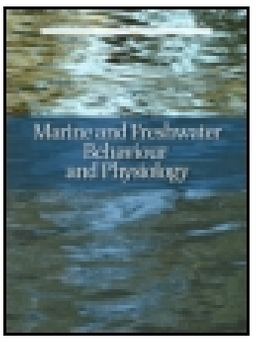

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Osmoregulation in elasmobranchs: a review for fish biologists, behaviourists and ecologists

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Abstract
This article provides a broad review of osmoregulation in elasmobranchs for non-specialists, focusing on recent advances. Marine and euryhaline elasmobranchs in seawater regulate urea and other body fluid solutes (trimethylamine oxide (TMAO), Na^+, Cl^-) such that they remain hyper-osmotic to their environment. Salt secretions of the rectal gland and excretions in the urine compensate for continuous inward diffusion of environmental salts. Freshwater and euryhaline elasmobranchs in fresh water synthesise less urea and retain less urea and other body fluid solutes compared to marine elasmobranchs and thus have relatively lower osmolarity. Electrolyte uptake at the gills and kidney reabsorption of salts maintain acid–base balance and ionic consistency. The role of the gills, kidney, liver and rectal gland in elasmobranch osmoregulation is reviewed. The ontogeny of osmoregulatory systems in elasmobranchs and the contribution of drinking and eating processes in maintaining osmotic consistency are discussed. Recommendations for future research are presented.

Keywords: *Elasmobranch, shark, gill, rectal gland, urea, osmoregulation*

Introduction

Elasmobranchs are predominantly marine, although some 10% are estuarine, 2% are euryhaline and 1% are obligate in fresh water (Martin 2005). Studies of osmoregulation in elasmobranchs have been reported in the literature over the last seventy-five years; however, there have been significant advances in our understanding of the mechanisms underlying elasmobranch osmoregulation in the past decade. Although there have been several recent reviews of elasmobranch osmoregulation (Hazon et al. 2003; Evans et al. 2004, 2005), these have restricted focus to selected aspects of osmoregulation and tended to be highly technical. This article provides a broad review of osmoregulation in elasmobranchs

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for non-specialists, particularly fish biologists, behaviourists and ecologists with limited training in the biochemistry and physiology of osmoregulation.

Osmoregulation depends on the relationship between the solute-to-solvent concentrations of the internal body fluids (extracellular and intracellular) and the outside medium that surrounds the animal. Unless the internal body fluids and the outside medium have the same solute concentration, water will enter the body when its fluids contain a higher concentration of solute and leave the body when the surrounding medium contains a higher concentration. Electrolytes will similarly diffuse through the body down concentration gradients. These considerations hold true at both the extracellular and intracellular level. Thus, marine animals face problems of dehydration and the elimination of excess salts, while freshwater animals must conserve their salts and eliminate excess water.

Marine and euryhaline elasmobranchs in salt water reabsorb and retain urea and other body fluid solutes (Table I) such that osmolarity remains hyper-osmotic to their surrounding seawater; consequently they experience little or no osmotic loss of water (Smith 1931; Thorson 1962). Any water that is gained by osmosis across the gills is quickly balanced by renal excretion. The continuous inward diffusion of Na^+ and Cl^- (salt) from the environment is compensated for by salt excretory mechanisms in the rectal gland and kidney (Burger and Hess 1960; Burger 1965; Haywood 1973; Piermarini and Evans 2000). In contrast, marine teleosts remain slightly hypo-osmotic to the surrounding sea water, experiencing some water loss, and maintain osmotic consistency by actively drinking seawater and secreting excess salts via the gills and kidney (see reviews by Evans 1993 and Evans et al. 2005).

Freshwater and euryhaline elasmobranchs in fresh water, balance osmotic water gain by increased urinary excretion (Thorson et al. 1967; Goldstein and Forster 1971a, reviewed by Evans et al. 2004). They also synthesise less urea as well as retain less urea, Na^+ and Cl^- than marine individuals such that osmolarity remains relatively low (Table I), but still greater than the surrounding fresh water (Thorson et al. 1967; Thorson 1970; Goldstein and Forster 1971a, 1971b; Poulsen 1981; Wood et al. 2002; Tam et al. 2003; Anderson et al. 2005). Diffusional losses of Na^+ and Cl^- are balanced by electrolyte uptake at the gills, and salt reabsorption by kidney tubules (Goldstein and Forster 1971a; Gerst and Thorson 1977; Piermarini and Evans 2000; Piermarini et al. 2002; Wood et al. 2002; Tresguerrers et al. 2005). In contrast, freshwater teleosts remain slightly hyper-osmotic to the surrounding fresh water and osmoregulate by drinking relatively little water, excreting large amounts of dilute urine, obtaining water and salts via the gills and also deriving some salts from their diet (see reviews by Evans 1993 and Evans et al. 2005).

The present work provides a broad review of osmoregulation in elasmobranchs for non-specialists. The role of the gills, kidney, liver and rectal gland in elasmobranch

Table I. Examples of Na^+, Cl^-, and urea concentrations (mM) in several elasmobranchs from salt and fresh water. SW = salt water; FW = fresh water.

Species and environment	Na^+	Cl^-	Urea	Reference
Dasyatis sabina (SW)	310	300	394	Piermarini and Evans (1998)
Dasyatis sabina (FW)	212	208	196	Piermarini and Evans (1998)
Carcharhinus leucas (SW)	289	296	370	Pillans and Franklin (2004)
Carcharhinus leucas (FW)	208	203	192	Pillans and Franklin (2004)
Squalus acanthias (SW)	286	246	351	Burger and Hess (1960)
Potamotrygon sp. (FW)	178	146	1.2	Wood et al. (2002)

osmoregulation is summarised. The ontogeny of osmoregulatory systems in elasmobranchs and the contribution of drinking and eating processes in maintaining osmotic consistency are discussed. Recommendations for future research areas are presented that have particular significance for fish biologists, behaviourists and ecologists.

Plasma solutes and osmoregulation

Elasmobranchs utilise nitrogenous organic compounds and inorganic ions in large part to maintain osmotic consistency with their environment. This is in contrast to teleosts, which principally use only sodium and chloride ions to maintain osmotic consistency through functions of the gill, kidney and by drinking processes (sees reviews by Evans 1993 and Evans et al. 2005). All elasmobranchs examined to date, except the freshwater potamytrygonid rays, are ureotelic; that is, they synthesise and excrete urea as an end product of nitrogen metabolism (Wood 1993; Wood et al. 2002; reviewed by Hazon et al. 2003). In marine elasmobranchs, plasma osmolarity is high, largely because body fluid concentrations of organic nitrogenous compounds, such as urea and trimethylamine oxide (TMAO), are high. Plasma urea levels generally comprise over 30% of total plasma osmolarity (Table II). Elasmobranch plasma TMAO levels are typically 75 mM (see review by Evans et al. 2004). Intracellular concentrations of TMAO approach 200 mM, approximately 50% of urea levels, a ratio that is generally consistent in most elasmobranchs (Hochachka and Somero 2002). In marine environments, elasmobranch body fluid inorganic ions, both monovalent (sodium and chloride) and divalent (magnesium, sulphate and calcium), are regulated at concentrations below seawater levels (Robertson 1975, 1976; Pillans et al. 2004).

In euryhaline elasmobranchs occupying fresh water, osmolarity is relatively low compared to their marine counterparts, largely because body fluid concentrations of organic nitrogenous compounds, such as urea and TMAO are relatively low (Smith 1931; Thorson 1962; Thorson et al. 1973; Piermarini and Evans 1998; Pillans and Franklin 2004; Pillans et al. 2004). For example, euryhaline bull sharks, *Carcharhinus leucas*, acclimated to fresh water have urea and TMAO levels of about a half and one-third of their marine counterparts, respectively (Table III). In freshwater environments, euryhaline

Table II. Comparison of the percentage contribution of Na^+, Cl^- and urea to serum osmolarity in elasmobranchs collected from or acclimated to salt water and fresh water. Values provided are expressed as a percentage of total plasma osmolarity. SW = salt water; FW = fresh water; NR = not reported in study.

Species and environment	Na^+	Cl^-	Urea	Reference
Scylorhinus canicula (SW)	28.7	30.6	32.1	Hazon and Henderson (1984)
Scylorhinus canicula (50% SW)	36.6	37.0	16.3	Hazon and Henderson (1984)
Carcharhinus leucas (SW)	28.8	28.8	35.6	Thorson et al. (1973)
Carcharhinus leucas (FW)	37.1	33.2	25.6	Thorson et al. (1973)
Carcharhinus leucas (SW)	27.1	27.7	34.7	Pillans and Franklin (2004)
Carcharhinus leucas (FW)	32.4	31.6	29.9	Pillans and Franklin (2004)
Raja erinacea (SW)	NR	28.4	39.2	Goldstein and Forster (1971a)
Raja erinacea (50% SW)	NR	30.6	33.3	Goldstein and Forster (1971a)
Dasyatis sabina (SW)	30.0	29.0	38.1	Piermarini and Evans (1998)
Dasyatis sabina (FW)	34.1	33.4	31.5	Piermarini and Evans (1998)
Potamotrygon sp. (FW)	58.2	53.9	0.39	Griffith et al. (1973)
Potamotrygon sp. (FW)	55.6	45.6	0.38	Wood et al. (2002)

Table III. Plasma ionic and urea concentrations (mM) in marine and freshwater *C. leucus*.

	Source data: Urist (1962)*		Pillans et al. (2004)**	
Solute	*C. leucas* (marine)	*C. leucas* (Freshwater)	*C. leucas* (Saltwater-acclimated)	*C. Leucus* (Freshwater-acclimated)
Na	223.4	200.12	304 ± 3	221 ± 4
K	9.0	8.2*	5.8 ± 3	4.2 ± 0.2
Ca	4.5	3.0	4.4 ± 0.3	3.0 ± 0.1
Mg	2.9	2.0	1.8 ± 0.1	1.3 ± 0.1
Cl	236.0	180.5	315 ± 3	220 ± 4
HCO_3	5.1	6.0	NR	NR
SO_4	0.6	0.5	NR	NR
PO_4	2.0	4.0	NR	NR
TMAO	NR	NR	47.3 ± 4.5	19.1 ± 1.3
Urea	333.0	132.0	293 ± 9	151.0 ± 5.0

NR = not reported in study.
* Data based on four bull sharks collected in southeastern Nicaragua from Rio San Juan near El Castillo. Statistical analysis was not performed on plasma serum compositions between freshwater and marine sharks.
** Data based on 28 bull sharks collected from the Brisbane River, Australia, and acclimated to both 100% salt water and fresh water over 17 days in the lab, with 7 days in the final salinity. There was a significant difference in plasma osmolarity, urea, TMAO, sodium, potassium, calcium, magnesium and chloride ion concentrations measured between freshwater and saltwater acclimated sharks.

elasmobranch extracellular fluid monovalent and divalent inorganic ion concentrations are maintained at lower levels than in marine habitats (Piermarini and Evans 1998; Pillans and Franklin 2004; Pillans et al. 2004). For example, in fresh water, bull sharks have sodium, chloride and magnesium ion concentrations about 12, 13 and 15% of levels below marine bull sharks (Table III).

Obligate freshwater *Potamotrygon* rays have urea concentrations of less than one-hundredth that of marine elasmobranchs (Table I) (Goldstein and Forster 1971b; Wood et al. 2002) and urea contributes less than 1% to their overall plasma osmolarity (Table II). *Potamotrygon* rays generally regulate their blood salts and osmolarity at levels similar to those of freshwater teleosts, but lower than those of marine elasmobranchs (Gerst and Thorson 1977; Mangum et al. 1978; Wood et al. 2002).

The contribution of the various osmolytes to osmoregulation differs between the extra- and intracellular compartments. For example, when the little skate, *Raja erinancea*, is acclimated to reduced salinities, concentrations of its extracellular organic compounds (urea, TMAO, amino acids) and inorganic salts (Na^+, K^+, Cl^-) reduce approximately equally (King and Goldstein 1983; reviewed by Goldstein and Perlman 1995). But, the skate's intracellular organic compounds decrease more than twice as much as its inorganic salts (King and Goldstein 1983; reviewed by Goldstein and Perlman 1995). This is likely because organic compounds compose a larger fraction of the skate's total intracellular (60%) versus extracellular (40%) osmolarity. However, amino acids contribute a large fraction of intracellular organic osmolarity in *R. erinacea* (19% intracellular versus 1% extracellular) (Robertson 1975; Forster and Goldstein 1976; Boyd et al. 1977; King and Goldstein 1983; reviewed by Goldstein and Perlman 1995).

It is commonly cited that marine elasmobranchs maintain osmolarity at levels iso-osmotic or slightly hyper-osmotic to the surrounding seawater. However, the present review failed to identify examples in the published literature in which marine elasmobranchs were maintaining blood iso-osmotic, rather than hyper-osmotic, with the surrounding medium.

Haywood (1973) exposed Pyjama cat sharks, *Poroderma africanum*, fed twice weekly (well-fed) and once per month (poorly-fed) to salinity ranges of 18–47.5‰. Well-fed sharks maintained serum hyper-osmotic to the surrounding sea water; however, poorly-fed sharks maintained serum iso-osmotic at low salinities and slightly hypo-osmotic at high salinities (Haywood 1973). Thus, it appears that, for at least the species examined to date, marine elasmobranchs generally maintain body fluids hyper-osmotic to the surrounding medium, but starvation may affect the level at which elasmobranchs osmoregulate resulting in instances of iso- or hypo-osmotic osmoregulation. The effects of diet on osmoregulation are further discussed in section 8 of this article.

Urea – biosynthesis, retention and reabsorption

High levels of urea found in marine elasmobranchs result from the difference between the rate of biosynthesis and loss across the gills and renal routes.

Gill retention of urea

Due to the large surface area of their gills, elasmobranchs lose urea to the environment via basic diffusional processes (Wood et al. 1995). For example, the gills of the spiny dogfish, *Squalus acanthias*, account for over 90% of the total urea lost from the shark's body (Wood et al. 1995). Despite this, the rate of urea diffusion across the elasmobranch gill is relatively low compared to teleosts; but, this has only been extensively studied in *S. acanthias* (Boylan 1967; Wood et al. 1995; Part et al. 1998; Fines et al. 2001; Walsh and Smith 2001). For example, gill permeability of urea by the rainbow trout, *Oncorhynchus mykiss*, exceeds that of *S. acanthias* by as much as 60 fold (Fines et al. 2001).

The mechanism responsible for the low permeability of *S. acanthias* gills to urea is not fully understood; however, it may relate to structural mechanisms limiting diffusion of urea. Mouritsen and Jorgensen (1994) suggested that the basolateral gill membrane of *S. acanthias* possibly minimises entry of urea due to a high cholesterol-to-phospholipid ratio in basolateral membrane vesicles (Figure 1). Cholesterol is known to reduce urea permeability (Mourtisen and Jorgenson 1994; reviewed by Wilkie 2002 and Evans et al. 2005) and Fines et al. (2001) demonstrated that *S. acanthias* has the highest reported basolateral membrane cholesterol-to-phospholipid molar ratio (3.68). Hill et al. (2004) found that the gill apical membrane of *S. acanthias* has a low permeability to urea as well as water. Diffusional fluxes across the basolateral membrane of *S. acanthias* were 1.5 to 2-folds higher than across the apical membrane, suggesting that the apical membrane is even less permeable to urea than the basolateral and an effective barrier to urea (Hill et al. 2004). However, alternative mechanisms may account for the elasmobranch gill's low rate of urea loss because permeability values obtained by Hill et al. (2004) were not low enough to account for total urea loss measured *in vivo* by others (Boylan 1967; Fines et al. 2001; Part et al. 1998). Hill et al. (2004) hypothesised that the mucous layer over the gills and/or differential intracellular concentrations of molecules that readily cross the apical membrane likely also contributes to the gill's low permeability to urea.

Active transport of urea from the gill back into the plasma may also contribute to the low rate of branchial urea loss measured in *S. acanthias* (Figure 1). Recent studies (Wood et al. 1995; Part et al. 1998; Fines et al. 2001) provide evidence of active, back-transport of urea across the basolateral membrane into the plasma of *S. acanthias*. Current evidence suggests that the urea gradient across the apical membrane is lowered by active transport of intracellular urea across the basolateral membrane via a Na^+: urea antiporter, energised by

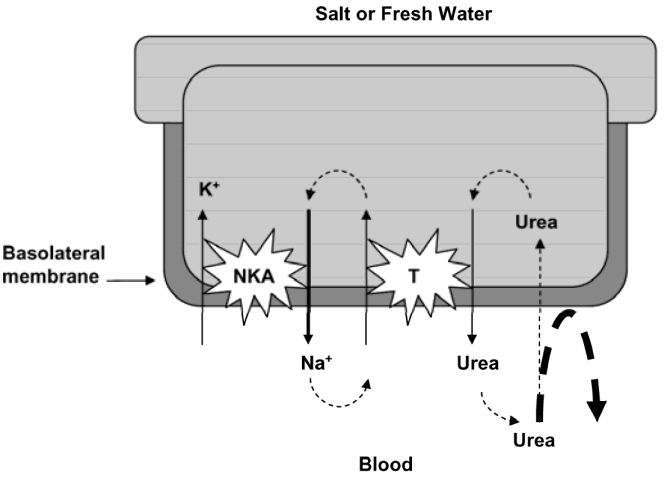

Figure 1. Hypothesised model of urea retention and transport at the elasmobranch gill. Basolateral gill membrane of *S. acanthias* minimises entry of urea (thick dashed arrow) due to a high cholesterol-to-phospholipid ratio. Intracellular urea that leaks back into the gills is transported across the basolateral membrane via a Na^+: Urea antiporter (T), energised by the continual removal of Na^+ from the gill via basolateral Na^+, K^+-ATPases (NKA). Figure re-drawn and used with permission from Evans et al. 2005.

the continual removal of Na^+ from the gill via basolateral Na^+, K^+-ATPases (Fines et al. 2001; reviewed by Wilkie 2002 and Evans et al. 2005).

Wood et al. (1995) infused *S. acanthias* with urea and ammonium chloride. Urea infusions had little effect on whole animal urea efflux whereas infusion with ammonium chloride (substrate for glutamine synthetase: a key nitrogenous trapping enzyme for urea production in elasmobranchs) increased urea efflux. Wood et al. (1995) measured significant activity levels of glutamine synthetase in the gills of *S. acanthias* and as a result hypothesised that urea synthesis could also possibly be occurring directly within the elasmobranch gill; however, this has never been tested experimentally. Possible urea synthesis in the gills could create a higher concentration of urea in the intermediary gill layer supporting active transport of urea back into the plasma (Wood et al. 1995; Hazon et al. 2003). As suggested by Wood et al. (1995), assays for the complete compliment of ornithine-urea (OUC) cycle and uricolytic enzymes in the elasmobranch gill are needed to evaluate such a hypothesis.

Thus, structural differences between the apical and basolateral gill membranes (Boylan 1967; Mourtisen and Jorgenson 1994, Fines et al. 2001; Hill et al. 2004) as well as active transport of urea into the plasma from the gills probably account for the low rate of urea flux measured across the gills in the spiny dogfish (Wood et al. 1995; Part et al. 1998; Fines et al. 2001).

Urea production

Elasmobranchs, except potamytrygonid rays, have an active ornithine–urea cycle (OUC) in the liver (Anderson 1995; 2001; Walsh and Mommsen 2001). The OUC is the dominant pathway of urea production in elasmobranchs (Schooler et al. 1966). Carbamoyl phosphate synthetase III (CPS III) is the first enzyme of the OUC with proximate substrates glutamine

and bicarbonate (Anderson 1980; Mommsen and Walsh 1991). Glutamine synthetase is the key initial nitrogen trapping enzyme for urea production via the CPS III-based OUC (Mommsen and Walsh 1989, Wood 1993). Anderson et al. (2005) demonstrated that when euryhaline bull sharks are transferred from a fresh to seawater environment, their plasma urea levels increase largely as a result of a 2.7-fold increase in hepatic urea biosynthesis. Acclimation of the marine elasmobranchs, lesser spotted dogfish, *Scyliorhinus canicula* (Hazon and Henderson 1984) and *R. erinacea* (Goldstein and Forster 1971b), up to 50% salt water produce a significant decrease in urea production, suggesting that marine species entering reduced salinities osmoregulate in part by reduced urea biosynthesis. Freshwater *Potamotrygon* rays lack activity levels of the liver enzymes necessary for the urea biosynthesis needed for adequate osmoregulation in a marine environment (Gerst and Thorson 1977). The white-edge whip ray, *Himantura signifier*, is obligate in fresh water and resides in the Batang Hari river basin in Jambi, Sumatra (Tam et al. 2003). Unlike freshwater potamytrygonid rays, *H. signifier* is occasionally exposed to brackish water along the river during certain periods of the year and has a functional OUC in the liver (Tam et al. 2003). Upon acclimation in the laboratory to the increases in salinity, *H. signifier* increases its rate of urea synthesis by up-regulating its OUC enzymes (Tam et al. 2003) (Table IV). However, Tam et al. (2003) showed that its rate of urea synthesis is two-fold less than in the blue-spotted fan tail ray, *Taeniura lymma*, a Sumatran marine ray (Tam et al. 2003) (Table IV).

It remains uncertain if urea synthesis may be occurring in extrahepatic tissue. Tam et al. (2003) reported for the first time the presence of a complete OUC in the lining of the stomach of two elasmobranchs, *H. signifier* and *T. lymma* (Table IV). Based on the activities of CPS III, the capacity of the OUC in the stomach of *H. signifier* was approximately 70% that in the liver (Tam et al. 2003). Tam et al. (2003) suggested that an OUC in the elasmobranch stomach could possibly generate a source of urea that could be utilised for osmoregulation. Steele et al. (2005) measured OUC enzyme activity in *R. erinacea* exposed to 100 and 75% seawater. The OUC enzymes were detected in the skeletal muscle of the skates; however, their activities did not change when the skate was acclimated from 100 to 75% seawater, despite concentrations of urea and TMAO decreasing. Steele et al. (2005) hypothesise that the presence of a functional OUC in the skeletal muscle of *R. erinacea* may play a role in the skate's overall osmoregulation.

Grimes et al. (1985) identified the presence of ureolytic bacteria of the genus *Vibrio* in the organs and tissues (particularly the kidney, liver and spleen) of 28 neritic sharks, suggesting a strong bacteria–host relationship. Knight et al. (1988) described the presence of ureolytic bacteria in the intestinal tract and liver of elasmobranchs and found that C14-labelled urea was metabolised by the bacteria, but not the shark tissue. They speculated that these bacteria may play a role in regulating shark tissue urea concentrations and osmolarity; however, the possible effects of these bacteria to elasmobranch osmoregulation has yet to be tested.

Reabsorption of urea by the kidney

In marine and euryhaline elasmobranchs in seawater, renal reabsorption of urea contributes to high serum urea levels. The kidney tubules of marine and euryhaline elasmobranchs in seawater are capable of reabsorbing most of the urea that is filtered through the glomeruli. Approximately 70–99% of filtered urea is reabsorbed from primary urine (Kempton 1953, reviewed by Hazon et al. 2003). Renal excretion accounts for 4–20% of total urea loss in elasmobranchs examined (Evans and Kormanik 1985; Wood et al. 1995); however, the

Table IV. Activities carbamoylphosphate synthetase III (CPS III) in the liver and stomach of *Himantura signifier* following progressive increases in salinity (0.7–20‰) and in *Taeniura lymma* at a constant salinity of 30‰. Marine elasmobranchs have an active OUC and synthesise urea through CPS III primarily for osmoregulation. CPS III uses glutamine as a nitrogen donor and is activated allosterically by N-acetyl-L-glutamate.

	H. signifier liver			*T. lymma* liver	*H. Signifier* stomach			*T. lymma* stomach
Enzyme + substrate and effector	0.7‰	10‰	20‰	30‰	0.7‰	10‰	20‰	30‰
CPS G	0.04 ± 0.01	0.13 ± 0.05	0.10 ± 0.02	0.13 ± 0.02	0.03 ± 0.01	0.01 ± 0.005	0.15 ± 0.07	0.0011 ± 0.0072
CPS G + A	0.15 ± 0.04	0.24 ± 0.03	0.35 ± 0.05	0.54 ± 0.06	0.10 ± 0.05	0.03 ± 0.01	0.45 ± 0.18	0.0059 ± 0.0026

Source: Tam et al. (2003).
CPS = carbamoylphosphate synthetase; G = glutamine; A = N-Acetyl-L-glutamate.
Values expressed are means of enzyme activity (μmol min^{-1} g^{-1} tissue wet mass) ± SEM.

kidney is a relatively minor site of urea loss compared to the gills (discussed in *Gill retention of urea*, p. 213).

In marine elasmobranchs and euryhaline species in salt water, reabsorption of urea by the kidney against a concentration gradient may involve active transport, possibly coupled to the movement of sodium (Schmidt-Nielsen et al. 1972; Hays et al. 1977; Morgan et al. 2003a, 2003b). Urea active transporters have been identified in the kidneys of *S. acanthias* (Smith and Wright 1999), *R. erinacea* (Morgan et al. 2003a, 2003b) and the Atlantic stingray, *Dasyatis sabina* (Janech et al. 2003). Morgan et al. (2003b) provide evidence for two separate urea transporters in the dorsal and ventral sections of the kidney and propose that these two mechanisms of urea transport are critical for renal urea reabsorption in *R. erinacea*. Morgan et al. (2003a) cloned and sequenced a 779 base pair cDNA for a urea transporter (SkUT) in the kidney of *R. erinacea*. Using SkUT as a probe, a northern analysis revealed three signals in the kidney of the skate (Morgan et al. 2003a). Upon exposure to a dilution of the external environment, levels of all three SkUT transcripts were significantly diminished in the kidney in the range of 44–77%, tissue osmolarity decreased and urea concentration decreased (Morgan et al. 2003a). Thus, it appears that *R. erinacea* acclimates to reductions in environmental salinity in part by decreasing active renal reabsorption of urea (Morgan et al. 2003a) and increasing renal clearance via increasing urine flow (Payan et al. 1973). Although two active urea transporters have been identified in *R. erinacea*, it is not known if urea active transporters are solely responsible for urea reabsorption in the little skate and whether similar mechanisms are found in other elasmobranchs (Schmidt-Nielsen et al. 1972; Hays et al. 1977; Smith and Wright 1999; Hazon et al. 2003).

Friedman and Hebert (1990) described a passive model of renal urea reabsorption in *S. acanthias* (Figure 2). The model is based on the presence of the countercurrent arrangement of nephron segments in the kidney bundle zone. The countercurrent arrangement is composed of four segments from a single nephron grouped to form two loops, which together with a fifth segment are enclosed by a continuous peritubular sheath (Friedman and Hebert 1990). The model requires: (1) a proximal nephron segment highly permeable to water; (2) a relatively distal segment with a high rate of active salt transport but impermeable to water and urea; (3) a loop through a sinus zone segment with high hydraulic but low urea permeability, thus allowing osmotic equilibration of water that increases tubular urea concentrations; and (4) a relatively terminal nephron segment within a bundle zone that is highly permeable to urea but not water, resulting in passive diffusion of urea from tubular fluid into the interstitial fluid within the bundle zone (Figure 2). Friedman and Hebert (1990) identified in the kidney of *S. acanthias* a countercurrent arrangement and a diluting nephron segment in the dorsal bundle of the peritubular sheath that has a high rate of active salt absorption and low water and urea permeability (satisfying the second requirement listed above in their model). Despite this observation and Friedman and Herbert's model, a system of passive renal urea reabsorption remains to be experimentally validated.

Elasmobranchs examined to date appear to acclimate to reduced environmental salinity in part by reduced urea synthesis (e.g. Tam et al. 2003) and/or a higher renal clearance of urea through increased urine flow (Smith 1931; reviewed by Hazon et al. 2003 and Anderson et al. 2005). When elasmobranchs are acclimated to dilute environments, urine flow rate increases twenty to fifty fold (Evans et al. 2004), resulting in increased Na^+, Cl^-, Mg^{2+}, SO_4^{2-} and urea excretion (Smith 1931; Goldstein et al. 1968; Goldstein and Forster, 1971a; Wong and Chan 1977; Sulikowski and Maginniss 2001; Hazon et al. 2003). Renal urea clearance is dependent upon glomerular filtration rate (GFR) (Janech et al. 2003;

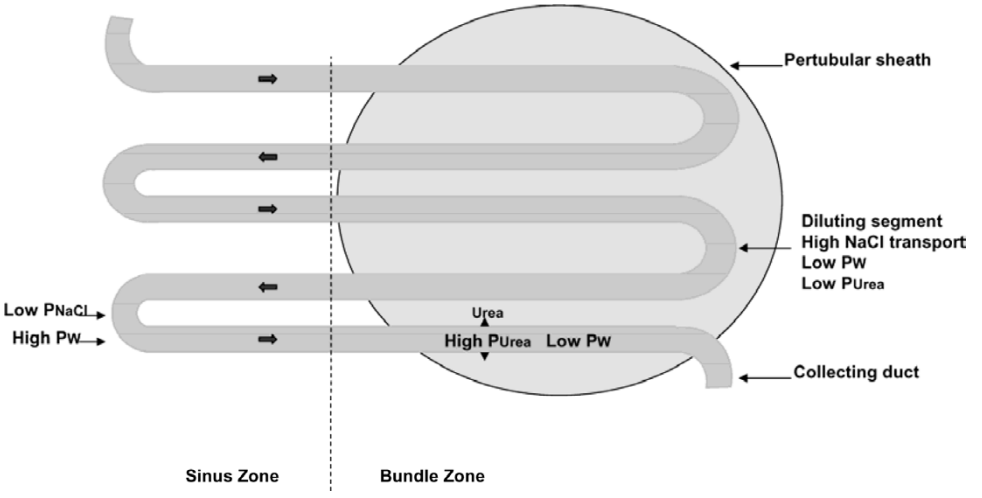

Figure 2. Hypothesised model of passive renal urea reabsorption in *S. acanthias* based on the presence of the countercurrent arrangement of nephron segments in the kidney bundle zone. The countercurrent arrangement is composed of four nephron segments grouped to form two loops and together with a fifth segment are enclosed by a continuous peritubular sheath. The diluting nephron segment in the dorsal bundle of the peritubular sheath has a high rate of active salt absorption and low water and urea permeability, while the segment in the sinus zone has low salt and high water permeability. The distal tubule has high urea and low water permeability, permitting diffusion of urea from the tubular fluid into intrabundle space (small black arrow heads). Small black broad arrows indicate direction of axial tubular fluid flow. Water, salt and urea permeability designated as P_W, P_{NaCl} and P_{Urea}, respectively. Figure re-drawn and used with permission from Friedman and Hebert 1990.

discussed in Anderson et al. 2005). *Potamotrygon* rays do not reabsorb urea (Goldstein and Forster 1971a).

Salt regulation

Rectal gland salt secretions

Marine and euryhaline elasmobranchs in seawater experience diffusion of Na^+ and Cl^- into the body from the surrounding environment, where the concentrations are higher. These diffusional gains are balanced by rectal gland secretions of Na^+ and Cl^- (Burger and Hess 1960; Oguri 1964; Conte 1969; Haywood 1975). The rectal glands of stenohaline freshwater elasmobranchs are non-functional and the rectal glands of euryhaline elasmobranchs moving from salt to fresh water have been shown to decrease in weight and length (Oguri 1964; Goldstein and Forster 1971a; Gerst and Thorson 1977; Thorson et al. 1978; Piermarini and Evans 1998; Pillans and Franklin 2004). Piermarini and Evans (1998) showed that the rectal gland weights to body weights (RGBW) ratios of euryhaline *D. sabina* in fresh water were 20% lower than *D. sabina* in salt water. Pillans and Franklin (2004) found no statistical difference in rectal gland weight to body length (RGBL) ratios between bull sharks captured in fresh water and salt water from the Brisbane River; however, they did find a difference in RGBL ratios between Brisbane River and Lake Nicaragua bull sharks. *Carcharhinus leucas* from Lake Nicaragua had a significantly smaller

rectal gland at the same length as Brisbane River sharks. Pillans and Franklin (2004) suggest that this pattern may result from Lake Nicaragua sharks inhabiting fresh water for longer periods than Brisbane River sharks. However, the bull sharks studied ranged in size from 86 cm to 2.23 m and as a result the compared RGBL patterns may be confounded by the different body sizes examined. Anderson et al. (2003) demonstrated that smaller *S. canicula* have proportionately larger rectal glands than larger individuals, speculating that smaller sharks require larger rectal glands because of the proportionately larger influx of salt from the environment. They further recommend that studies comparing the osmoregulatory function of individual organs use animals of similar size or apply correction factors to avoid scaling errors.

The active transport enzyme, Na^+, K^+-ATPase, plays an important role in the salt ion regulatory functions of mitochondrion-rich cells and has been identified in the elasmobranch gill and rectal gland (McCormick 1995; Marshall and Bryson 1998; Evans et al. 1999; Piermarini and Evans 2000; Pillans et al. 2004). Activity of Na^+, K^+-ATPase results in part from changes in the relative abundance of the enzyme (Blanco and Mercer 1998; Piermarini and Evans 2000). Na^+, K^+-ATPase activities in the rectal glands of marine and euryhaline elasmobranchs in seawater are high (Piermarini and Evans 2000; MacKenzie et al. 2002; Pillans et al. 2004). For example, Pillans et al. (2004) found that Na^+, K^+-ATPase activities are about 1.5-fold higher in the rectal glands of saltwater compared to freshwater bull sharks. Piermarini and Evans (2000) demonstrated that the activity and abundance of Na^+, K^+-ATPase in the rectal gland of saltwater *D. sabina* was approximately double that of its freshwater counterpart, providing further evidence of the rectal gland's role in marine elasmobranch salt secretion.

Salt regulation and acid–base balance at the gills

The elasmobranch gill is involved with salt ion uptake and acid–base balance, particularly important for maintaining ionic homeostasis in a freshwater environment (Perry 1997; Wilson et al. 1997; Piermarini and Evans 2000; Piermarini et al. 2002; Wood et al. 2002; Evans et al. 2005; Tresguerrers et al. 2005). A working model of salt uptake at the gill of elasmobranchs in freshwater has been proposed (Piermarini and Evans 2001; Piermarini et al. 2002; reviewed by Evans et al. 2005) (Figure 3). The number of Na^+, K^+-ATPase rich cells localised on the gill lamellae of freshwater *D. sabina* are approximately seven times greater than in their marine counterparts, and more than twice as high as saltwater-acclimated *D. sabina*; thus providing evidence for the gill's role in salt ion uptake in a freshwater environment (Piermarini and Evans 2000). Further, Na^+, K^+-ATPase rich cells also appear to be associated with acid secretion (Piermarini and Evans 2001; Tresguerres et al. 2005). This involves hydrogen ion excretion in an electroneutral exchange for environmental sodium, via a Na^+/H^+ exchange mechanism driven by Na^+, K^+-ATPase (Figure 3). Basolateral Na^+, K^+-ATPase actively pumps gill intracellular sodium ions across the basolateral membrane into the plasma, maintaining low intracellular sodium concentrations, which is favourable for apical sodium uptake from the environment in exchange for hydrogen ion excretion (Piermarini and Evans 2001; Evans et al. 2004). The elasmobranch gill also contains another type of mitochondrion-rich cell, distinct from that of Na^+, K^+-ATPase, with the active transport enzyme, vacuolar proton-ATPase (V-H^+-ATPase) (Figure 3) (Wilson et al. 1997; Piermarini and Evans 2001; Piermarini et al. 2002; Tresguerres et al. 2005). V-H^+-ATPase appears to be associated with chloride ion uptake and bicarbonate ion excretion: important for osmoregulation and acid–base regulation (Piermarini and Evans 2001; Evans et al. 2004; Tresguerres et al. 2005).

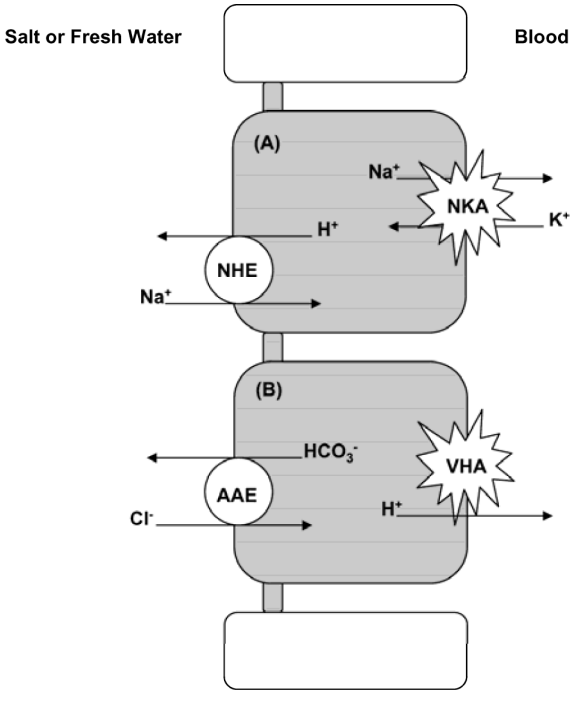

Figure 3. Hypothesised model of acid–base and NaCl transport in the elasmobranch gill. A distinct mitochondira-rich cell (A) with Na^+, K^+-ATPase is likely the site of hydrogen ion excretion in an electroneutral exchange for environmental sodium. Basolateral Na^+, K^+-ATPase (NKA) actively pumps gill intracellular sodium ions across the basolateral membrane into the plasma, maintaining low intracellular sodium concentrations, favourable for apical sodium uptake in exchange for hydrogen excretion via a sodium/hydrogen exchange (NHE) mechanism. Another distinct mitochondrion rich cell (B) with V-H^+-ATPase is likely the site of chloride ion uptake and bicarbonate ion excretion. Basolateral V-H^+-ATPase (VHA) seems to actively pump hydrogen ions across the basolateral membrane, creating an intracellular bicarbonate concentration, favourable for apical bicarbonate secretion and chloride ion uptake via an apical anion exchanger (AAE). Figure re-drawn and used with permission from Piermarini and Evans, 2001 and The Company of Biologists Ltd.

Basolateral V-H^+-ATPase seems to actively pump hydrogen ions across the basolateral membrane, creating an intracellular bicarbonate concentration that is favourable for apical bicarbonate secretion and chloride ion uptake via an apical anion exchanger (Figure 3). Piermarini and Evans (2001) found that the activity and abundance of V-H^+-ATPase is about five times higher in the gills of *D. sabina* in fresh versus salt water, suggesting its role in active NaCl uptake and excretion of HCO_3^-. Based on infusions of *S. acanthias* with base ($NaHCO_3$), Tresguerres et al. (2005) demonstrated the importance of V-H^+-ATPase in base secretion at the gill of *S. acanthias* and proposed that V-H^+-ATPase are inserted in the basolateral membrane under alkalotic stress, functioning to rid the cell of excess hydrogen ions generated by hydration of carbon dioxide by intracellular carbonic anhydrase. Thus, the elasmobranch gill appears to be important for salt ion uptake and acid–base balance as Na^+, K^+-ATPase and V-H^+-ATPase rich cells are sites of Cl^- uptake/HCO_3^- excretion and Na^+ uptake/H^+ excretion respectively (Piermarini and Evans 2001; Evans et al. 2004; Tresguerres et al. 2005).

Branchial elimination of salts in marine elasmobranchs has generally been considered to be insignificant (see review by Evans et al. 2004). Haywood (1975) demonstrated that when both the urinary system and rectal gland of *P. africanum* was ligated, the shark was still able to regulate its levels of sodium and chloride to normal equilibrium concentrations. He hypothesised that the gills may be excreting excess salt in compensation for the ligated rectal gland. Studies since Haywood (1975) have not been able to experimentally verify branchial salt excretion. For example, Wilson et al. (2002) found that when the rectal gland was removed from *S. acanthias* exposed to a saltwater environment, gill Na^+, K^+-ATPase-rich cell number and activity did not increase as would be expected if branchial excretion of salts was occurring in compensation for the rectal gland. Acclimation of freshwater *D. sabina* to salt water was associated with a decrease in branchial Na^+, K^+-ATPase activity (Piermarini and Evans 2000). Tresguerres et al. (2005) infused *S. acanthias* intravenously with salt solutions and found no evidence for gill excretion of salt, while plasma osmolarity and salt concentrations remained constant. They reasoned that the extra salt load was being secreted by the rectal gland and not the gills (Tresguerres et al. 2005). Pillans et al. (2004) found no difference in branchial Na^+, K^+-ATPase activity when bull sharks were acclimated from freshwater to saltwater; however they did find an increase in Na^+, K^+-ATPase activity in the bull shark's rectal gland, further suggesting that excess salt elimination was not occurring at the gills, but rather the rectal gland. Thus, the elasmobranch gill appears to be important for salt uptake and acid–base balance, while the rectal gland appears to be the primary site for salt excretion (Piermarini and Evans 2000; Wood et al. 2002; Hazon et al. 2003).

Kidney salt excretion

The kidney likely also plays a role in the regulation of sodium chloride (Friedman and Herbert 1990; Lacy and Reale 1991; Hazon et al. 2003; Evans et al. 2004). Elasmobranchs have the capacity to alter kidney function in response to environmental salinity. When elasmobranchs are acclimated to dilute environments, urine flow rate increases twenty to fifty fold (Evans et al. 2004), resulting in increased Na^+, Cl^-, Mg^{2+}, SO_4^{2-} and urea excretion (Smith 1931; Goldstein et al. 1968; Goldstein and Forster 1971a; Wong and Chan 1977; Sulikowski and Maginniss 2001; Hazon et al. 2003). In the saltwater environment, sodium and chloride ions are reabsorbed, but to a much lesser extent than urea, likely via a sodium potassium countercurrent co-transporter (reviewed by Evans et al. 2004).

The removal of the rectal glands in *S. acanthias* and *P. africanum*, inhabiting a saltwater environment, did not result in significant changes of plasma salt ion levels (Burger 1965; Haywood 1975, Wilson et al. 2002), suggesting possible compensatory salt excretory roles by the gills (discussed in *Salt regulation and acid–base balance at the gills*, p. 219) and/or the kidneys. Evans et al. (2004) noted that the urine total osmotic concentration is below that of the plasma indicating that the elasmobranch kidney is probably not able to create a net secretion of salt and therefore cannot solely compensate for the rectal gland after its removal. Although marine and euryhaline elasmobranchs in seawater can excrete gains of salt in part by urinary output, the kidney is not the major site of salt excretion as has been demonstrated for the rectal gland.

Haematocrit and water content

As elasmobranchs acclimate to changes in environmental salinity, total water content and the distribution of water among the fluid containing compartments is maintained fairly

constant despite changes in osmotic pressure of the body fluids. There appears to be no measurable change in haematocrit (percentage by volume of red blood cells) in elasmobranchs moving between fresh and salt water. Piermarini and Evans (1998) demonstrated that haematocrit did not change significantly when freshwater *D. sabina* were exposed to 100% salt water in the lab for 8 days; however, plasma osmolarity, Na^+, Cl^- and urea increased significantly. Thorson et al. (1973) and Pillans et al. (2004) provided evidence that bull sharks found under fresh and marine conditions have virtually equal haematocrit values, suggesting that diffusional gains of water are compensated for, likely in part by increased urinary output. Acclimation to the freshwater environment does not appear to cause extracellular plasma volume to increase (Thorson 1961; Goldstein et al. 1968; Thorson et al. 1973). For example, Goldstein et al. (1968) transferred the lemon shark, *Negaprion brevirostris*, to 50% seawater over a period of 7 days and maintained it there for 7 days. A drop in plasma urea concentration and a nearly 3-fold increase in urea clearance from the body fluids were documented; but, there was no significant change in haematocrit. These observations provide evidence that euryhaline elasmobranchs and possibly marine elasmobranchs acclimated to reduced salinities maintain water balance when moving between salt and fresh water; however, haematocrit provides a partial picture of the maintenance of body fluid compartments. To better understand water balance, extra and intracellular tracer and marker studies are needed.

Osmoregulation in developing elasmobranchs, neonates and juveniles

Studies on the ontogeny of osmoregulatory systems in elasmobranchs are limited and warrant further research. However, it appears that, for at least the species investigated to date, elasmobranchs have the ability to osmoregulate from at least birth and some species may develop this ability prior, in a similar manner as the adults. Embryos of the big skate, *Raja binoculata*, retain high urea and TMAO concentrations independent of their mothers (Read 1968a). Embryos from both *R. binoculata* and *S. acanthias* contain OUC enzymes with activities independent of maternal control (Read 1968b). In the viviparous smooth-hound shark, *Mustelus* sp., and eagle ray, *Myliobatis* sp., the young are bathed in urea-rich uterine fluid throughout gestation (Price and Daiber 1967). Urea concentrations in the developing pups' blood and in uterine fluid are higher than those of the mother's blood (Price and Daiber 1967). In *S. acanthias*, developing embryos are exposed to seawater when gestation is only about one-quarter complete (Price and Daiber 1967) and Jones and Price (1974) demonstrated that *S. acanthias* pups are capable of appropriately regulating plasma urea and chloride with changes in salinity. *Raja erinacea* embryos contain significant levels of OUC enzymes as well as synthesise and retain urea, as well as other osmolytes, in order to osmoregulate with changes in external salinity (Steele et al. 2004). These osmoregulatory mechanisms in *R. erinacea* are in place as early as 4 months, around the time at which the egg capsule opens and the embryo becomes bathed by the external environment (Steele et al. 2004). Thorson and Gerst (1972) showed that serum urea levels of uterine pups of the euryhaline bull shark resemble those of the mother and change accordingly as she passes from fresh to salt water and back. Neonatal or juvenile bull sharks can live in either fresh or saltwater at birth, and juveniles caught in totally freshwater habitats have serum parameters (including urea) similar to those of adult bull sharks in the same environment (Thorson et al. 1973; Pillans and Franklin 2004). Pillans and Franklin (2004) and Pillans et al. (2004) demonstrated that juvenile bull sharks have the osmoregulatory plasticity to acclimate to changes in environmental salinity. Pillans and Franklin (2004) suggest that this ability to

osmoregulate from birth has ecological implications since euryhaline juveniles may be able to optimally exploit a freshwater or marine habitat based on lower predator densities and/or higher food availabilities.

Drinking

Marine teleosts drink to maintain ionic and water balance, with excess ions being excreted (reviewed by Evans 1993 and Evans et al. 2005). Elasmobranchs have the physiological ability to drink and two sharks, *S. canicula*, and the Japanese dogfish, *Triakis scyllia*, have been shown to do so following rapid transfer from a hypo- to a hyper-osmotic medium, which requires an increase of plasma osmolarity (Armour et al. 1993; Anderson et al. 2002). Following transfer from a hypo- to a hyper-osmotic environment, *S. canicula*, increased its drinking rate (Anderson et al. 2002). Further, Armour et al. (1993) found that following transfer to 130% seawater, *S. canicula* fed a low protein diet had high plasma concentrations of Na^+ and Cl^- and a large volume of seawater in the its gut, suggesting that the dogfish may have been drinking to maintain blood volume. This suggests that at least some elasmobranchs control drinking rate to regulate osmolarity in response to rapidly changing exposures to salinity. Hormonal control of drinking rate in elasmobranchs is an active area of research (see reviews by Hazon et al. 2003; Evans et al. 2004; Gelsleichter 2004; Takei and Loretz 2005); however, beyond the scope of this review.

Diet

While the environmental salinity undoubtedly exerts the major influence on the regulation of body fluids in elasmobranchs, diet also influences these vital processes. Haywood (1973) demonstrated that infrequently fed *P. africanum* could not adequately osmoregulate under acclimation to varying degrees of salinity in the laboratory; however, well-fed sharks could. Reduction in food intake resulted in reduced metabolic urea production to the extent of depressing serum osmolarity resulting in degrees of hypo-osmotic regulation (Haywood 1973). Armour et al. (1993) found that *S. canicula* fed a low protein diet had significantly decreased blood production of urea in normal (100%) salt water compared to fish fed a high protein diet. Plasma urea concentration required to regulate iso-osmotically to the environment was achieved through a substantial decrease in urea clearance (Armour et al. 1993). In 130% salt water, dogfish fed on a low protein diet were unable to appropriately increase plasma urea concentration to effectively osmoregulate; however, dogfish fed a high protein diet were capable (Armour et al. 1993). Armour et al. (1993) further demonstrated that in 130% salt water, dogfish fed both high and low protein diets maintained high blood concentrations of sodium and chloride ions. High plasma concentrations of Na^+ and Cl^-, plus the presence of a large volume of sea water in the gut implies that these sharks may have been drinking. In a study by Wood et al. (2002), potamotrygonid rays were acclimated in the laboratory to their native, ion-poor waters of the Rio Negro, Brazil, as well as to ion-rich hard water. Unidirectional Na^+ and Cl^- flux rates were measured with radiotracers. The rays experienced negative salt balance in their native ion-poor waters (salt efflux rate exceeded influx rate from the water). Efflux rates for both Na^+ and Cl^- were lower in the ion-poor waters compared to ion-rich hard waters, yielding much lower balance points (external Na^+ or Cl^- levels at which influx and efflux were equal). However, the rays were not eating, so Wood et al. (2002) proposed that they may require food-derived salts from their environment for successful adaptation to the Rio Negro. Further investigation is

needed to understand how starved marine elasmobranchs, no longer able to appropriately osmoregulate via the OUC, effectively raise plasma osmolarity by deriving salts from eating or drinking in conjunction with minimising salts and/or urea excretions.

Future considerations

The present article reviews current gaps in the field of elasmobranch osmoregulation that warrant further research. These include: (1) the mechanism(s) responsible for the low permeability of elasmobranch gills to urea; (2) the possible role of ureolytic bacteria in elasmobranch osmoregulation; (3) the ontogeny of osmoregulatory systems in elasmobranchs; and (4) the relative importance of environmentally derived salts from drinking or eating in starved marine elasmobranchs.

Osmoregulation in elasmobranchs continues to be an area of active research; but, the majority of studies are laboratory based. Although elasmobranch behaviour is governed by many external and internal stimuli, field studies are needed to determine if elasmobranch movements and activity patterns are, in part, governed by their ability to osmoregulate in various habitats. For example, it has been suggested that juvenile bull sharks may selectively exploit freshwater or marine habitats based on lower predator density and/or higher food availability (Pillans and Franklin 2004). New acoustic telemetry studies allow opportunities to tag elasmobranchs as well as other species with transmitters able to record position and environmental variables, such as water quality (e.g. Sundström et al. 2001; Simpfendorfer et al. 2004). Thus, various elasmobranchs can be simultaneously tracked to compare behaviours and movements with respect to factors such as salinity, water temperature, pH, oxygen levels as well as measured presence or absence of prey and predators.

Habitat modifications, such as pollution, may impact elasmobranch osmoregulation. High waterborne silver exposure has been shown to result in respiratory and osmoregulatory failure in spiny dogfish (DeBoeck et al. 2001). Elasmobranchs are ecologically important components in virtually every marine habitat (Compagno 1990). Thus, future research should seek to understand how current habitat modifications (e.g. changes in salinity, increases in temperature, addition of toxins) affect elasmobranch osmoregulation and how this may in turn impact their feeding, movements, development and reproduction.

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