Marine Biology 23, 121—127 (1973)

Hypo-osmotic Regulation Coupled with Reduced Metabolic Urea in the Dogfish *Poroderma africanum*: An Analysis of Serum Osmolarity, Chloride, and Urea

G. P. Haywood

Department of Zoology, University of Cape Town; Rondebosch, Cape Town, Republic of S. Africa

Abstract

"Pyjama sharks" *(Poroderma africanum)* were exposed to a wide range of salinities, over which blood serum was analysed for osmolarity, chloride and urea concentrations. Fish were divided into two groups, those fed twice weekly (high intake), and those fed once a month (low intake). Both groups were exposed to the same salinity range. "High intake" fish showed the characteristic elasmobranch osmolarity picture, with serum values slightly hyper-osmotic at all times. "Low intake" fish, however, showed a degree of hypo-osmotic regulation. Serum values for both groups overlapped at very low salinities. Serum urea was also affected by diet, so that again two distinct sets of values were produced, again with overlap at the lower salinities. When previously well-fed fish were starved over a period of one month, serum urea and osmolarity decreased simultaneously. Consequently, it is felt that serum osmolarity is directly related to serum urea levels. Serum chloride was not found to be affected by diet, both groups showing the same change in blood values when exposed to the same change in salinity. It is shown, however, that a reduction in food intake, over a period of more than a fortnight, can reduce metabolic urea to the extent of depressing serum osmolarity and, hence, shift the ionic and osmotic equilibrium between the fish and the sea water. This may result in varying degrees of hypo-osmotic regulation.

Introduction

It is generally recognised that marine elasmobranchs, like their teleost counterparts, contain blood salts at about one-half sea-water concentration, so that serum-chloride values lie in the region of 250 mM/l (Chaisson, 1930; Smith, 1931; Burger and Hess, 1960; Burger, 1962; Price, 1967; Price and Creaser, 1967). Unlike teleosts, however, marine elasmobranchs have evolved the technique of reabsorbing and retaining urea in the tissues and body fluids (Smith, 1936; Kempton, 1953), so that serum osmolarity remains just greater than that of the external sea water. This greatly reduces their osmotic problem, so that they do not need to drink sea water (Smith, 1931) and excrete salts, as do teleosts (Keys and Willmer, 1932; Smith, 1932). However, in common with teleosts, they still have the problem of a natural and continuous diffusion of salts into the fish from the external sea water, where the concentration is higher. This is compensated for by salt excretion in the urine, and also by secretions of the rectal gland (Burger and Hess, 1960; Burger, 1962, 1965). It seems that there is also salt transfer at the gill epithelium (Maetz and Lahlou, 1966; Payan and Maetz, 1970, 1971).

When placed in diluted sea water, the osmolarity of an elasmobranch's blood falls and, conversely, when these fishes are exposed to concentrated sea water, the osmolarity rises (Scott, 1913, Chaisson; 1930; Margaria, 1931; Burger, 1965; Price and Creaser, 1967). This has been attributed largely to changes in concentration of serum urea (Smith, 1936; Price and Creaser, 1967; Goldstein *et al.*, 1968; Goldstein and Forster, 1971; Vlaming and Sage, 1973), but Burger (1965) found that previously starved spiny dogfish (*Squalus acanthias*), on being fed once more, showed no appreciable rise in tissue-urea concentrations, although serum osmolarity rose. A possible explanation for this is discussed below.

Whilst most workers have agreed on the effect of salinity changes upon the osmolarity of elasmobranch blood, the effect upon blood-salt levels seems less clear. Price and Creaser (1967) stated that, when exposed to a low salinity, the serum chloride level in the skate *Raja eglanteria* was depressed, although not greatly. Burger (1962, 1965), however, has indicated that, in the spiny dogfish *Squalus acanthias*, serum chloride is resistant to dilution of the medium, although he has shown that chloride can drop to about 220 mM/l. Recent work on the little skate *R. erinacea* (Goldstein and Forster, 1971), the sting-ray *Dasyatis sabina* (Vlaming and Sage, 1973) and the lemon shark *Negaprion brevirostris* (Goldstein *et al.*, 1968) support the present findings and those of Price and Creaser (1967).

It was decided to base this investigation of ionic regulation on chloride rather than sodium since, at the time of experimentation, the equipment and technique for sodium determination was not considered satisfactory. Also, serum shows higher values for sodium than for chloride in most elasmobranchs and, therefore, chloride can be used to give a minimal index of salt value.

Blood samples from *Poroderma africanum* over the whole salinity range were analysed for trimethylamine

oxide, after Dyer (1945), but only traces were found; therefore, urea is considered the most important osmoregulatory component in this system, after sodium chloride.

Materials and Methods

Poroderma africanum (Scyliorhinus africanus) were collected by rod and line during the early stages of the work (1971), but this was found to be unsatisfactory due to hook damage to the fish; subsequently, all fish were caught by hand whilst SCUBA diving. Fish were obtained from both the Atlantic and Indian Ocean sides of the Cape Peninsula, in water depths ranging from 5 to 20 m.

All fish were kept in a large stock tank until required, when they were transferred to smaller experimental tanks. Both stock and experimental tanks were supplied with thermostatically-controlled running sea water, power-filtered through polyester fibre and activated charcoal, and sterilized under ultraviolet light (UV). Periodic monitoring of the sea water revealed a tendency for the pH to drop slightly from its initial value of 7.9 during recycling. This was counteracted by periodic addition of small amounts of fresh sea water and buffered deionized water. The medium was diluted in this way, and concentrated by evaporation.

The size and weight of fish were not specifically chosen, but most fell into the size range 0.5 to 0.8 m, and weighed 1 to 3 kg. Both sexes were used, but males were predominant. Since fish were being used for further research, not reported here, they were divided into two batches — those for chloride analysis and further work, and those for analysis of serum urea and osmolarity. Both sexes contributed equally to the serum-chloride results, but the batch used for urea and osmolarity assay was comprised entirely of males. Ten fish were used for the blood-chloride assay, and 12 for the urea and osmotic-strength determinations.

Whilst in the stock tank, fish were regularly fed fresh teleost meat and small crustaceans. The water temperature was 11 °C.

Experimental fish were exposed to sea water (13 °C), whose salinity was gradually altered in both directions, over the maximum tolerated range. Osmotic pressure and chloride concentration of the sea water in the stock and experimental tanks were checked regularly and, after acclimation of fish at known values for 60 to 72 h, blood samples were taken by syringe from the junction between the cardinal sinus and the sinus venosus. Samples of 1 ml or less were taken each time, and at intervals of never less than 2 days, to minimise any shock effect. By so doing it was possible to use fish for several experiments, and indeed return them to the stock tank after an experimental period to recover fully and feed normally prior to re-use. All blood samples were immediately transferred, to glass centrifuge tubes and allowed to clot for 24 h at 8 °C in a refrigerator, for serum separation. Serum was expressed from the clotted blood by centrifuging, and was either analysed immediately or returned to cold storage. Serum was always analysed for osmolarity, chloride, and urea concentrations within 48 h of separation.

All changes in medium salinity were made very slowly — in the order of 20 to 30 mM/l Cl over 2 days; the fish were then allowed the stated acclimation time. From observations of a few fish, from which samples were taken within 24 to 36 h of salinity change, it seems that a minimal period of 48 h is required for acclimation, provided the salinity change is not in excess of the above values.

Osmotic strength was determined cryoscopically with a Knauer platinum-thermistor osmometer (accuracy ± 5 mOsm/l), chloride by mercuric nitrate titration after Schales and Schales (1941), (accuracy ± 5 mM/l), and urea colorimetrically after Pré *et al.* (1968) (accuracy ± 1%). Colorimetric absorbance at 460 nm was read initially on a Unicam SP 800A UV spectrophotometer, but later on a Unicam SP 600 series-2.

Experimental fish were divided into two groups: "low intake", which were each fed about 0.25 to 0.5 kg fresh teleost meat once a month, and "high intake" fish, which were fed the same amount twice a week. Blood samples from "low intake" fish were only taken during the third and fourth week of each monthly period, and samples from "high intake" fish were taken a day or so before each feed. The species is sluggish and, consequently, little energy is expended by fish in the experimental tanks. "Low intake" fish seemed adequately supplied with food, although slight visceral shrinkage was apparent towards the end of each feeding cycle.

To show the effect of starvation on previously well-fed fish, 4 males, not previously used, were kept in normal sea water (osmolarity 1050 mOsm/l) and fed 0.25 to 0.5 kg teleost meat every other day, for a period of 1 week. The last day of feeding was taken as "Day 0"; from then onwards the fish were not fed, but blood samples were taken at regular intervals of 3 to 5 days, over a period of a month. After this, 2 fish were fed again, whilst 2 remained as unfed controls. Blood samples from all 4 fish were again taken a day after this feeding.

Results

Fig. 1 shows the effect of the external chloride concentration upon serum chloride. *Poroderma africanum* was found to tolerate a wide range of external chloride concentrations, the lower limit being about 290 mM/l (18.0‰) and the upper about 775 mM/l (47.5‰). At these two extremes, the serum chloride

was 200 mM/l and 315 mM/l, respectively. In normal sea water (545 to 555 mM/l Cl), the serum had a chloride value of about 260 mM/l, which decreased to 220 mM/l when the external salinity was 375 mM/l Cl. Serum chloride rose to 315 mM/l when the fish were immersed in water of about 700 mM/l Cl. Diet had no effect upon serum chloride and, consequently, both groups were included in the above curve.

water (1060 mOsm/l upwards), this difference increased to 120—140 mOsm/l, and the regression coefficients for this part of the graph were shown to be significantly different ($P < 0.01$). The upper tolerance limit was 1320 mOsm/l.

A similar trend was shown by serum urea concentrations. Over a range of external media, from 700 to 1320 mOsm/l, blood values for the two groups were

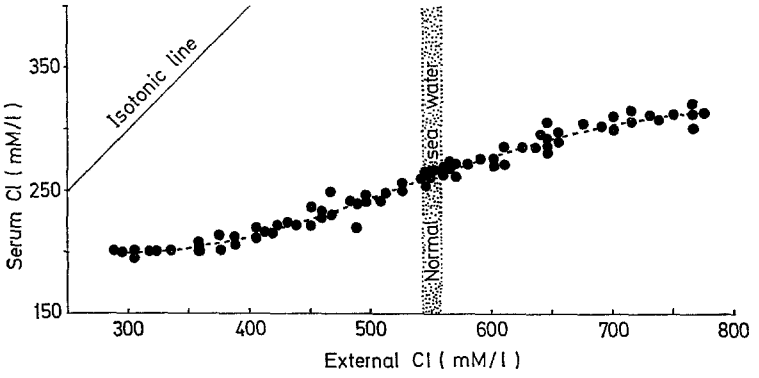

Fig. 1. *Poroderma africanum.* Variation in serum chloride with modification of external salinity

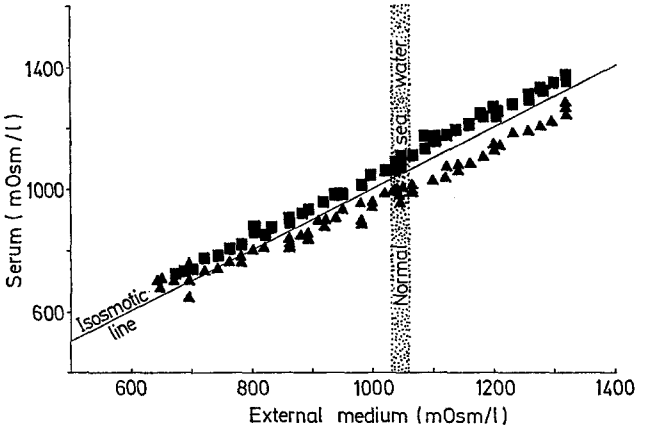

Fig. 2. *Poroderma africanum.* Combined effects of diet and salinity upon serum osmolarity. Squares: "high intake" fish; triangles: "low intake" fish

The results of variation in external medium upon serum osmolarity are shown in Fig. 2. A clear difference can be seen between "high intake" and "low intake" fish. At the lower tolerance limit of 650 mOsm/l, fish from both groups showed similar serum osmolarities, in the order of 700 mOsm/l but, as the external medium was raised towards 850 mOsm/l, the two groups showed significant differences (slope difference $P < 0.05$). Above an external osmolarity of 850 mOsm/l, the two sets of points were well separated, with a serum difference of 60 to 100 mOsm/l. In concentrated sea

quite distinct (Fig. 3). Serum urea was shown to range from 215 to 550 mM/l, with "normal sea water" values of 455 to 465 mM/l and 405 to 415 mM/l for "high intake" and "low intake" fish, respectively. When replotted on a semi-logarithmic scale, a straight-line relationship was produced. This was similar to Fig. 2, and the initial slope difference was again found to be significantly different ($P < 0.001$). Whilst the semi-logarithmic plot did not show statistically significant differences in regression coefficients, it can nevertheless be seen that Fig. 3 shows a visible dis-

placement of serum values, by approximately 50 mM/l at normal sea water, and by up to 80 mM/l, in higher external media.

Linking of serum osmolarity and urea is shown more strongly in Fig. 4, which presents the results from 4 male fish over a month's starvation period, after previous feeding. All fish showed subsequent progressive drops in both serum osmolarity and urea concentration, to the extent that, after 13 to 14 days, serum osmolarity fell below the isosmotic line and continued to decrease until about 20 to 20 mOsm/l below, at which point it began to level out. Subsequent feeding of 2 fish was followed almost immediately by a corresponding rise in their serum osmolarity and urea values, whilst the 2 control fish showed unaltered values.

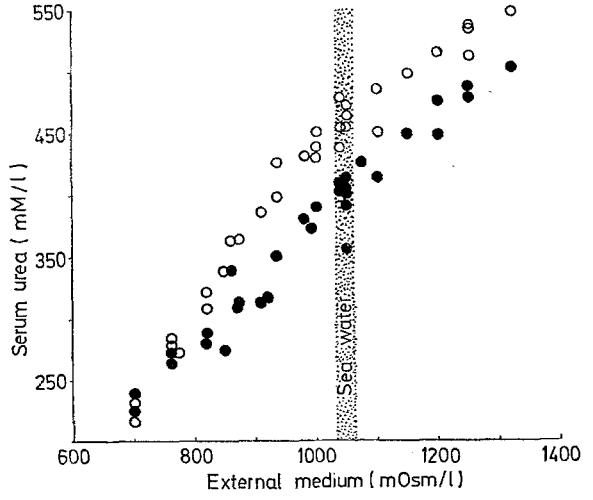

Fig. 3. *Poroderma africanum.* Combined effects of diet and salinity upon serum-urea levels. Open circles: "high intake" fish; black circles: "low intake" fish

Discussion

The serum-chloride trace indicates a degree of tolerance of salinity change by *Poroderma africanum* over the more central region of the exposed range. Whilst the low gradient indicates active regulation of body fluids over this region, the regulation is more intense and noticeable towards the extremities of the range. Consequently, less effort at active regulation of ionic and osmotic agents between body fluids and external medium seems expended over the more usual salinities than for those near the ends of the range. The range of the species also extends almost as far above sea water as below — indicating an ability to tolerate hyper- or hypo-saline conditions equally well. Although these findings are related to laboratory conditions, they are interesting in view of the fact that *P. africanum* is considered stenohaline, few (if any) individuals being encountered in brackish water. Over the more central portion of the range, it would seem that chloride regulation is more actively carried out at the cellular or intra-cellular level, than at the fish-sea water interface. At the extremities of the tolerated range, more effort is made to regulate body fluids so as to reduce the already high ionic and osmotic stress at the cellular level. This is also assisted, to a certain extent, by reduced permeability to certain ions, including chloride (Haywood, unpublished), for this species and others (Maetz and Lahlou, 1966; Carrier and Evans, 1972).

Burger (1962, 1965) indicated that, in the case of the spurdog *Squalus acanthias*, "plasma chloride is resistant to dilution of the medium". He pointed out that short

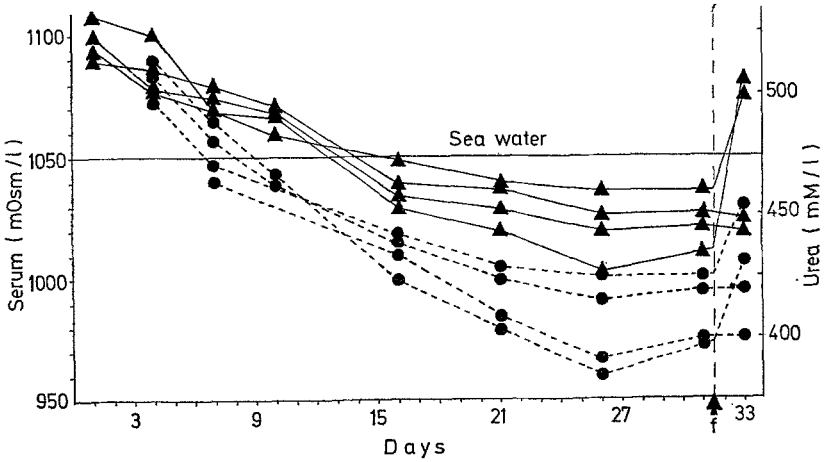

Fig. 4. *Poroderma africanum.* Effect of prolonged starvation upon 4 previously well-fed fish. Circles: serum urea; triangles: serum osmolarity; f: point at which 2 fish were re-fed. Positioning of the urea scale with respect to the sea-water value and osmolarity scale is purely arbitary, and is included to show only how the two criteria vary together

periods of up to 24 h in 87 to 95% sea water did not change plasma chloride, and fish which were kept in running diluted sea water for 3 to 9 days were able to stabilize plasma chloride, although it was found to drop to 220 mM/l in many cases. This latter fact seems in itself a contradiction of the earlier statement. It should also be remembered that the running sea water used was of fluctuating salinity "for the most part in the 72% to 82% range, with excursions up to 6 hours in 60% and 92%" (Burger, 1965). Consequently, the salinity at any given time appears to have been fluctuating and, since Burger has further stated that it took 48 h of dilution of the medium to affect fully the fish's internal composition, it is felt that the experimental parameters used by Burger were possibly not severe enough to show any low-salinity effects on the fish.

Price and Creaser (1967) showed that, for the clearnose skate *Raja eglanteria*, exposure to dilutions of medium produced depressions in serum chloride as well as urea concentration. When exposed to higher salinity, serum chloride was found to increase, although only in small amounts. Again 48 h was required for attainment of osmotic and ionic equilibrium with the environment, after a change of more than 2.4‰ or 40 mM/l Cl of the medium. Goldstein and Forster (1971) have shown that, in the little skate *R. erinacea*, transference gradually into half-strength sea water, from full-strength, resulted in reductions of plasma urea and chloride by 45 and 30%, respectively. Similar experiments with the lemon shark *Negaprion brevirostris* (Goldstein *et al.*, 1968) resulted in reduction of plasma urea and chloride by 55 and 20%, respectively. In the sting-ray *Dasyatis sabina*, Vlaming and Sage (1973) reported that "Plasma NaCl concentrations in *Dasyatis* are reduced to a greater extent by environmental dilution than are urea levels, until the external medium osmolarity falls below 350 mOsm per l".

Present results show that serum osmolarity and urea concentration vary with external salinity, in keeping with previous findings. It was shown by Scott (1913) that, when an elasmobranch is placed in diluted sea water, its osmotic pressure falls and, conversely, when in concentrated water, rises. However, all previous measurements of serum osmolarity show serum values slightly higher than the medium, and it has become generally accepted that serum is always maintained slightly hyper-osmotic to the sea water (Burger and Hess, 1960; Burger, 1965), in order to effect a slight endosmosis. As a result, marine elasmobranchs are not considered to drink the medium (Smith, 1931), as do marine teleosts.

The variation in serum urea and serum osmolarity seem closely linked with respect to the superimposed diet effect. According to Thorson *et al.* (1967), elasmobranch body fluids have shown urea nitrogen in the range of approximately 750 to 1300 mg/100 ml. If expressed in similar units, "high intake" fish showed urea nitrogen levels of 1260 to 1290 mg/100 ml, and fish on "low intake", about 1120 to 1150 mg/100 ml, in normal sea water. Whilst both sets of values lie within Thorson *et al.*'s figures, a difference of about 140 mg/100 ml is visible between the two groups. By comparison with the results of several workers presented in "Fish Physiology" (Hoar and Randall, 1969), *Poroderma africanum* appears to have serum urea levels a little higher than other species quoted (320 to 440 mM/l), there being a considerable amount of species variation.

Both urea and osmolarity show a tendency to stabilize at the lowest salinities. Watts and Watts (1966) have indicated that urea synthesis in elasmobranchs is stimulated by dilution of the medium, which might explain the slight "tail off" — although this should surely become apparent before such drastic dilutions are achieved. The effect of medium dilution upon urea production does not appear to be as noticeable for this species as the effect of diet.

So far little previous work has been done on actual feeding effects upon serum osmolarity and urea levels. Indeed, no previous thought appears to have been given to how elasmobranchs may fare under conditions of famine, and the consequential effects upon body fluids. From present findings, it is concluded that, when fed a regular and abundant meal, *Poroderma africanum* are able to maintain a high serum urea concentration and a correspondingly hyperosmotic serum (about 50 to 60 mOsm/l), at all salinities. However, with more infrequent feeding, the osmotic pressure of the serum falls below that of the medium. Furthermore, the link between diet and serum osmotic pressure is demonstrated in the experimental results presented in Fig. 4. This is to be expected, since serum urea results from metabolism of ingested proteins.

It has been shown, for *Poroderma africanum*, that the two serum osmolarity lines produced from the two diet groups represent fish at two extremities of a scale. Whilst it was found quite easy to keep fish alive without food for periods in excess of 2 months, adverse effects were noticed after about the sixth week and, consequently, fish being fed only once a month ("low intake") were deemed to be at "starvation" level (i.e. the absolute minimal feeding required to just maintain life, without producing abnormal body functions). From the results, as expressed in Fig. 4. it seems that, during the starvation period, there is a gradual decline in both serum urea and osmolarity until the two level off, due probably to metabolism of body protein. It would, therefore, also appear that for fish fed only once a month, serum values rise somewhat directly after feeding, but then decline again over the starvation interval. Since this effect was somewhat anticipated, blood samples were only taken just before each feed, as previously mentioned, in order

to obtain serum values from the fish after all digestion was completed and no more urea was being synthesized (in the case of "low intake" fish). In retrospect, it can be seen that fish which are only fed once a fortnight, or indeed at any intermediate interval, will have blood urea and osmolarity values somewhere between those of the two diet groups. Consequently, fish under varying conditions of food abundance may well show blood values over the whole intermediate range. It is very likely that food scarcity would initiate a search by the fish, which would possibly move to another area. It is not considered likely that fish would be exposed frequently to such long periods without food, but it should, however, be born in mind that underwater observations, coupled with laboratory observations, indicate that *P. africanum* is extremely gregarious and sluggish. Naturally favoured habitats are dark crevices beneath rocks — indicative that, rather than stalking food, the species seems content to wait for the arrival of prey. Furthermore, all laboratory findings are for a water temperature of 13 °C and, at many times, sea-water temperatures around the peninsula are in the region of 15° to 17 °C, which argues for higher metabolic and digestive rates under such conditions. Thus, urea production might be expected to be more intensive but shorter-lived under natural conditions.

In contrast, Creaser (1965), showed that, for the clearnose skate *Raja eglanteria*, 6 days of starvation does not lower serum urea levels, and Price and Creaser (1967), stated that skates which were not fed for a period of 71 h showed no signs of urea depletion. Burger (1965) found no appreciable rise in the serum osmolarity of *Squalus acanthias* which were each fed 500 g of herring over a period of 3 days, after a 5-day starvation period. He concluded that "apparently, the 500 g herring was not converted measurably into urea". However, present findings indicate that this is not so for *Porodermo africanum*, since the feeding of 2 dogfish with 250 g teleost meat, after starvation, produced immediate rises in serum urea and osmolarity. It seems that a starvation period of only 5 days is insufficient to cause an appreciable drop in blood urea levels in these dogfish, although slight falls in both urea and osmolarity can be seen.

Present findings indicate, therefore, that for the dogfish *Poroderma africanum*, there seems to be little effect of diet upon serum chloride, whilst serum urea and osmolarity are definitely affected. Within 4 or 5 days of the cessation of feeding, blood urea and osmolarity show distinct reduction and, within 14 days, the blood becomes isosmotic and even hypo-osmotic to sea water. This results in fish maintaining a hypo-osmotic balance with the external sea water, rather than the normal slightly hyper-osmotic situation. Under such conditions, one would expect water loss from the fish which could only be balanced by drinking of the medium. It is possible that they regulate more similar to marine teleosts than "typical" marine elasmobranchs.

Summary

1. The striped dogfish *Poroderma africanum* was found to tolerate a wide range of salinities, from about 18.0 to 47.5‰, under laboratory conditions.

2. Serum chloride alters with external salinity, varying from 200 mM/l in the most dilute sea water, to 315 mM/l in the most concentrated.

3. Both serum osmolarity and urea are affected by changes in external salinity, and there is also a pronounced diet effect on both. "Low intake" fish regulated both serum osmolarity and urea at lower levels than normally-fed fish over the majority of the exposure salinity range. This produced hypo-osmotic regulation over a large range of the salinities for infrequently-fed fish.

4. Serum urea and osmolarity appear to be directly related, so that infrequent feeding produces lower levels of metabolic urea resulting, in turn, in lower serum osmolarity. Both factors decrease steadily and simultaneously during a period of starvation; however, on re-feeding the fish, both increase immediately.

5. In conclusion, it has been shown that, whilst the external medium controls the actual composition of body fluids, the level at which regulation between these fluids and the external medium occurs is also affected by the availability of metabolic urea and, hence, availability of food.

Acknowledgements. My sincere thanks to Professor A. C. Brown for much help and advice; also to Professor Day and other members of staff. I am indebted to the Biochemistry Department for the use of their Unicam SP 800A U.V. spectrophotometer, and to Mr. A. Fricke, for much help in the collecting of specimens. Grateful thanks also to the C.S.I.R. for financial assistance.

Literature Cited

Burger, J. W.: Further studies on the function of the rectal gland in the spiny dogfish. Physiol. Zoöl. **35**, 205—217 (1962).

— Roles of the rectal gland and the kidneys in salt and water excretion in the spiny dogfish. Physiol. Zoöl. **38**, 191—196 (1965).

— and W. N. Hess: Functions of the rectal gland in the spiny dogfish. Science, N.Y. **131**, 670—671 (1960).

Carrier, J. C. and D. H. Evans: Ion, water and urea turnover rates in the nurse shark, *Ginglymostoma cirratum*. Comp. Biochem. Physiol. **41A**, 761—764 (1972).

Chaisson, A. F.: The changes in the blood concentration of *Raja erinacea* produced by modification of the salinity of the external medium. Contr. Can. Biol. Fish. **5**, 479—491 (1930).

Creaser, E. P.: Fluctuations in two osmoregulatory components, urea and chloride, of the clearnose skate *Raja eglanteria* Bosc 1802, upon modification of the salinity of the external medium, M.S. Thesis. University of Delaware 1965.

Dyer, W. J.: Amines in fish muscle — I. Colorimetric determination of trimethylamine as the picrate salt. J. Fish. Res. Bd Can. **6**, 351—358 (1945).

Goldstein, L. and R. P. Forster: Osmoregulation and urea metabolism in the little skate *Raja erinacea*. Am. J. Physiol. **220**, 742—746 (1971).

—, W. W. Oppelt and T. H. Maren: Osmotic regulation and urea metabolism in the lemon shark *Negaprion brevirostris*. Am. J. Physiol. **215**, 1493—1497 (1968).

Hoar, W. S. and D. J. Randall (Ed.); Fish physiology. Vol. 1. Excretion, ionic regulation, and metabolism, 465 pp. New York: Academic Press 1969.

Kempton, R. T.: Studies of the elasmobranch kidney — II. Reabsorption of urea by the smooth dogfish, *Mustelus canis*. Biol. Bull. mar. biol. Lab., Woods Hole. **104**, 45—56 (1953).

Keys, A. B. and E. N. Willmer: "Chloride-secreting cells" in the gills of fishes with special reference to the common eel. J. Physiol., Lond. **76**, 368—381 (1932).

Maetz, J. et B. Lahlou: Les échanges de sodium et de chlore chez un elasmobranche, *Scyliorhinus*, mesurés à l'aide des isotopes ^{24}Na et ^{36}Cl. J. Physiol., Paris **58**, p. 249 (1966).

Margaria, R.: The osmotic changes in some marine animals. Phil. Trans. R. Soc. **107**, 606—620 (1931).

Payan, P. et J. Maetz: Balance hydrique et minérale chez les elasmobranches: arguments en faveur d'un contrôle endocrinien. Bull. Inf. Sci. Technol. C.E.A. **146**, 77—96 (1970).

— — Balance hydrique chez les elasmobranches: arguments en faveur d'un contrôle endocrinien. Gen. comp. Endocr. **16**, 535—554 (1971).

Pré, J., P. Giraudet et P. Cornillot: Ultramicrodétermination directe automatique et manuelle de l'urée dans les liquides biologiques. Path. Biol., Paris **16**, 895—899 (1968).

Price, K. S.: Fluctuations in two osmoregulatory components, urea and sodium chloride of the clearnose skate *Raja eglanteria*. Bosc 1802 — II Upon natural variations of the salinity of the external medium. Comp. Biochem. Physiol. **23**, 77—82 (1967).

— and E. P. Creaser: Fluctuations in two osmoregulatory components, urea and sodium chloride, of the clearnose skate *Raja eglanteria*. Bosc 1802 — I Upon laboratory modification of external salinities. Comp. Biochem. Physiol. **23**, 65—76 (1967).

Schales, O. and S. S. Schales: A simple and accurate method for the determination of chloride in biological fluids. J. biol. Chem. **140**, 879—884 (1941).

Scott, G. G.: A physiological study of the changes in *Mustelus canis* produced by modifications in the molecular concentration of the external medium. Ann. N. Y. Acad. Sci. **23**, 1—73 (1913).

Smith, H. W.: The absorption and excretion of water and salt by the elasmobranch fishes. II Marine elasmobranchs. Am. J. Physiol. **98**, 269—310 (1931).

— Water regulation and its evolution in fishes. Rev. Biol. **7**, 1—26 (1932).

— The retention and physiological role of urea in the Elasmobranchii. Biol. Rev. **11**, 49—82 (1936).

Thorson, T. B., M. C. Cowan and D. E. Watson: *Potamotrygon* spp.: elasmobranchs with low urea content. Science., N.Y. **158**, 375—376 (1967).

Vlaming, V. L. and M. Sage: Osmoregulation in the euryhaline elasmobranch, *Dasyatis sabina*. Comp. Biochem. Physiol. **45A**, 31—44 (1973).

Watts, D. C. and R. L. Watts: Carbamoyl phosphate synthetase in the Elasmobranchii. Osmoregulatory function and evolutionary implications. Comp. Biochem. Physiol. **17**, 785—798 (1966).

Author's address: Mr. G. P. Haywood
Department of Zoology
University of Cape Town
Private Bag Rondebosch
Cape Town
Republic of S. Africa

Date of final manuscript acceptance: September 27, 1973. Communicated by E. O. Bayagbona, Lagos and J. H. S. Blaxter, Oban