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Respiration and nitrogen excretion by the squid *Loligo forbesi*

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Abstract

Respiration and nitrogen excretion rates of mature adult *Loligo forbesi* were investigated at the Roscoff Laboratory (North Brittany, France) during individual short-term incubation experiments in January 1986. The squids were in post-digestive condition and not actively swimming. Both oxygen uptake and nitrogen excretion are continuous processes. The metabolic rates of this active nektonic species (145 ml kg^{-1} h^{-1} oxygen uptake, 18.56 μg g^{-1} h^{-1} ammonia excretion) are distinctly higher than those of benthic cephalopods. Proteins constitute the main metabolic substrate for energetic needs. Besides ammonia, urea is also continuously released, in amounts ranging from 5 to 16% of ammonia-excretion values.

Introduction

Cephalopods are carnivorous animals, whose modes of life range from active pelagic squids to less-active benthic octopuses. Metabolic rates have been investigated for several species, and appear to be influenced by state of activity and feeding. In the squid *Loligo opalescens,* the oxygen consumption rate averages 254 ml O_2 kg^{-1} h^{-1} at rest (standard metabolic rate), and 862 ml O_2 kg^{-1} h^{-1} during swimming activity (O'Dor 1982). Likewise, in *Octopus vulgaris,* routine oxygen uptake (80 ml O_2 kg^{-1} h^{-1}) is 2.4 times lower than the active metabolic rate (Wells et al. 1983a). Both squids and octopuses are known to accumulate a low oxygen debt after effort (O'Dor 1982, Wells et al. 1983b). Feeding condition and digestion also affect the exchange rates. Regular food intake raises the respiratory rate by over 40% (Wells et al. 1983b), whereas starvation induces decreased oxygen consumption (Boucher-Rodoni and Mangold 1985, 1988).

Cephalopods are ammonotelic organisms, nitrogen end-products being eliminated mainly as soluble ammonia (Potts 1967). Urea has, however, been reported to represent another significant source of waste nitrogen in some species (Delaunay 1931, Hoeger et al. 1987). Like oxygen uptake, ammonia excretion is affected by activity and feeding (Boucher-Rodoni and Mangold 1985, 1988, Hoeger et al. 1987). A five-fold increase has been reported for the squid *Illex illecebrosus* during exercise (Hoeger et al. 1987). At the beginning of fasting, ammonia excretion is reduced, lipid being also used as a metabolic substrate in addition to protein (Boucher-Rodoni and Mangold 1985, 1988).

Loligo forbesi is an active nektonic predator which, like other teuthoids (Bradbury and Aldrich 1969, O'Dor 1982), often rests quietly on the bottom. We measured oxygen consumption and nitrogenous excretion of quiet individuals in post-digestive condition, i.e., the standard metabolic rates of this species.

Materials and methods

The experiments were carried out at the Station Biologique, Roscoff, France, in January 1986. *Loligo forbesi* were caught by squid-jigging and kept in a large outside tank (48 m^3), connected with the open sea at high tide. The captive squid could thus maintain their natural feeding habits. Each squid was transferred into a smaller rubber-walled indoor tank the evening preceding the first series of experiments and overnight between experiments. For recording, the squid was placed in a 63-litre experimental tank with a close-fitting top, and allowed to acclimate for 2 or 3 h during flushing with sea water. Short-term incubation experiments (50 to 60 min total duration) were started by shifting from open-circuit to closed-circuit water-circulation (Eheim pump, placed downstream of the oxygen probe), ensuring a satisfactory mixing of the sea water in the experimental tank. Oxygen tension and water temperature were recorded continuously (Orbisphere microprocessor oxygen indicator). At 10 min intervals, 30 ml of sea water were sampled for immediate analysis of ammonia (Technicon autoanalyz-

er, colorimetric indophenol method, Solórzano 1969) and urea (Technicon autoanalyzer, diacetyl-monoxime reaction, Aminot and Kerouel 1982). Five millilitres of sea water were deep-frozen for later determination of primary amines (fluorescamine method, Udenfriend et al. 1972). The temperature of the sea water ranged from 10° to 12.5 °C, but did not increase by more than 0.5 °C during the course of one incubation.

For long-term experiments (8 to 20 h), the circuit was kept open at a flow rate of 1 365 ml min^{-1}, allowing a complete renewal of the tank sea water in 4 h. The input water was oxygenated in an intermediary container. Oxygen consumption, ammonia and urea excretion were recorded continuously from the outflow. The temperature remained stable at 12.3 °C.

During incubation, the squids generally rested quietly at the bottom of the tank, but sometimes they floated or gently swam to and fro during part of the experimental period. They were either in post-digestive or in early fasting condition, i.e., fed for the last time the day preceding the first series of experiments. Experiments were ended when oxygen fell below 80% saturation. Our results, therefore, correspond to standard metabolism (weak activity and digestion completed).

Table 1. *Loligo forbesi.* Regression coefficients of oxygen consumption (ml g^{-1}), during 50 to 60 min closed-circuit experiments. Two incubations (I and II) were performed each day

Date (incubation)		Slope	*r*	*n*
Squid A (♀)				
Jan. 9	(I)	0.0024	0.998	6
	(II)	0.0027	0.991	4
10	(I)	0.0026	0.994	6
	(II)	0.0025	0.989	5
Squid B (♀)				
Jan. 11	(I)	0.0026	0.998	6
	(II)	0.0029	0.998	4
12	(I)	0.0023	0.999	7
	(II)	0.0021	0.999	6
Squid C (♂)				
Jan. 14	(I)	0.0022	0.996	5
	(II)	0.0026	0.995	5
15	(I)	0.0022	0.998	5
	(II)	0.0022	0.996	5

Results

Oxygen consumption

Oxygen consumption of two mature female *Loligo forbesi* (Squids A and B) and one mature male (Squid C) followed a linear mode during closed-circuit incubations over a total experimental duration of 50 to 60 min, as indicated by the highly significant linear regression and correlation coefficients in all experiments (Table 1). The slopes of the regression lines are not significantly different from each other; thus, the results from the three squids were pooled (Fig. 1). The mean oxygen-consumption rates are listed in Table 2.

Oxygen consumption did not appear to be significantly different in mature females and males. One of the females (Squid B) started to lay eggs 1.5 h after the end of the last incubation and, even in this prespawning individual, oxygen consumption was not dramatically affected (Table 2).

To ascertain the linearity of the metabolic response, oxygen consumption was recorded continuously for 20 h in an open-circuit experimental tank (Squid D). Fig. 2 shows that oxygen consumption, which was constant over short periods of time, underwent fluctuations related to variations in illu-

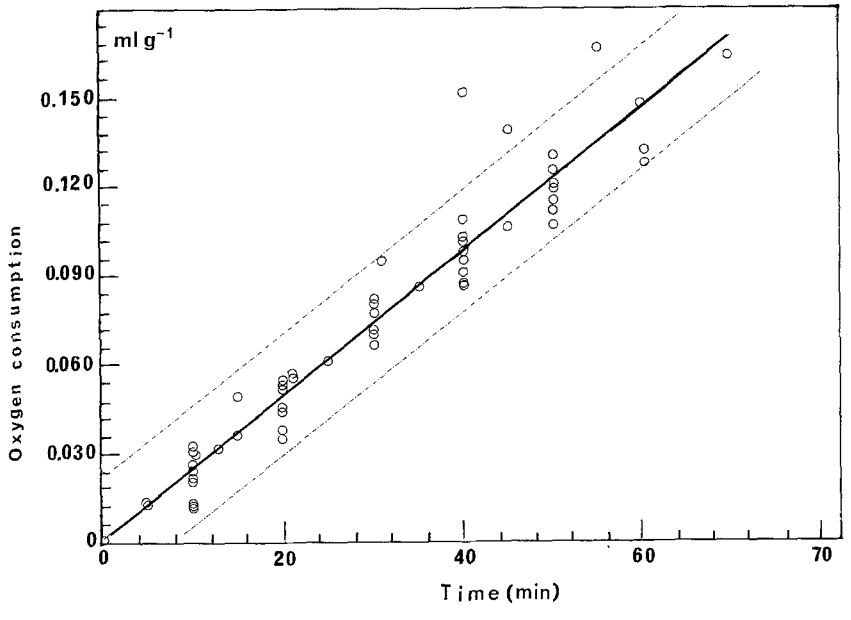

Fig. 1. *Loligo forbesi.* Oxygen consumption (ml g^{-1}) during short-term incubation (pooled results from Squids A, B, C; $r=0.976$, $n=80$, slope = 0.0024). Dashed lines show 95% confidence intervals around the mean

Table 2. *Loligo forbesi.* Mean oxygen uptake, ammonia and urea excretion rates for four experimental squids (one or two incubations per date). O:N ratio calculated as μmol g^{-1} h^{-1}. Squid D results drawn from continuous open-circuit recordings. Bd wt: body weight

(Bd wt) date	Oxygen		Ammonia		Urea		O:N
	(ml g^{-1} h^{-1})	*n*	(μg g^{-1} h^{-1})	*n*	(μg g^{-1} h^{-1})	*n*	
Squid A (♀)							
(738 g) Jan. 8 (a)*	0.175	1	14.4	1			15.7
9 (a)	0.149±0.003	6	20.55±1.89	5	2.64±0.15	5	9.7
9 (a)	0.169±0.022	4	17.67±2.12	4	2.73±0.50	4	10.7
10 (a)	0.140±0.007	6	12.78±1.66	5	2.76±0.11	6	12.9
10 (a)	0.169±0.009	5	14.01±0.83	3	3.11±0.10	5	15.4
Squid B (♀)							
(745 g) Jan. 11 (a)	0.171±0.005	6	30.91±1.21	5	2.88±0.14	6	6.8
11 (a)	0.133±0.016	4	21.90±2.03	4	2.63±0.22	4	6.9
12 (d)	0.135±0.002	7	19.26±0.85	6	3.29±0.12	7	8.7
12 (a)	0.135±0.004	6	20.48±0.79	6	4.00±0.10	5	8.1
Squid C (♂)							
(1 004 g) Jan. 14 (a)	0.154±0.004	5	21.03±0.97	5	1.96±0.05	5	9.4
14 (a)	0.117±0.013	5	12.02±0.25	5	1.95±0.03	5	13.5
15 (a)	0.156±0.010	5	18.68±0.94	5	1.79±0.03	5	10.1
15 (a)	0.158±0.007	5	14.26±0.37	5	1.51±0.15	4	13.8
Squid D (♂)							
(937 g) Jan. 25 (a)	0.121±0.003	11	11.28±0.30	12	1.83±0.09	12	13.4
25 (b)	0.171±0.003	12	22.60±0.84	5	2.17±0.21	5	9.4
25 (c)	0.104±0.003	8					
26 (d)	0.134±0.003	8					

* Afternoon (a), evening (b), night (c), morning (d) rates

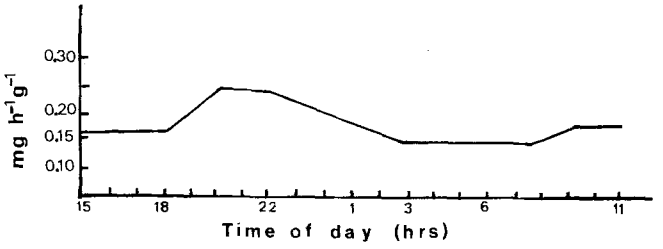

Fig. 2. *Loligo forbesi.* Oxygen consumption rates (mg g^{-1} h^{-1}) over 20 h continuous recording (Squid D)

mination. Transition from daylight to dim light, and from total darkness to dim light increased the rate of oxygen consumption. In darkness, the respiratory metabolism decreased (Fig. 2, Table 2). In the afternoon and in the morning, the level was similar to that of squid in short-term experiments (Table 2).

Excretion of ammonia, urea and primary amines

Ammonia

As in other cephalopod species, ammonia excretion in *Loligo forbesi* is a linear process (Fig. 3). The regression and correlation coefficients (Table 3) confirm that excretion was continuous over the experimental period. The values of the slopes of the regression lines, however, are not as constant as those for oxygen consumption, evidence that the excretion rates were variable, perhaps in relation to the feeding condition of the squid (from post-digestion at the beginning of the experimental series to early starving).

The atomic O:N ratio, i.e., oxygen consumption:ammonia excretion (Table 2), indicates that, as in other cephalopods (Boucher-Rodoni and Mangold 1985, 1988, Hoeger et al. 1987), the main energetic needs of *Loligo forbesi* are met by proteins.

Urea

Urea excretion rates were 5 to 16% as high as rates for ammonium, and urea was also excreted continuously (Fig. 3; Tables 2 and 3). Mature females excreted more urea than mature males (Table 2).

Primary amines

In some experiments, primary amines were excreted parallel to ammonia and urea (Table 3), whereas in others their concentration varied with no relation to ammonia and urea release. No conclusions can be drawn from these results.

Excretion of ammonia and of urea were continuously recorded over a period of 8 h, in open-circuit conditions, during the beginning of the 20 h oxygen consumption recording (Squid D). Fig. 4 confirms that ammonia and urea excretion were both continuous processes. The variations recorded were always parallel for both products (Fig. 4),

Table 3. *Loligo forbesi.* Regression coefficients of products of nitrogenous excretion ($\mu g\ g^{-1}$) during 50 to 60 min closed-circuit experiments

Date	Ammonia			Urea			Primary amines		
	slope	r	n	slope	r	n	slope	r	n
Squid A (♀)									
Jan. 9	0.333	0.975	7	0.049	0.996	7	no data	–	–
9	0.329	0.990	5	0.056	0.968	5	no data	–	–
10	0.232	0.976	6	0.041	0.998	7	0.024	0.934	7
10	0.216	0.996	3	0.056	0.999	6	−0.024	−0.599	5
Squid B (♀)									
Jan. 11	0.476	0.995	6	0.047	0.996	7	0.020	0.885	4
11	0.411	0.995	5	0.053	0.990	5	0.020	0.763	5
12	0.304	0.999	7	0.049	0.993	8	0.008	0.922	8
12	0.337	0.993	7	0.067	0.997	6	0.021	0.822	7
Squid C (♂)									
Jan. 14	0.323	0.997	6	0.033	0.998	6	0.018	0.839	6
14	0.206	0.998	6	0.033	0.999	6	0.014	0.763	4
15	0.282	0.997	6	0.031	0.982	6	0.009	0.605	6
15	0.227	0.995	6	0.031	0.982	6	0.006	0.612	5

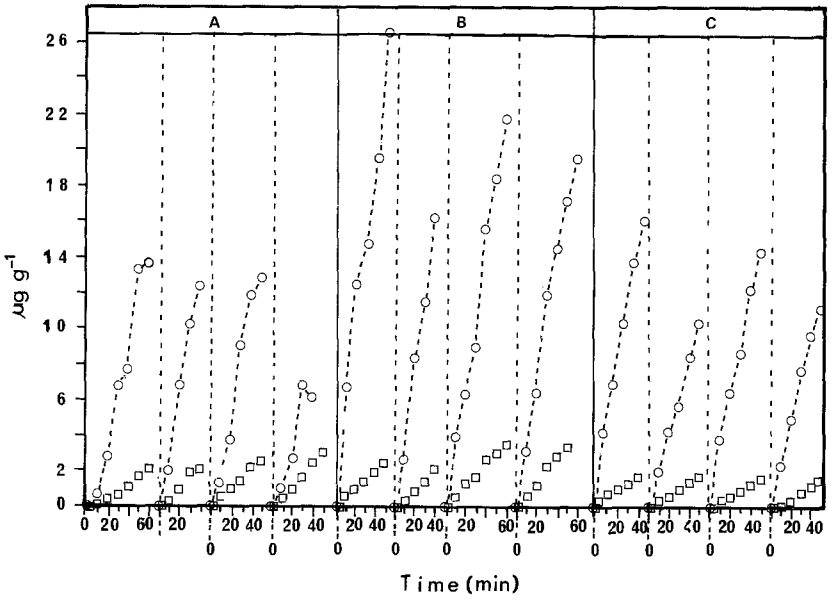

Fig. 3. *Loligo forbesi.* Ammonia (○) and urea (□) release ($\mu g\ g^{-1}$) during individual incubations of Squids A, B and C

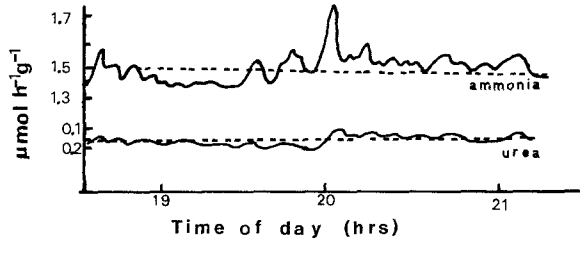

Fig. 4. *Loligo forbesi.* Ammonia and urea exretion rates (μmol $g^{-1}\ h^{-1}$) over 8 h continuous recording (Squid D)

with some irregular spikes of low amplitude coinciding for both ammonia (max. 0.2 to 0.3 μmol N-$NH_4\ g^{-1}\ h^{-1}$) and urea (max. 0.03 μmol N-urea $g^{-1}\ h^{-1}$).

In the late afternoon, ammonia and urea excretion rates averaged 1.5 and 0.14 μmol $g^{-1}\ h^{-1}$, respectively, increasing to 1.68 and 0.17 μmol $g^{-1}\ h^{-1}$ after 20.00 hrs. Similar to oxygen consumption, ammonia and urea excretion rates increased in dim light.

Discussion and conclusions

In the squid *Loligo forbesi,* both respiration and excretion are linear processes in quiet, non-digesting individuals. The O:N ratio, used as an indicator of the main substrate oxidized for energy in many marine invertebrates (Conover and Corner 1968, Regnault 1981, Stickle and Bayne 1982, O'Dor et al. 1984, Boucher-Rodoni and Mangold 1985, 1988, Hoeger et al. 1987), confirmed that metabolic needs are met mainly by protein in adults. Segawa and Hanlon (1988) reported oxygen consumption and ammonia excretion rates from young *L. forbesi* (hatchlings <45 d; 9.4 to 115.3 mg

body weight), and showed that lipids seem to be the major metabolite during the immediate post-hatching period, protein being used as substrate thereafter.

Respiratory metabolism was modified by activity and feeding state. At rest and during post-digestion, the oxygen consumption of *Loligo forbesi* (145 ml kg^{-1} h^{-1} at 10° to 12 °C) was distinctly higher than that reported for both *Octopus vulgaris* (60 to 80 ml kg^{-1} h^{-1} at 15 °C; Wells et al. 1983a, Boucher-Rodoni and Mangold 1985), and *Sepia officinalis* (85 ml kg^{-1} h^{-1} at 15 °C; Boucher-Rodoni unpublished results). In another nektonic species, *Illex illecebrosus,* the respiratory rate is 126 ml kg^{-1} h^{-1} (Hoeger et al. 1987), and even higher values (254 ml kg^{-1} h^{-1}) have been recorded for *Loligo opalescens* (O'Dor 1982). The difference in metabolic rates for the two *Loligo* species probably arises from the different size of the adults investigated: 26 to 64 g total body weight for *L. opalescens,* 738 to 1 004 g for *L. forbesi.*

Ammonia is the main nitrogen end-product in cephalopods. Its rate of excretion in *Loligo forbesi* (18.56 μg g^{-1} h^{-1}) is higher than in *Octopus vulgaris* (7.85 μg g^{-1} h^{-1}) and *Sepia officinalis* (4.5 μg g^{-1} h^{-1}, Boucher-Rodoni and Mangold 1988), but is close to that recorded for *Illex illecebrosus* (19.96 μg g^{-1} h^{-1}, Hoeger et al. 1987). Thus, both oxygen uptake and ammonia excretion rate appear to be higher in active nektonic than in benthic cephalopods.

Preliminary results concerning daily variations revealed that oxygen consumption and nitrogenous excretion of *Loligo forbesi* vary simultaneously. They seem to be subject to a circadian rhythm, respiration and excretion increasing at dawn and dusk. This rhythm is possibly related to the crepuscular habits of the species in the wild, which is maintained even when the squids are not fed during experimentation.

Besides ammonium, urea was also a significant excretion product in *Loligo forbesi,* comprising 6 to 16% of the ammonia excretion. A higher proportion (24%) of urea has been reported for *Illex illecebrosus* (Hoeger et al. 1987). Data on blood urea are somewhat contradictory in the literature, some authors claiming (von Fürth 1900, Emmanuel 1957), others disclaiming (Lindemann 1900, Delaunay 1931) its presence. In the urine of octopods, Delaunay reported urea to comprise 2 to 5% and ammonia 50% of the total non-protein nitrogen, whereas Emmanuel and Martin (1956) recorded a value of 73% for ammonia.

The linearity of the response for ammonia and urea excretion in *Loligo forbesi* implies that continuous diffusion occurs through the gills. The variations observed in the ammonia and urea water concentration during the 8 h excretion-rates recording might be the result of excretory activity of the renal sacs. However, their amplitude was low, and they more probably reflect irregularities in the mixing of the sea water in the laboratory tanks. Ammonia and urea release by the renal sacs is possibly associated with a more pronounced muscular activity: a marked increase in ammonia and urea concentration was recorded in the sea water during handling of the squid, probably as a result of strong contraction of their mantle muscles.

Thus, the essential part of ammonia excretion takes place by diffusion through the gills. In fish, the amount of ammonia excreted by the kidneys is very small compared to that eliminated through the gills (Forster and Goldstein 1969), even when the urine is acidic (which is also the case in cephalopods). Accepting this as valid for ammonia, it still remains an open question as far as urea is concerned. Both ammonia and urea have a similar coefficient of diffusion; however, the former passes through biological membranes faster than does urea. Goldstein et al. (1964) showed for the fish *Myocephalus scorpius* that more than two-thirds of the blood ammonia was lost during branchial circulation, whereas no detectable decrease in urea concentration could be discerned. There are two possible explanations for continuous urea excretion in squid: either the gill epithelium of squid is more permeable to urea than that of fish, or the urea is leaking passively and continuously through the renal sacs. In *Illex illecebrosus,* Hoeger et al. (1987) reported continuous urea and ammonia excretion during periods of rest, but during exercise urea excretion was much more variable; these results support the hypothesis that urea is accumulated in the renal sacs and is lost continuously during resting periods, while muscular activity induces periodical marked voiding of the sacs.

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