NITROGENOUS WASTE EXCRETION IN THE INTERTIDAL ROCK GUNNEL (PHOLIS GUNNELLUS L.): THE EFFECTS OF EMERSION

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The rock gunnel (Pholis gunnellus Linnaeus) is an intertidal inhabitant on the rocky shores of the northern Atlantic, mainly from Cape Cod and farther North. It is found under rocks and debris at the low tide line, and is exposed to the air during the tidal cycle. Like other intertidal teleosts, Pholis exhibits adaptations that allow it to survive exposure to the air, including a mucus-covered skin, and strong pectoral fins which allow it to prop itself up and wriggle over the substrate while emersed (personal observations; see also Davenport and Sayer, Comp. Biochem. Physiol. 84A:189-194, 1986). Many of these intertidal species can tolerate at least 24 hours of emersion and some, up to 5 days in a humid environment (Davenport and Sayer, op. cit.).

Nitrogenous waste excretion in immersed aquatic teleosts occurs across the gills, with ammonia being the predominant form, but with urea playing varying roles depending on the species (Davenport and Sayer, op. cit.). Upon emersion several strategies may be used. Upon longer term emersion, nitrogen excretion shifts from ammonotelism to ureotelism as a means of water conservation (e.g. Gordon et al., J. Exp. Biol. 72:57-75, 1978). During shorter term emersion, some authors suggest that nitrogen excretion ceases (Gordon et al., op. cit.), while others suggest that excretion continues, albeit at a reduced rate (Davenport and Sayer, op. cit.). In the latter case, if waste nitrogen production continues at the same rate, then nitrogen should accumulate and be eliminated later after reimmersion into sea water. In order to determine which of these several teleostean patterns of nitrogen excretion is used by Pholis, we examined ammonia and urea excretion in sea water for 24 hours before and after a 24 hour emersion period.

Pholis was collected during the summers of 1986 and 1987 at low tide line on the shores of Frenchman Bay, Mount Desert Island, ME. Fish (weighing 3 to 15g.) were maintained in running sea water at 15° C. and starved for several days prior to the experiments to standardize nitrogen excretion. for the control periods of nitrogen excretion, fish were placed in 1 liter plastic aquaria in 200 ml of fresh aerated sea water (SW1). 3 ml samples of the sea-water bath were removed at the intervals indicated, and immediately frozen for analysis. The fish were emersed after 24 hours, by placing them in similar aquaria with seawater-saturated Kimwipes on the bottom. After 24 hours, the fish were reimmersed in 200 ml of sea water for 24 hours (SW2) and seawater samples were removed as for the control. Ammonia and urea in the sea water were determined as previously reported (Kormanik and Evans, J. Exp. Biol. 125:173-179, 1986), and excretion rates were calculated. In a second series of experiments, blood concentrations of several constituents were determined after 24 hour emersion, and at the end of a 24 hour post-emersion control (SW2). Fish were anesthetized on ice and blood collected in microhematocrit tubes by severance of the tail. Due to the small size of the fish, only hematocrit and one or two assays could be performed on any sample. Blood data therefore represent several groups of fish sampled at different times. Na^+ and K^+ were determined by flame photometry. Other assays were performed as previously described. All data are presented as mean $\pm$ standard error, n =

Table 1. Ammonia and urea excretion in Pholis gunnellus before and after 24 hour emersion. Efflux values are expressed in umole $100g^{-1}$ h^{-1}, over the time period indicated. Values for SW2 were compared to those of SW1.

Ammonia efflux (n = 9)

Time (hour)	0–1	1–2	2–3	3–6	6–24
SW1	25.2 ± 8.8	28.1 ± 7.5	30.8 ± 6.4	24.0 ± 4.4	16.7 ± 3.9
	---------- 24 hour emersion ----------				
SW2	109** ± 3	74.4** ± 1.5	69.4** ± 11.6	47.2** ± 3.9	$29.5^{n.s.}$ ± 6.6

Urea efflux

Time (hour)	0–3	3–6	6–24
SW1	2.46 ± 1.33	2.64 ± 1.38	3.19 ± 1.55
	---------- 24 hour emersion ----------		
SW2	6.47* ± 1.64	$4.19^{n.s.}$ ± 1.65	10.5** ± 2.4

* – $p < 0.05$
** – $p < 0.01$
n.s. – $p > 0.05$

number of fish. Statistical comparisons were one-tailed, using Student's t-test for unpaired data.

The results of the ammonia and urea excretion experiments are presented in Table 1. Control ammonia excretion rates were typical for aquatic teleosts, and declined slightly with time. Control urea excretion rates are about 10% of ammonia rates on a molar basis, and therefore represent about 20% of total nitrogen excretion, again fairly typical of ammonotelic aquatic teleosts (Davenport and Sayer, op. cit.). After 24 hour emersion and reimmersion in sea water, ammonia excretion rates were over four-fold higher than pre-emersion levels. Rates then decreased over the 24 hour period and at the end, while still elevated, were not significantly different from those of the pre-emersion controls. Urea excretion rates after emersion were also several fold higher than the controls, and did not show a decline to control levels after the 24 hour emersion period, but rather remained elevated even after 24 hours in SW2.

Table 2. Blood concentrations in Pholis gunnellus before and after 24 hours of emersion (see text for details). All data are compared to the controls, with significance levels the same as for Table 1. N = 2 to 5.

	Controls	after 24 hour emersion	SW2
Urea (mM)	8.6 ± 2.7	14.7* ± 1.7	8.9 n.s. ± 3.1
Na^+ (mM)	182 ± 3	201 * ± 4	181 n.s. ± 0
K^+ (mM)	11.6 ± 0.6	11.7 n.s. ± 1.1	8.1 n.s. ± 1.9
H'crit (%)	53.2 ± 0.9	56.4 n.s. ± 2.7	49.5 n.s. ± 2.8
Ammonia	470 ±150	560 n.s. ±300	---

Blood data are reported in Table 2. Blood ammonia concentrations we determined are few in number; it was difficult to get enough blood for our assay from these small fish. Blood ammonia appeared to increase slightly in the post-emersion fish compared to the controls, but the difference was not significant. Blood urea increased by 67% at the end of emersion compared to the concentration in the pre-emersion controls. At the end of the SW2 period, blood urea was back to normal, in spite of the still increased excretion rates (Table 1). Na^+ increased slightly in the post-emersion samples, but then returned to pre-emersion levels at the end of the SW2 period. Both K^+ and the hematocrit showed no increase. K^+ appears somewhat elevated in all samples (Table 2) which may have resulted from tissue fluid contamination as a result of this method of collection.

These data are instructive on several points. Firstly, urea (and possibly ammonia) accumulates in the tissues during air exposure. While hematocrit and blood Na^+ increase 6 and 10% respectively, presumably as a result of dehydration, the 67% increase in blood urea would suggest that in Pholis, nitrogen excretion is reduced during emersion and at least urea is retained. Both urea and ammonia excretion are enhanced after emersion, and since excretion rates for both in the SW2 period are at least twice that of the SW1 period, all of the ammonia and urea accumulating in the emersion period could be eliminated within 24 hours after reimmersion in sea water. However, while blood urea returned to normal after 24 hours in sea water (SW2), urea excretion was still elevated, suggesting that not all of accumulated nitrogen was eliminated after this prolonged emersion period. Thus Pholis gunnellus, unlike Blennius pholis (Davenport and Sayer, op. cit.) resembles other intertidal teleosts by storing nitrogenous wastes during emersion and then eliminating them after reimmersion in sea water with a burst of excretion.
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