacity is reduced in the deep-sea where light limits visual predator–prey interactions (Childress, 1995; Seibel et al., 1997). Among shallow-living crustaceans, and presumably cephalopods as well, the energy required to maintain position in the water column is an insignificant portion of the energy budgeted for activity (Childress and Nygaard, 1974; Seibel et al., 1997). However, with increasing depth, the requirement for locomotion declines, and the relative cost of maintaining vertical position in the water column increases accordingly. Buoyancy mechanisms thus become cost-effective at greater depths.

Accumulation of methylamines may circumvent the locomotory penalty associated with neutral buoyancy. Some methylamine osmolytes are compatible with macromolecular (e.g., enzyme) function and are accumulated in the intracellular space (for review see Hochachka and Somero, 2002). Accumulation of methylamines would not displace muscle tissue as extracellular ammonium sequestration does. The methods employed previously for analysis of midwater cephalopod ammonium content did not distinguish between NH_4^+ and methylamines (Sanders and Childress, 1988). The possibility exists that, in some cases, methylamines rather than ammonium were recorded (Kelly and Yancey, 1999). The present study reanalyzed the cationic composition of several midwater squid species using single ion chromatography (Sanders and Childress, 1991) to quantify ammonium specifically. Inter- and intraspecific variation in ammonium content is discussed.

2. Methods

2.1. Collection

Most of the cephalopods analyzed were captured during 10 cruises aboard the R/V Point Sur and the R/V New Horizon between March 1993 and September 1996, primarily in an area 160 km west of Point Conception, CA ($34^\circ 37' \text{ N}$, $122^\circ 42' \text{ W}$ to $34^\circ 30' \text{ N}$, $123^\circ 20' \text{ W}$). Some specimens were collected off Oahu, Hawaii ($21^\circ 20' \text{ N}$, $158^\circ 20' \text{ W}$ to $21^\circ 35' \text{ N}$, $158^\circ 35' \text{ W}$). Most were collected using the opening/closing Mother Tucker trawl with a 10-m² mouth. The net was equipped with a 30-l thermally protecting cod end which reduced mechanical damage and heat shock to animals during recovery (Childress et al., 1978). Ship speed was kept very low (0.5–1 kt) to decrease turbulence and skin abrasion of specimens in the net. Additional specimens were collected in Monterey Bay, CA ($36^\circ 42' \text{ N}$; $122^\circ 02' \text{ W}$), using the ROV Ventana from the R/V Pt. Lobos (Monterey Bay Aquarium Research Institute). Specimens were weighed on a motion-compensated shipboard precision balance system (Childress and Mickel, 1980) prior to dissection. Tissue was extracted from the left ventral mantle of each cephalopod examined. Additional samples were taken from the fins and tail of some species. Fluid was also extracted from the coelomic chamber in several cranchiid squid species. All samples were frozen immediately in liquid nitrogen following dissection.

2.2. Tissue composition

For cation measurements, frozen tissue or coelomic fluid samples were thawed, deproteinated by dilution with methanol (1:1 ratio), and centrifuged at 8000 rpm for 10 min in a microfuge. Standard solutions and seawater samples were diluted in a similar manner. All samples and standards were diluted again, to a final dilution of 200×, with HPLC grade water. Cation concentrations were determined using single column ion chromatography (Sanders and Childress, 1991). The eluent was 3.2 mM nitric acid (filtered through a 0.45- μm filter, pumped at 1.0 ml min⁻¹). A Rheodyne valve with a 20- μl sample loop was used to introduce samples through a guard column and cation column (Alltech Cation/R), and ions were detected using an Alltech conductivity detector. Values of samples were obtained by comparison to known concentrations of standards. Total methylamine ($\sum\text{MA}^+$) values for *Bathyteuthis berryi* and *Bathyteuthis abyssicola* were determined by comparison to a trimethylamine (TMA) standard. Although the identity of those peaks has not been positively determined, the retention time was within 0.4 min of the TMA standard.

Because cation concentrations were determined from mantle tissue homogenates diluted by wet mass, actual fluid ammonium concentrations cannot be calculated. The values reported by Clarke et al. (1979) were based on fluid expressed from muscle tissue and so are not directly comparable to the present results. The values reported here for cranchiid squids

Table 1
Cationic composition (mM) of pelagic cephalopods

Species	Mass	<i>n</i>	Na ⁺	K ⁺	NH ₄ ⁺	MA ⁺
<i>Tissue ammoniacal</i>						
Chiroteuthid Clade						
<i>Chiroteuthis calyx</i> adult	45.6, 48.0	2	92.8, 136.5	20.9, 26.8	296.2, 266.0	–
Juvenile-tail fluid	–	1	423.1	17.1	132.3	–
Juvenile-tail tissue	–	1	243.7	29.0	304.2	–
<i>Chiroteuthis imperator</i>	275.0	1	125.3	30.5	276.6	–
<i>Mastigoteuthis inermis</i>	21.1	1	70.7	19.0	175.7	–
<i>Joubiniteuthis portieri</i>	12.7	1	77.3	12.8	217.0	–
<i>Grimalditeuthis bonplandi</i>	68.5	1	215.6	30.0	216.4	–
Histioteuthidae						
<i>Histioteuthis heteropsis</i>	0.68–40.5	8	17.2–187.8	13.4–24.5	89.3–277.0	–
<i>Histioteuthis hoylei</i>	15.3, 500.0	2	34.4, 41.3	11.2, 41.0	69.2, 289.5	–
<i>Coelomic fluid</i>						
Cranchiidae						
<i>Helicocranchia pfefferi</i>	10.1	1	38.0	1.950	271.0	–
<i>Megalocranchia fisheri</i>	6.59–47.9	3	0.0–105.9	0.0–43.7	333.9–556.9	–
<i>Nonammoniacal</i>						
Bathyteuthidae						
<i>Bathyteuthis abyssicola</i>	37.7	1	122.9	36.5	8.14	258.3
<i>Bathyteuthis berryi</i>	1.15	1	101.7	36.9	18.3	165.8

Measurements are for mantle muscle tissue unless otherwise stated. Unidentified cations found in bathyteuthid squids are presumed to be methylamines (MA⁺) and are quantified against a trimethylamine standard.

are based on fluid extracted from the coelomic chamber and so are comparable to previously published measurements (Clarke et al., 1979). Protein was measured spectrophotometrically according to Lowry et al. (1951) using bovine serum albumin as the standard. Minimum depth of occurrence (MDO), the depth below which 90% of the individuals of a given species were captured, was determined from net capture and submersible observation data (Roper and Young, 1975; Hunt, 1996; Seibel et al., 1997).

3. Results

3.1. Cation determinations

Cation concentrations for 11 species of pelagic squids are reported in Table 1. Ammonium was the dominant cation in all squids measured with the exception of *B. berryi* and *B. abyssicola*. Instead, both of these species contained large quantities of an unidentified cation (Fig. 1; Table 1). We believe the unidentified cation is actually that reported as ammonium in a previous study (Robison, 1989). That study reported 263 mM ammonium in tentacle muscle of *B. berryi*, while here, we report NH_4^+ concentrations ranging from 8 to 18 mM in bathyteuthid mantle muscle. The unidentified peak in our analysis had a retention time similar (within 0.4 min) to trimethylamine (TMA) standard (Fig. 2), but Seibel and Walsh (2002) found negligible concentrations of TMA in *B. berryi*, using the chemical method of Weckell and Barnett (1991). When compared to a TMA standard, the concentration of the unidentified peak ranged from 166 to 258 mM. A few other species also had large unidentified peaks (Fig. 1). *Mastigoteuthis inermis* had two such peaks whose retention times were not closely aligned with any of the chosen

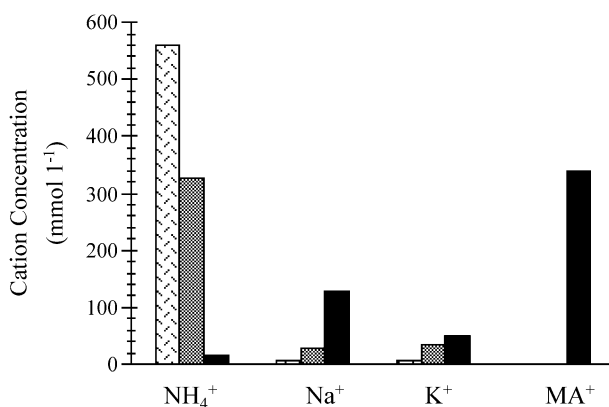


Fig. 1. The concentrations of ammonium, sodium, and potassium are shown for three species representing three different functional (and phylogenetic) groups of midwater squids. *Megalocranchia fisheri* (hatched bars) represents the family Cranchiidae, a unique family that sequesters ammonium fluid in a specialized coelomic chamber. *Histioteuthis heteropsis* (grey bars) sequesters ammonium fluid in vacuoles within the muscle tissue in the mantle and arms. *B. abyssicola* (filled bars), previously believed to sequester ammonium in its muscle tissue, has only low concentrations of ammonium. However, large concentrations of an unidentified compound (ΣMA^+) were found in mantle tissue of bathyteuthids.

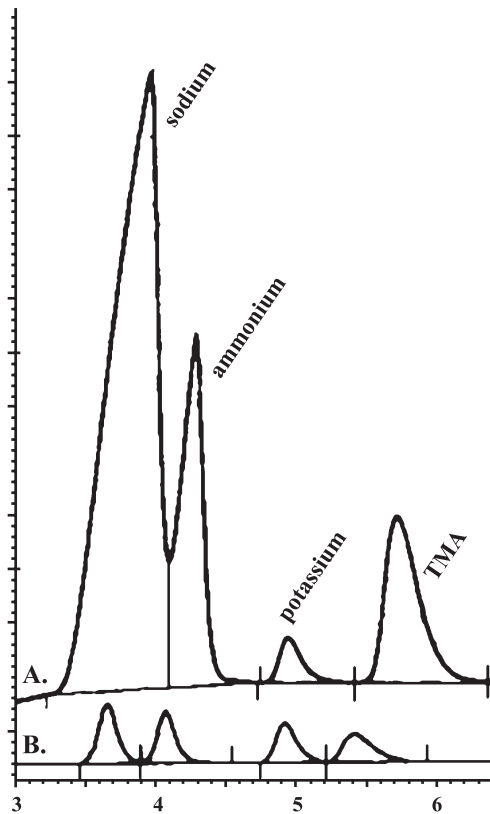


Fig. 2. *C. calyx*. HPLC traces showing the peaks and elution times for tail fluid (A) relative to 20 mM standards (B) of Na^+ , NH_4^+ , K^+ , and trimethylamine (TMA). Peaks correspond to values listed in Table 1. The TMA peak (A) is not positively identified but the elution time corresponds closely to that of the trimethylamine standard (B).

standards. *Histioteuthis hoylei* also had large quantities of some cation in addition to ammonium. Coelomic fluid from cranchiid squids contained only ammonium.

Histioteuthis heteropsis was the only species measured in sufficient numbers to detect trends across a range of body size. Ammonium concentration was inversely correlated with sodium concentration ($[\text{Na}^+] = 234 - 0.80[\text{NH}_4^+]$; $p < 0.01$; $r^2 = 0.78$; Fig. 3) and positively correlated with body mass (M ; $[\text{NH}_4^+] = 110.5 M^{0.23 \pm 0.08}$; $p = 0.03$; Fig. 4). Accordingly, sodium declined with increasing body mass ($[\text{Na}^+] = 165.6 M^{-0.53 \pm 0.13}$; $p < 0.01$).

3.2. Protein

Mean mantle muscle protein content (% wet mass, Table 2; Fig. 5) ranged from just 0.12% in the deep-living gelatinous octopod *Japetella heathi* to 25.88% in the epipelagic squid *Stenoteuthis oualaniensis*. Published protein values for shallow-living squids range from 14.5% to 20.9% (Lee, 1994). Protein content in mantle tissue of

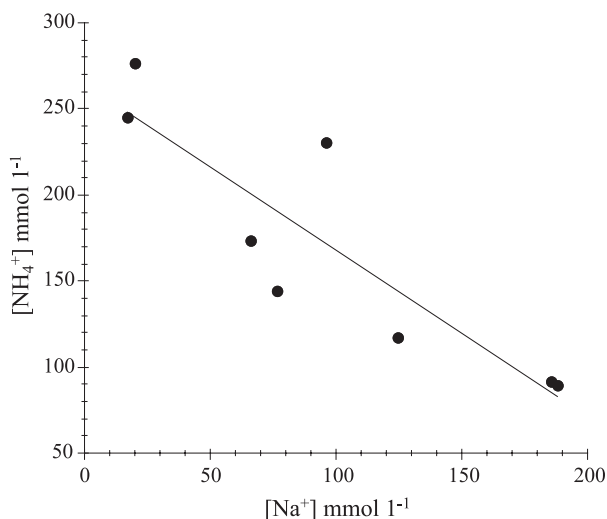


Fig. 3. *H. heteropsis*. Concentrations of mantle tissue ammonium are significantly inversely correlated with sodium concentration ($[\text{Na}^+] = 234 - 0.80[\text{NH}_4^+]$; $p < 0.01$; $r^2 = 0.78$).

squids previously categorized as “muscular” (Voight et al., 1994) ranged from 7.42% in one specimen of *Abraliopsis falco* to 25.88% for *S. oualaniensis*. Protein content in tissue-ammoniacal squids was significantly lower than in muscular squids ($p < 0.01$), ranging from 1.84% in *Grimalditeuthis bonplandi* to 6.27% in *Octopoteuthis deletron*. *Bathyteuthis berryi* had protein content on par with muscular squids (11.58%). With *B. berryi* excluded, a highly significant decline was observed in mantle muscle protein

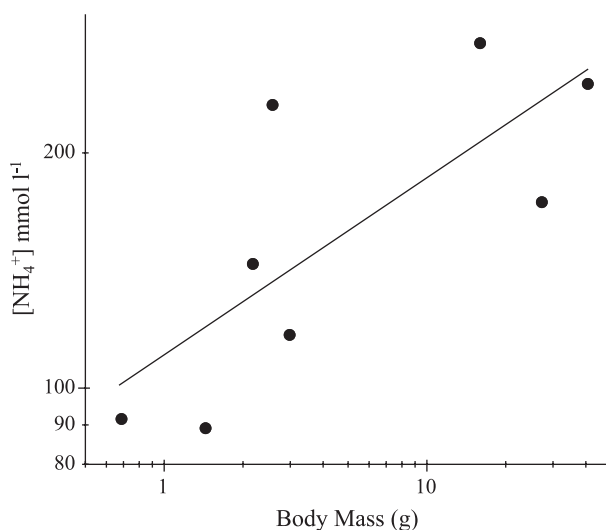


Fig. 4. *H. heteropsis*. Ammonium concentration increases as a function of body mass ($[\text{NH}_4^+] = 110.5M^{0.23 \pm 0.08}$; $p = 0.03$, $r^2 = 0.49$).

Table 2

Protein content (% wet mass) of pelagic cephalopods in relation to minimum depth of occurrence (MDO)

Species	<i>n</i>	MDO (m)	Mass (g)	Protein
<i>Tissue ammoniacal squids</i>				
Chiroteuthid clade				
<i>Chiroteuthis calyx</i>	4	300	38.4–70.1	5.52±0.82
(arm IV)	4			2.07±0.43
<i>Mastigoteuthis inermis</i>	2	375	4.06–7.32	3.82, 3.33
<i>Joubiniteuthis portieri</i>	1	500	44.9	2.90
<i>Grimalditeuthis bonplandi</i>	1	500	100	1.84
<i>Octopoteuthis deletron</i>	1	200	8.2	6.27
(fin)	1		9.28	
Histoteuthidae				
<i>Histoteuthis heteropsis</i>	2	300	0.5, 10.9	1.47, 2.43
<i>Muscular squids</i>				
Ommastrephidae				
<i>Stenoteuthis oualaniensis</i>	1	10	110	25.88
Gonatidae				
<i>Gonatus onyx</i>	2	100	2.5–6.1	11.42, 12.91
Enoploteuthidae				
<i>Abraliopsis falco</i>	2	50	0.5–1.1	7.42, 10.25
Bathyteuthidae				
<i>Bathyteuthis berryi</i>	1	800	1.7	11.58
<i>Octopod clade</i>				
Bolitaenidae				
<i>Japetella heathi</i>	2	700	33.1–54.7	0.39, 0.12
(arm I)	1	33.1	2.29	
<i>Eledonella pygmaea</i>	1	800	40	0.27
Vampyroteuthidae				
<i>Vampyroteuthis infernalis</i>	2	600	4.2–46.1	0.16, 0.32

Measurements are for mantle muscle unless otherwise stated.

content with increasing minimum depth of occurrence (MDO; $\text{protein} = 20e^{-0.005\text{MDO}}$; $p < 0.01$; $r^2 = 0.83$; Fig. 5). Note that this analysis treats each species as if they were phylogenetically independent, so the statistical significance is certainly exaggerated. However, a recent analysis using independent contrasts has confirmed a significant decline in cephalopod swimming capacity with increasing habitat depth as indicated by oxygen consumption rates and activities of metabolic enzymes (Seibel and Carlini, 2001). The protein contents in mantle muscle may be expected to follow this pattern suggesting that phylogeny is not the driver of this apparent pattern.

4. Discussion

The present analysis confirms the presence of large quantities of ammonium-rich fluid within the mantle musculature or coelom of most midwater cephalopod families (Table 1; see Voight et al., 1994 for review of previously measured values).

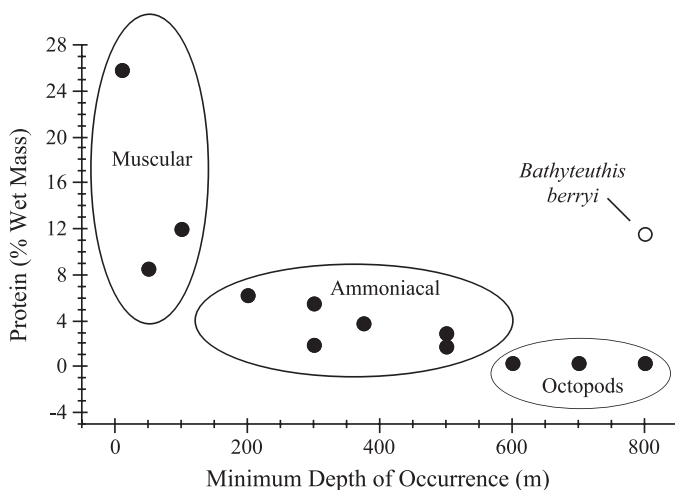


Fig. 5. Mean mantle muscle protein content in cephalopods (see Table 2) as a function of minimum depth of occurrence, the depth below which 90% of individuals of a given species were captured (protein = $20e^{-0.005MDO}$; $p < 0.01$; $r^2 = 0.83$, regression line not shown). This analysis treats each species as a phylogenetically independent data point, so the significance of the relationship is certainly exaggerated (Seibel and Carlini, 2001). *B. berryi* is represented by an open circle and not included in the regression analysis above. All others are shown as filled circles. Species with similar strategies for maintaining vertical position in the water column are grouped in ellipses. Muscular squids are generally negatively buoyant, although lift is provided by lipid accumulation in some species. Ammoniacal squids accumulate ammonium in the muscle tissue, and pelagic octopods have a high water content and exclude sulfate ions. These groupings generally follow phylogenetic relationships (Table 2).

Joubiniteuthis portieri, a member of the family Joubiniteuthidae within the chiroteuthid clade, was also found to contain large quantities of ammonium (217 mM) in its mantle muscle. Joubiniteuthid squids were previously suspected of containing ammonium based on anatomical observations, but no direct measurements had been made (Voight et al., 1994).

4.1. Bathyteuthids

Despite a report to the contrary (Robison, 1989), we did not find significant quantities of ammonium within the musculature of *B. berryi* or *B. abyssicola*. Rather, we found large concentrations of a presumably nitrogenous cation, likely a methylamine, in both species. These compounds may have been mistaken for ammonium using the methods employed previously (Clarke et al., 1979; Lipinsky and Turoboyski, 1983; Robison, 1989). The HPLC retention time for the compound corresponds closely to trimethylamine (within 0.4 min), and a variety of methylamines have been reported in cephalopods (Lin et al., 1983). However, Seibel and Walsh (2002) found only low TMA concentrations, although high quantities of TMAO were present in *B. abyssicola* mantle muscle. Several other genera possessed substantial concentrations of unidentified cations but in addition to the predominant ammonium ion.

We suspect that the unidentified compounds in bathyteuthid and other squids are located intracellularly, while only ammonium fluid is sequestered in specialized vacuoles.

We found only ammonium in the sequestered coelomic fluid of cranchiid squids. Furthermore, the muscle tissue of bathyteuthid squids does not appear to contain sufficient vacuolated tissue to sequester a cation in such high concentrations (Roper, 1969). An intracellular localization of methylamine compounds is significant because locomotory performance would not be compromised by muscle tissue displacement. The cations must then be compatible with macromolecular function. Some nitrogen-containing cations [e.g., tetramine ($\text{CH}_3)_4\text{N}^+$] are compatible intracellular solutes while others (e.g., TMA) are not (Hochachka and Somero, 2002).

The mantle tissue of bathyteuthids is thick and muscular compared to ammoniacal species (Roper, 1969). Protein was near 12% of wet mass in *B. berryi*, a value within the range found for shallower-living muscular squids (Table 2; Fig. 4). Protein content in locomotory muscle tissue of midwater animals generally corresponds closely to locomotory capacity (Childress and Nygaard, 1974; Bailey and Robison, 1986; Childress et al., 1990). It is interesting in this light that bathyteuthids have large oily digestive glands that may provide sufficient lift to attain neutral buoyancy (Denton and Gilpin-Brown, 1973). Thus, large ammonium concentrations may not have been selected for during the evolution of *Bathyteuthis* spp., and the unidentified cation is probably a metabolic by-product accumulated as a compatible osmolyte rather than as a buoyancy mechanism.

Seibel and Walsh (2002) suggested that phosphatidyl-choline hydrolysis required for lipid storage produces free choline that serves as a precursor for TMA/TMAO production. By a similar mechanism, the large oily digestive gland of bathyteuthids may lead to the accumulation of the novel compound observed here. Gonatid squids accumulate large digestive gland lipid stores that may also serve a buoyancy role (Clarke et al., 1979; Arkhipkin and Bjorke, 1999). However, in these species, retained lipids are used to fuel an extended period of egg brooding, while neutral buoyancy is a secondary benefit (Seibel et al., 2000b; Hunt and Seibel, 2000).

The well-developed musculature in bathyteuthid squids suggests the possibility of high locomotory capacity relative to ammoniacal squids. This is supported by the presence of giant nerve fibers innervating the mantle muscle and analysis of the statocyst that suggests the ability to move swiftly and turn rapidly in all planes (Young, 1989). Using a remotely operated vehicle (ROV Tiburon, Monterey Bay Aquarium Research Institute), we observed *B. berryi* on two separate occasions. Both times, it was hovering motionless near neutral buoyancy. However, as the vehicle approached, both specimens demonstrated rapid escape responses that prevented capture (for video footage see www.mbari.org/midwater/bathyteuthis). Many other midwater squids (e.g., Chiroteuthidae and Histioteuthidae) are also frequently encountered by submersibles but do not possess the capacity to evade capture (Vecchione et al., 1992; Hunt, 1996). The relatively strong locomotory abilities of bathyteuthids are surprising given their deep habitat depth (>800 m) and low metabolic rates (Seibel et al., 1997; 2000a). Nothing is known of the predatory habits of bathyteuthid squids.

4.2. Intraspecific variation in NH_4

Most midwater squids undergo an ontogenetic descent whereby successive developmental stages occupy progressively greater depths (Roper and Young, 1975; Hunt,

1996; Hunt and Seibel, 2000; Voight, 1995). In some cases, ontogenetic descent is accompanied by changes in ammonium distribution or concentration. For example, deep-living adults of *Chiroteuthis calyx* accumulate ammonium primarily in the enlarged ventral arms (Clarke et al., 1979) resulting in a head-up position in the water column that facilitates “fishing” but presumably limits mobility (Denton and Gilpin-Brown, 1973). The juveniles in shallower water, on the other hand, are thought to adopt a more horizontal position by virtue of concentrated ammonium in the elongate “neck” with greater density in the mantle and arms. As such, it is believed that the center of gravity and buoyancy is fairly close together in these doratopsis larvae such that the juveniles can easily adopt any position thus enhancing mobility (Denton and Gilpin-Brown, 1973). However, juveniles possess a long ornate tail that was not considered in these earlier analyses, probably because it is typically broken off and not recovered during net-capture. We show here that ammonium fluid is contained in vesicles in the tail tissue as well as in the fluid-filled pouches in the tail. Thus, some distance separates the centers of buoyancy and gravity, and the juveniles lack appreciable agility. Interestingly, the tail closely resembles the siphonophore *Nanomia bijuga*, which possesses powerful nematocysts and presumably offers protection from predation via mimicry (Vecchione et al., 1992).

In *H. heteropsis*, ontogenetic descent results in reductions in locomotory capacity and increased use of fins relative to jet propulsion (Seibel et al., 2000a). Accordingly, relative buoyancy also appears to increase with size in this species. Ammonium concentrations in mantle tissue are positively correlated with body mass in *H. heteropsis* (Fig. 3). This trend suggests a gradual accumulation of ammonium throughout the lifetime of the animal. This is consistent with the suggestion that ammoniacal buoyancy may be adopted only late in life in some species, possibly related to sexual maturation (Voight et al., 1994). However, Lipinsky and Turoboyski (1983) found ammonium to vary widely in *Histioteuthis macrohista* with no relationship to size.

Denton and Gilpin-Brown (1973) estimated that ammoniacal squids would have to retain 40% of their lifetime nitrogen production as ammonium in order to achieve neutral buoyancy. Clarke et al. (1979) point out that stored ammonium nitrogen must merely offset the weight of protein (the predominant sinking component in squids). For ammoniacal squids, this averages out to about 300 mM ammonium throughout the body (that is, more than half the body is 500 mM NH_4^+ fluid). Assuming an O:N ratio of 11, a value reported for fed cephalopods (Boucher-Rodoni and Mangold, 1989), a lifetime averaged metabolic rate of $0.14 \mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$ (twice the routine rate reported by Seibel et al., 1997, for starved specimens) and body averaged ammonium concentrations of 300 mM, then ammoniacal squids would require nearly a year to achieve neutral buoyancy in the absence of direct ammonium accumulation (consumption of other ammoniacal species or direct NH_4^+ uptake). This estimate is consistent with our measurements for *H. heteropsis* and with an ontogenetic descent to deeper water where energy savings via attainment of neutral buoyancy may become relatively more important.

4.3. Conclusions

A trade-off exists between locomotory capacity and energetic savings via attainment of neutral buoyancy that is clearly illustrated by comparison of bathyteuthid squids with other

ammoniacal species. The ammoniacal species rely on large volumes of ammonium-rich fluids stored in the extracellular space in mantle tissue, thus displacing space for muscle machinery. The bathyteuthids on the other hand have retained strong mantle muscles and relatively high locomotory capacity by accumulating lipid and compounds, including trimethylamine oxide (Seibel and Walsh, 2002) that provide lift as a component of the normal intracellular milieu. This trade-off is also exemplified by the ontogenetic changes in depth and ammonium concentration in *H. heteropsis*. As selection for strong locomotory capacity decreases with increasing depth in the ocean (Childress, 1995), the penalty for investing in buoyancy mechanisms is reduced (Childress and Nygaard, 1974). Thus, locomotory capacity is traded directly for neutral buoyancy.

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