

# THE INFLUENCE OF AMBIENT TEMPERATURE ON THE FUNCTION OF THE NASAL SALT GLAND AND THE COMPOSITION OF CLOACAL FLUID IN THE PENGUIN *SPHENISCUS DEMERSUS*\*

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## ABSTRACT

The lateral nasal (salt-secreting) glands of *S. demersus* were found to be morphologically and histologically similar to the salt glands of marine birds in general. No significant differences were recorded in the ionic and osmotic composition of nasal and cloacal fluid that was collected at 18°C and 28°C respectively. The daily intake of 220 g of fish per bird, however, had a significant influence on the diurnal variation of the potassium values of the cloacal fluid. A very low flow-rate and high osmotic concentration of the nasal gland exudate was recorded, indicating the efficiency of the nasal glands as auxiliary osmoregulatory organs.

## INTRODUCTION

Since the discovery that the lateral nasal glands of some birds contribute to the excretion of inorganic ions (Schmidt-Nielsen, Jörgensen and Osaki 1958) only limited observations on the function of the salt glands of penguins have been made (Schmidt-Nielsen and Sladen 1958; McFarland 1959, 1960). Moreover, no work in this field has been done on *Spheniscus demersus*, the Cape or jackass penguin. Studies on the function of the salt gland under stress have been carried out on a number of other species, either by administering sodium chloride or acclimating birds to different concentrations of sea water (McLelland and Pickering 1969; Schmidt-Nielsen and Sladen 1958; Schmidt-Nielsen and Kim 1964; Holmes, Butler and Phillips 1961; Ellis, Goetemiller, De Lellis and Kablotsky 1963; Benson and Phillips 1964; Hughes 1970). No attempt has yet been made to study the effect of a rise in ambient temperature on the osmoregulation of a bird with a functional salt gland. One of the aims of this investigation was to obtain some information on this aspect. *S. demersus* was chosen as an experimental animal for it has no access to fresh water and can stay at sea for long periods. Also, its diet includes marine invertebrates (Rand 1960; McLean 1966) iso-osmotic to sea water, and in our laboratories birds have subsisted on a diet consisting exclusively of 220 g fish (*Sardinops ocellata*) per day, without losing weight or showing any ill-effects. In spite of this the birds produce a very watery, muciferous excreta, suggesting a highly efficient salt gland function.

## PROCEDURE

Salt glands were removed from six penguins, fixed in alcohol or formol-saline, sectioned at 3, 5 and 8  $\mu$  and stained either with Azocarmine and Azan or with Hematoxylin and Eosin.

Base-line values for osmolality and ionic content of blood plasma and cloacal fluid were determined on samples taken from nine normally hydrated animals shot at sea. The state of hydration of the birds resting or breeding on the islands is dependent on the time they have spent on land and this could not be determined easily.

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In order to study the effect of temperature upon the composition of cloacal fluid and nasal gland exudate, six mature birds were captured at random on Marcus Island, Saldanha Bay. The animals were housed in a temperature controlled room for two weeks to accustom them to their new environment. After this initial familiarization period the birds were exposed to a temperature of 18°C and 90% r.h. for four days, and thereafter to an ambient temperature of 20°C and 60% r.h. for a further four days. Each bird received 220 g of fish (*Sardinops ocellata*) daily at 1900h.

The frozen fish was thawed in sea water for one hour prior to feeding. The birds were kept in separate, plastic-coated, wire cages over stainless-steel trays filled to a depth of 1,5 cm with light mineral oil. This prevented evaporation of the cloacal fluid. Samples of cloacal and nasal fluid were collected twice daily, at 0800h and at 1830h, immediately before feeding time. After being fed, the birds were thoroughly washed down with tap water to remove all traces of fish, taking special care to clean the beak and external nares.

The nasal gland exudate was collected by inserting microcapillary tubes into the external nares as deeply as possible. The tubes were then sealed with plastic material, frozen and stored for later analysis.

Osmolalities were determined by the method of Gross (1954) after the tubes had been centrifuged at 500 r.p.m. for five minutes in a small clinical centrifuge.

TABLE 1

MEAN BODY AND SALT GLAND MASS (BOTH GLANDS) OF *S. demersus*

<i>n</i>	Mean body mass (kg)	Standard deviation	Mean salt gland mass (g)	Standard deviation
9	2,56	±0,938	1,14	±0,23

## RESULTS AND DISCUSSION

*Histology of the salt gland*

The mean weight of both salt glands sampled from nine birds was 1,154 g (mean body weight 2,55 kg—Table 1). The main ducts which open dorsally into the nasal cavity immediately posterior to the external nares, originate as secondary and tertiary ducts within the glands. The secondary ducts drain the individual lobes of which 12–16 can be seen in cross-section of the gland (Figure 1). This arrangement differs from reports on the herring gull which has two ducts serving each gland (Technau 1936) but is identical to the situation in the black gull (Doyle 1960) and *Gallus* (McLelland, Moorehouse and Pickering 1968). The tertiary ducts (diameter 22–80 $\mu$ ) are lined by 6–14 wedge-shaped, strongly striated secretory cells, with granular cyto-

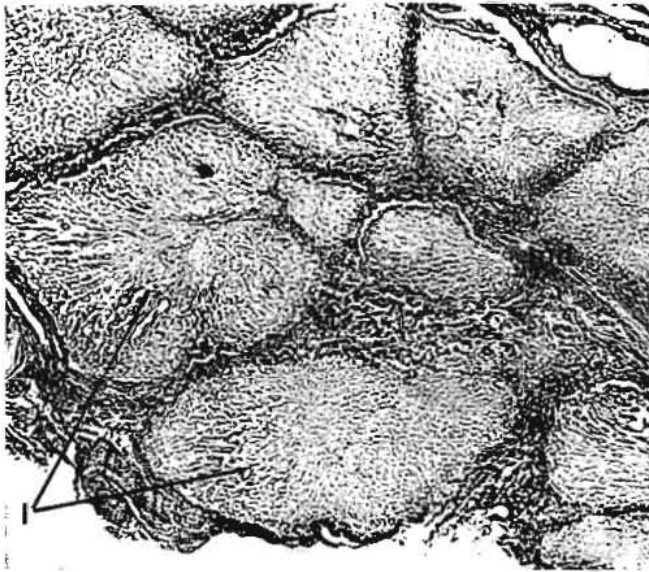


FIGURE 1

Section of salt gland illustrating single median duct (md) and lobes (l) ( $\times 400$ )

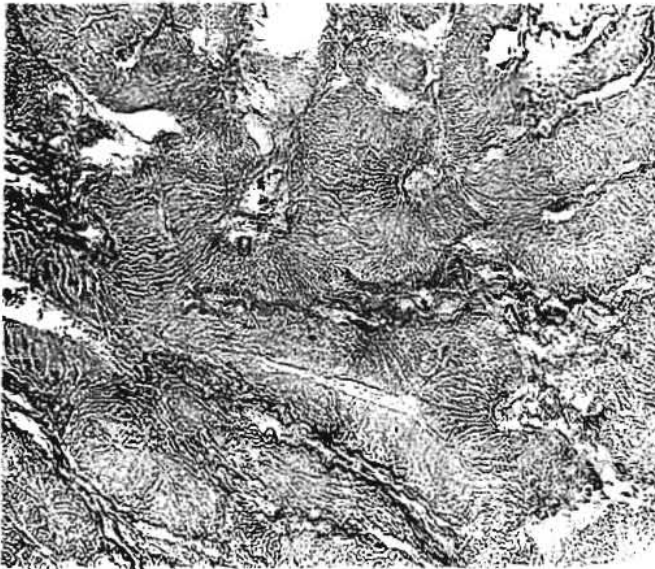


FIGURE 2

Section of salt gland illustrating striations (s) and granular plasma (g) of secretory cells. ( $\times 1000$ )



FIGURE 3

Salt gland: Positive reaction for adrenalin (a) in the connective tissue sheath (C) of the salt gland (Sg). ( $\times 400$ )

plasm (Figure 2). The striations and granularity of the cytoplasm is more strongly developed in the centrally situated secretory cells than in those on the periphery of the lobes. The striations have been shown to be deep infoldings of the cell membrane creating epicytoplasmatic spaces and thus enlarging the secretory area of the cells considerably (Doyle 1960; McLelland, Moorehouse and Pickering 1968). The granularity of the cytoplasm is due to concentration of mitochondria. The eosinophilia of the central regions of the lobes was very striking and is indicative of a large amount of cytoplasm in the cells of these areas. This was also observed by Scothorne (1959) in the domestic duck and suggests a higher rate of secretion by the cells in the central regions of the lobes.

The salt glands are encapsulated by a connective tissue sheath which penetrates the lobes in the form of trabeculae and surrounds the individual lobes as well as the central duct (Figure 1). Contrary to reports that no smooth muscle is present in the connective sheaths around the salt glands of the herring gull, I have found smooth muscle cells in the periphery of the sheath of *S. demersus*.

Staining for adrenergic and noradrenergic fibres revealed the presence of adrenergic fibres in the connective tissue sheath and these coincided closely with the areas where smooth muscle cells were present (Figure 3). These muscle fibres may be responsible for altering the pressure within the salt glands, thereby facilitating the expulsion of the contents of the secretory tubules, and may also act as a regulatory mechanism in the flow of blood through the gland.

Apart from the presence of muscle fibres and the adrenergic innervation of the periphery of the connective tissue sheath, the salt gland of *S. demersus* is almost identical to that of the herring gull (Fänge, Schmidt-Nielsen and Osaki 1958).

TABLE 2

OSMOLALITY AND ELECTROLYTE CONCENTRATION OF PLASMA, CLOACAL FLUID AND URINE OF *S. demersus*. PLASMA AND CLOACAL FLUID FROM BIRDS CAPTURED UNDER NATURAL CONDITIONS, URINE FROM CATHETERIZED URETERS COLLECTED UNDER ANAESTHESIA

			<i>n</i>	<i>Osmotic conc.</i> <i>mOsm/l</i>	<i>S.D.</i>	<i>Cl</i> <sup>-</sup> <i>mEq/l</i>	<i>S.D.</i>	<i>Na</i> <sup>+</sup> <i>mEq/l</i>	<i>S.D.</i>	<i>K</i> <sup>+</sup> <i>mEq/l</i>	<i>S.D.</i>
Plasma	..	..	9	298	± 31	106	± 7,0	153	± 6	8,8	± 2,8
Cloacal fluid	..	..	9	679	± 83	75	± 34,0	89	± 21	53,0	± 17,0
Urine	..	..	12	651	± 137	110	± 56,5	40	± 20	41,0	± 25,0

TABLE 3

COMPOSITION OF THE NASAL FLUID OF *S. demersus*, COLLECTED AT DIFFERENT AMBIENT TEMPERATURES (DAYS 1-4 AT 18°C, DAYS 5-8 AT 28°C) AND TIMES (0800H AND 1830H). MEAN VALUES ( $\bar{X}$ ), WITH STANDARD DEVIATIONS FROM THE MEAN (S.D.)

Days		Osmolality (mOsm/l)		Cl <sup>-</sup> (mEq/l)		Na <sup>+</sup> (mEq/l)		K <sup>+</sup> (mEq/l)	
		$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.
<i>Low temperature (18°C)</i>									
1	1830h	1408	± 105	1091	± 965	1072	± 691	42	± 26
2	0800h	1198	± 85	748	± 263	701	± 183	25	± 7
	1830h	All samples lost							
3	0800h	2409	± 1253	3196	± 2272	3920	± 3044	94	± 59
	1830h	831	± 69	472	± 109	920	± 243	43	± 24
4	0800h	1549	± 327	659	± 338	1550	± 264	54	± 14
	1830h	766	± 136	612	± 292	803	± 407	36	± 26
<i>High temperature (28°C)</i>									
5	0800h	1233	± 86	814	± 505	1955	± 900	34	± 21
	1830h	1264	± 132	783	± 314	897	± 133	23	± 8
6	0800h	1677	± 879	876	± 189	1279	± 899	37	± 18
	1830h	1533	± 1091	716	± 208	1220	± 717	31	± 27
7	0800h	1666	± 1143	854	± 342	1459	± 695	25	± 11
	1830h	1172	± 366	700	± 212	700	± 212	19	± 11
8	0800h	1154	± 41	755	± 243	996	± 95	28	± 8
	1830h	1262	± 29	721	± 225	1135	± 374	22	± 13

*The influence of ambient temperature on the function of the salt gland and on general water metabolism*

The range in electrolyte content of the cloacal fluid sampled from nine birds shot at sea was: sodium 60-131mEq/l, potassium 25-69mEq/l and chloride 36-144mEq/l. If it is accepted that these birds were fully hydrated and the osmotic concentration (549-748mOsm/l) of the cloacal fluid is compared to similar values reported for *Gallus* (115mOsm/l), *Melosittacus* (236mOsm/l) (Skadhauge 1972) and *Struthio* (±150mOsm/l) Louw, Belonje and Coetzee 1969) these values appear to be relatively high. This could have resulted from the reabsorption of water from the lower intestinal tract as recent research has established the existence of such a mechanism (Skadhauge 1972). Urine from cannulated ureters, however, showed equally high values (Table 2). The osmotic content was 332-865mOsm/l and the electrolyte content was: sodium 12-65mEq/l, potassium 10-80mEq/l and chloride 39-203mEq/l (n=12). It therefore seems as

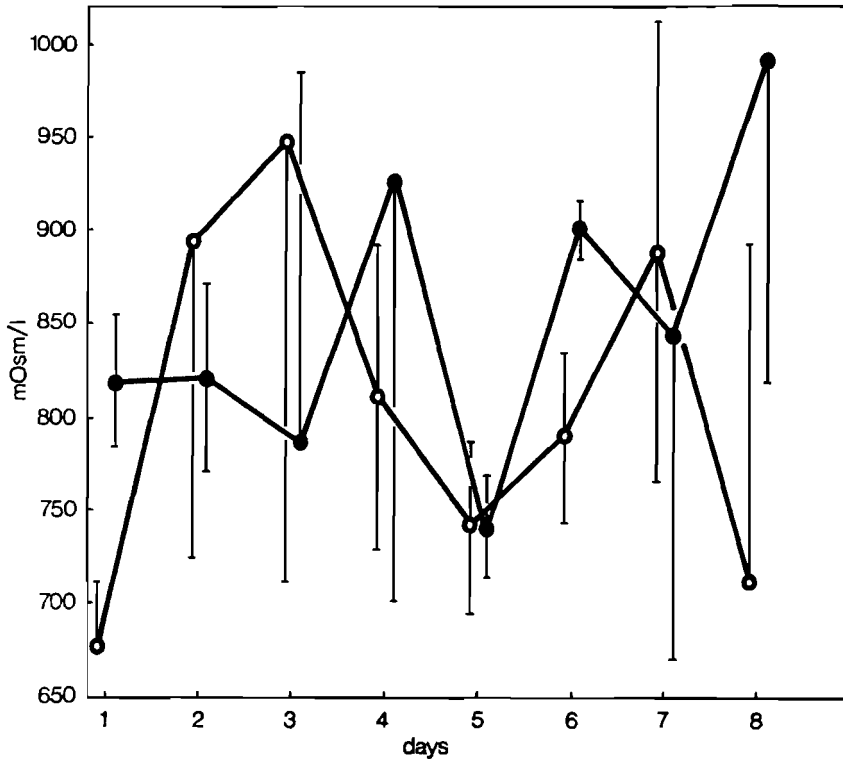


FIGURE 4

Osmotic composition of the cloacal fluid of *S. demersus* collected at different ambient temperatures and times.

Days 1-4 at 18°C, days 5-8 at 28°C.

- 0800h ± 12 hours after birds were fed
- 1830h immediately before birds were fed

if these high values are not due to extrarenal reabsorption of water but are a result of highly efficient renal function. Electrolyte contamination from faecal material is likely to be minor, as is reported for the kittiwake (Hughes 1970). Nevertheless, it is clear that the kidneys of *S. demersus* cannot alone ensure a favourable water balance as these birds have no access to fresh water. An extrarenal pathway for the excretion of excess electrolytes via the salt glands, especially under stress conditions, would seem to be needed.

In the present investigation it was, however, found that a 10°C rise in temperature and a 30% drop in relative humidity had no significant effect on the osmolality or the sodium, chloride and potassium concentrations of the salt gland exudate (Table 3).

The flow rate of salt gland excretion remained extremely low even after the temperature had been raised and it was often very difficult to obtain a sample. It is therefore possible that the excretion rate of the avian salt gland may have been over-estimated in the past as the rate of

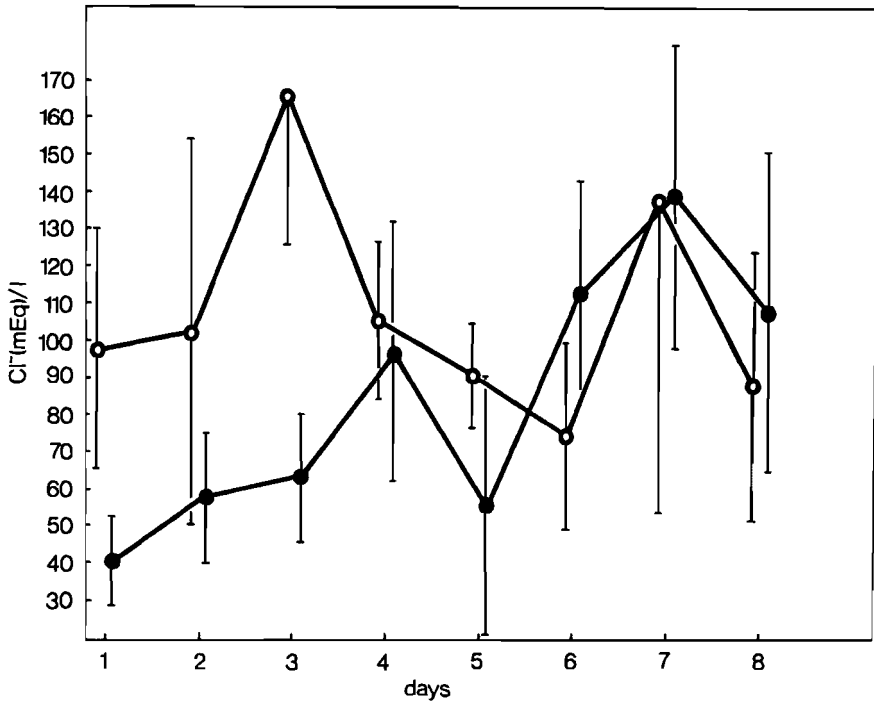


FIGURE 5

Chloride values of the cloacal fluid of *S. demersus* collected at different ambient temperatures and times. Day 1-4 at 18°C, days 5-8 at 28°C.

○ 0800h (12 hours after birds were fed)  
 ● 1830h (immediately before birds were fed.) } 10-11-1960  
 (14h) (12 hours)

secretion and general function has been studied almost exclusively under severe conditions of stress induced by sodium chloride loading or by acclimating animals to drinking progressively higher concentrations of sea water.

Examination of the data contained in Figures 4-7 shows that the rise in temperature had no effect upon the cloacal fluid concentrations, but that the time of sample collection had a significant effect on the concentrations of potassium (Figure 7) and to a lesser extent also on sodium and chloride concentrations (Figures 5 and 6). No effect was, however, detected on the osmolality of the cloacal fluid (Figure 4). This effect is probably due to electrolyte loading during feeding. In general the values obtained for nasal fluid concentrations (657-995mOsm/l) closely approximate the values reported for the salt gland exudate of the frigate bird, Laysian and black-footed albatrosses and are higher than those recorded for the king and emperor penguins by McFarland (1960).

The above results, however, may be due to the sampling technique as the nasal fluid was collected in the external nares where evaporation could have caused considerable concentration



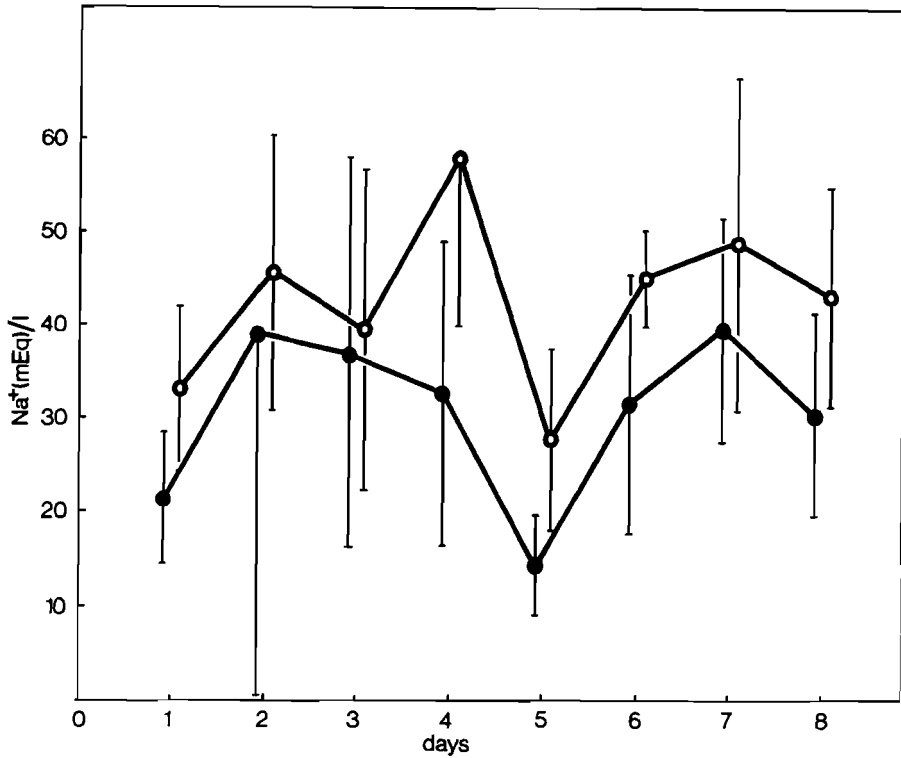


FIGURE 6

Sodium values of the cloacal fluid of *S. demersus* collected at different ambient temperatures and times. Days 1-4 at 18°C, days 5-8 at 28°C.

- 0800h ± 12 hours after birds were fed
- 1830h immediately before birds were fed

of the secretion. This was, however, unavoidable without surgical interference, which in turn may have affected the cloacal fluid composition through stress.

From these results it would appear that a 10°C rise in temperature and a 30% drop in relative humidity had no significant effect on the osmolality or sodium, potassium and chloride concentrations of both cloacal and nasal fluid, indicating that either the heat stress was not sufficient to induce water loss through thermal panting or that the individual variation and small sample size did not allow differences to be detected. It is, however, clear that the salt glands of *S. demersus* excrete mainly sodium chloride and that the potassium loading through feeding was excreted almost exclusively by the kidneys. *S. demersus* in this way differs from some lizards like *Iguana iguana*, *Diplosaurus dorsalis* and *Dromastus aegyptus*, where the potassium content of the salt gland secretion exceeds the sodium content. The electrolyte composition of the nasal salt gland exudate in *S. demersus* is, however, similar to that usually observed in marine birds (Schmidt-Nielsen, Borut, Lee and Crawford 1963).

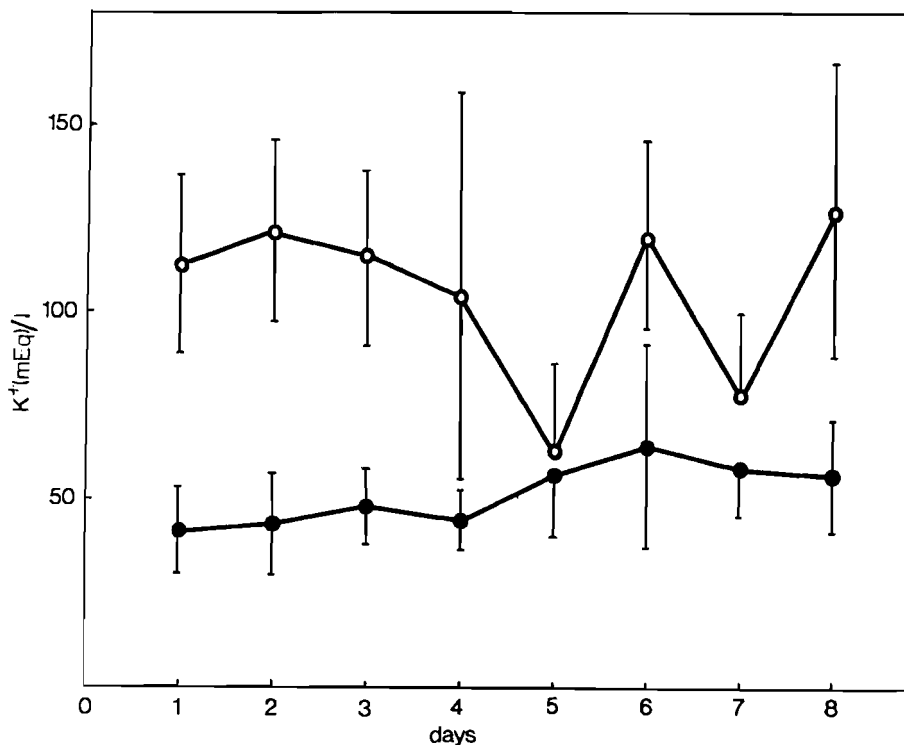


FIGURE 7

Potassium values for the cloacal fluid of *S. demersus* collected at different ambient temperatures and times. Day 1-4 at 18°C, days 5-8 at 28°C.

- 0800h ± 12 hours after birds were fed
- 1830h immediately before birds were fed

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