J. Membrane Biol. 212, 93–107 (2006)
DOI: 10.1007/s00232-006-0869-5

The Journal of
Membrane
Biology

The Physiology and Evolution of Urea Transport in Fishes

M.D. McDonald[1], C.P. Smith[2], P.J. Walsh[3]
[1]NIEHS Marine and Freshwater Biomedical Sciences Center, Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, Florida, 33149-1098, USA
[2]Faculty of Life Sciences, University of Manchester, Manchester, M13 9PT, UK
[3]Department of Biology, University of Ottawa, Ottawa, Ontario KIN 6N5, Canada

Received: 21 November 2005/Accepted: 20 July 2006

Abstract. This review summarizes what is currently known about urea transporters in fishes in the context of their physiology and evolution within the vertebrates. The existence of urea transporters has been investigated in red blood cells and hepatocytes of fish as well as in renal and branchial cells. Little is known about urea transport in red blood cells and hepatocytes, in fact, urea transporters are not believed to be present in the erythrocytes of elasmobranchs nor in teleost fish. What little physiological evidence there is for urea transport across fish hepatocytes is not supported by molecular evidence and could be explained by other transporters. In contrast, early findings on elasmobranch renal urea transporters were the impetus for research in other organisms. Urea transport in both the elasmobranch kidney and gill functions to retain urea within the animal against a massive concentration gradient with the environment. Information on branchial and renal urea transporters in teleost fish is recent in comparison but in teleosts urea transporters appear to function for excretion and not retention as in elasmobranchs. The presence of urea transporters in fish that produce a copious amount of urea, such as elasmobranchs and ureotelic teleosts, is reasonable. However, the existence of urea transporters in ammoniotelic fish is curious and could likely be due to their ability to manufacture urea early in life as a means to avoid ammonia toxicity. It is believed that the facilitated diffusion urea transporter (UT) gene family has undergone major evolutionary changes, likely in association with the role of urea transport in the evolution of terrestriality in the vertebrates.

Correspondence to: M.D. McDonald; email: dmcdonald@rsmas.miami.edu

Key words: Facilitated diffusion — Active transport — UT-A — Gill — Kidney — Excretion

Introduction

Apart from mammals, fish are the most intensively studied vertebrates with respect to urea transport processes. No doubt this is at least in part due to pioneering studies in the late 19th and early 20th centuries documenting the exceptionally large gradients for urea in cartilaginous fishes. More recently, this interest has been further stimulated by observations of significant urea production and excretion in at least some species of bony fishes as well. This review describes an overview of what is currently known about these transport processes in fishes in the context of their current physiological processes, and it also takes a retrospective look at how urea transport has evolved within the vertebrates from a piscine perspective. We build upon several past reviews and the reader is also referred to these (Walsh, 1997; Walsh et al., 2001a; Walsh & Smith, 2001; Wood et al., 2003).

Urea Transport in Red Blood Cells and Hepatocytes

A brief synopsis of urea transport in mammalian hepatocytes and red blood cells is first presented to provide a framework for discussion of mechanisms in these tissues in fish. Since in mammals the liver is the primary tissue of ureagenesis, there is a need for urea export and urea can potentially move out of hepatocytes down its concentration gradient via either a broadly specific aquaporin (AQP-9) or UT-A (Klein et al., 1999; Carby et al., 2003). However, since arginase (the specific enzyme generating urea) is cytoplasmic, there would not seem to be a need for

specific mitochondrial urea transport in mammalian hepatocytes. For mammalian red blood cells (RBCs), a UT-B is present instead of a UT-A (*see* 'Piscine Perspectives' below). It was initially suggested that the physiological role of urea transporters in RBCs was to prevent detrimental volume change as they pass through the *vasa recta* and experience vast changes in the concentrations of urea (Macey & Yousef, 1988). In this regard, there is a human condition where a UT-B gene mutation prevents UT-B protein expression. People with this mutation (Jk_{null}) are devoid of UT-B protein. If UT-B was important to maintain RBC integrity then one would expect evidence of damaged RBCs and hemolysis, yet Jk_{null} individuals are asymptomatic and only when challenged by thirsting do they show a mild reduction in their ability to concentrate urine (Sands et al., 1992). As Smith and Walsh (2001) point out, perhaps a more likely function of UT-B is that its presence prevents the rapid washout of the medullary urea gradient by circulating RBCs. The fact that UT-B has asymmetric transport properties, in that urea moves out of RBCs (efflux) more effectively than it moves in (influx) supports this hypothesis (Macey & Yousef, 1988). From this discussion it can be postulated that animals such as fish lacking the ability to concentrate their urine as greatly as mammals, and thus presumably with much lower standing concentrations of renal urea, may have no requirement for ''outwardly directed'' urea transporters in their RBCs. To date, the admittedly limited literature appears to bear out this lack of urea transporters in fish RBCs.

Fish Red Blood Cells

Relatively speaking, considerably more work has been carried out to address urea transport across RBC membranes than for hepatocytes in fish. For elasmobranchs, initial studies by Murdaugh, Robin and Hearn (1964), measuring lysis times of *Squalus acanthius* RBCs in several artificial solutions, suggested the presence of urea transporters. However, Rabinowitz and Gunther (1973) later rejected this hypothesis using more stringent experimental tests on *Squalus suckleyi*, including [^{14}C]-tracer methods. Kaplan et al. (1974) similarly reported no evidence for facilitated transport in erythrocytes from *Squalus acanthias* and *Raja erinecea*. Walsh et al. (1994b) extended this conclusion by showing no effect of phloretin or acetamide on the influx of urea into RBCs of the lesser spotted dogfish *Scyliorhinus canicula*. In a study of skate erythrocytes, Carlson and Goldstein (1997) seem to have established that urea in fact permeates via the lipid phase of the membrane. The evolutionary implications of the apparent lack of specialized urea transport in elasmobranch RBCs are discussed further below (*see* 'Piscine Perspectives').

In teleosts, studies of urea transport in RBCs are also relatively limited. Kaplan et al. (1974) showed the absence of facilitated transport of urea in erythrocytes of winter flounder (*Pseudopleuronectes americanus*) and goosefish (*Lophius piscatorius*). Walsh et al. (1994) discovered a small (25%) phloretin-sensitive component of urea uptake by redfish (*Scianops ocellatus*) RBCs but a lack of inhibition of urea transport by phloretin and acetamide in RBCs of the gulf toadfish (*Opsanus beta*). The latter finding agrees with the lack of a detectable UT homologous signal in *O. beta* RBC mRNA (Walsh et al., 2000).

Fish Hepatocytes

Study of urea transport in elasmobranch hepatocytes is limited to one work that supports a passive mode of urea transport (Walsh et al., 1994b). Given this observation and those on RBCs, however, it is tempting to conclude that with the obvious exception of possible gradients within specialized transport tissues (gills and kidney) aimed at major transepithelial movement, urea concentrations within the elasmobranch body are largely uniform. Thus, urea movement responds slowly to the overall poise set by synthesis and transport/leak at the animal-environment interface. Clearly, further molecular studies are required to determine more precisely the presence/absence of transporters in various tissues.

In teleosts, *O. beta* and *O. tau* (oyster toadfish) hepatocytes exhibited substantial inhibition of urea influx and efflux by phloretin (Walsh et al., 1994b; Walsh & Wood, 1996). Surprisingly, however, Northern analysis of *O. beta* liver did not detect UT-homologues (Walsh et al., 2000). It is possible that broadly specific AQPs accomplish urea transport in fish hepatocytes. Certainly, more species of both elasmobranchs and teleosts need to be examined. Especially in the case of the toadfishes, we may be creating a biased view of hepatic urea transport since this is an atypical group in terms of ureagenesis and ureotely relative to other teleosts where ammoniotely predominates.

One further interesting aspect of urea production in both elasmobranchs and teleosts is that the urea-producing enzyme, arginase, is located primarily in mitochondria in many species (Mommsen & Walsh, 1989; Anderson & Walsh, 1995; Jenkinson, Grody & Cederbaum, 1996; Wright et al., 2004) and not in the cytoplasm as seen in mammals and other vertebrates with CPSase I. It is possible that urea efflux from mitochondria in fish thus requires a specialized transporter. Another interesting facet is that this may be true for not only liver, but extra-hepatic tissues such as muscle, where as much as 50% of urea production can occur in several species of fish (Anderson, 2001; Steele, Yancey & Wright, 2005; Kajimura et al., 2006).

Urea Transport for Excretion

Elasmobranchs

Städeler and Frérichs (1858) are documented to be the first to discover that "colossal quantities" of urea could be found throughout the tissues of elasmobranchs (reviewed by Smith, 1936). The intense research that followed established early on that sharks, rays and skates retain $\sim$ 300–600 mmol·l^{-1} urea to keep their plasma osmolality slightly above that of seawater for the purpose of osmoregulation. To hold these elevated concentrations of urea within their body fluids and tissues against a massive gradient (urea concentration in seawater is < 0.01 mmol $\cdot$ l^{-1}), elasmobranchs need an effective way to both produce urea and retain it within their bodies. Accordingly, much like terrestrial organisms, elasmobranchs have a fully functional ornithine-urea cycle (O-UC) and maintain a tight regulation on urea excretion and leakage, using urea transporters found in the kidney and gill.

The Kidney

Early measurements of urine urea concentrations indicated that they are approximately one-third those of blood, a finding that initiated concentrated study with respect to renal urea transport in the elasmobranch kidney (Denis, 1913; Smith, 1931a, b, 1936; reviewed by Hickman & Trump, 1969). Kempton (1953) found that a substantial amount of urea was filtered across the glomeruli, however, most if not all (70–99.5%) of the urea was then reabsorbed by the kidney tubules, resulting in the relatively low urine urea concentrations. Classic studies on the reabsorptive mechanism uncovered that the specificity of the system was such that urea and the urea analogues methylurea and acetamide were reabsorbed by the renal tubules but thiourea, a third urea analogue, was not (Schmidt-Nielsen & Rabinowitz, 1964). A high correlation was found between urea and Na^+ reabsorption; both substances being transported in a ratio of 1.6 urea:1 Na^+ that persisted even with manipulation of extracellular fluid volume (Schmidt-Nielsen, Truniger & Rabinowitz, 1972). This finding suggested the involvement of an active, Na^+-coupled urea transporter, much like what has been measured in mammalian kidney proximal tubules, although not cloned to date (Isozaki, Verlander & Sands, 1993, Isozaki et al., 1994a, b; Sands, Martial & Isozaki, 1996; Kato & Sands, 1998). However, continued work on elasmobranchs demonstrated that, when administered intravenously, phloretin, a potent inhibitor of facilitated urea diffusion by what we now know as either UT-A, UT-B or UT-C transporters, resulted in a 30% decrease in both urea and Na^+ reabsorption (Hays et al., 1977). At the time, this finding added fuel to the already heated debate on whether renal urea reabsorption in elasmobranchs was by Na^+-coupled secondary active transport or facilitated diffusion.

Recently, however, molecular analysis has uncovered a gene that showed high homology to mammalian UT-A2 facilitated diffusion urea transporters in dogfish shark, *Squalus acanthias*, kidney and brain (shUT; Smith & Wright, 1999). Since this breakthrough, UTs have been found in other elasmobranchs (i.e., three transcripts (3.1, 2.8, 1.6 kb) of SkUT in the little skate, *Raja erinacea*, Morgan, Ballantyne and Wright, 2003a; *Triakis*UT in the dogfish *Triakis syllia*, Hyodo et al., 2004), shifting the general opinion in support of urea reabsorption occurring by facilitated diffusion, as originally suggested by Boylan (1972) and then elaborated on by Friedman and Hebert (1990). This theory is strongly based on the complicated architecture of the elasmobranch kidney, which has a nephron arrangement that results in not one loop, as in the mammalian kidney, but several, forming a complicated countercurrent system believed to be important in fluid regulation. The arrangement creates two distinct anatomical zones; loops I and III are enclosed by a peritubular sheath creating the "bundle zone" and loops II and IV are unconfined within the blood sinus or the "sinus zone" (Lacy et al., 1985). The facilitated diffusion theory is also based on the then assumption that there is differential urea permeability across the various nephron segments (Boylan, 1972; Friedman & Hebert, 1990). This is now supported by a recent study that localized *Triakis*UT (also expressed in the brain and liver) to the dogfish renal collecting duct (*i.e.*, the last segment in the bundle zone) and no other renal segment (Hyodo et al., 2004).

However, this evidence does not rule out Na^+-coupled urea transport in the elasmobranch kidney and it now appears that both facilitated diffusion and Na^+-coupled transport may be responsible for renal urea transport, much like the mammalian kidney. Using an *in vitro* isolated brush border (apical) membrane vesicle preparation, Morgan and coworkers (Morgan, Wright & Ballantyne, 2003b) found phloretin-sensitive, non-saturable urea transport in the bundle zone supporting their work showing skUT expression in this zone of the kidney (Morgan et al., 2003a; Fig. 1*A*). In the sinus zone, they found a phloretin-sensitive, Na^+-linked urea transporter that showed Michaelis-Menton saturation kinetics with a low K_m suggestive of a high affinity transporter (K_m = 0.70 mmol·l^{-1} and a V_{max} = 1.18 μmol·h^{-1}·mg^{-1}). Morgan et al. (2003a) also found skUT expression in the sinus zone and together these data suggest the presence of both a phloretin-sensitive skUT and perhaps a Na^+-urea cotransporter in the apical membrane of sinus zone nephron segments (Fig. 1*B*). Interestingly, these

findings support both early studies by Schmidt-Nielson et al. (1972) and Hays et al. (1977) showing both Na^+ dependence and phloretin sensitivity.

The primary site of regulation of urea tissue levels in elasmobranchs is the kidney, not the gill (Goldstein & Forster, 1971a, b; Payan, Goldstein & Forster, 1973) and one of the main reasons for regulation is environmental dilution. Elasmobranchs are typically marine, however, some species have the osmoregulatory capacity for tolerating reduced environmental salinities. As stated above, urea retention, accomplished by specific transporters in the gill and kidney, is an essential component of the osmoregulatory strategy of marine elasmobranch fishes. However, in more dilute environments, the osmotic pressure of the environment is reduced and, correspondingly, euryhaline elasmobranchs must decrease urea levels. Studies report that elasmobranchs moving into more dilute environments can reduce their urea levels so that they are 30–70% lower than their marine counterparts (Smith, 1931b; Urist, 1962; Thorson, 1967; Price & Creaser, 1967; Goldstein & Forster, 1971a, b; de Vlaming & Sage, 1973; Forster & Goldstein, 1976; Chan & Wong, 1977; Cooper & Morris, 1998; Sulikowski & Maginniss, 2001; Morgan et al., 2003a), yet these fish still maintain very high concentrations of urea in their plasma (100–250 $mmol \cdot l^{-1}$) compared to freshwater teleosts (1–2 $mmol \cdot l^{-1}$; Wood, 1993; McDonald & Wood, 1998). The reverse is also true; a 58% increase in plasma urea concentrations has been measured when the euryhaline Atlantic stingray, *Dasyatis sabina*, that normally resides in freshwater, is transferred into seawater (Piermarini & Evans, 1998).

In general, acclimation to more dilute environmental conditions results in an increase in glomerular filtration, to help rid the fish of excess water, and a decrease in renal tubular urea reabsorption, resulting in an increase in the renal elimination of urea (Smith, 1931b). To date, Morgan and coworkers (2003a) have been the only group to demonstrate that the reduction in urea reabsorption could be due to a reduction in the expression of renal urea transporters. Namely, upon exposure to 50% seawater, there was a significant decrease in the tissue concentration of urea in both bundle and sinus zones of the kidney in the little skate, *Raja erinacea* (Morgan et al., 2003a). At the same time, the levels of all three skUT transcripts were significantly diminished in the kidney by up to 3.5-fold, with the 3.1 kb isoform appearing to show more sensitivity to the environmental change.

The Gill

It was in 1967 when J. Boylan made the observation that the elasmobranch gill epithelium, a structure of only one to two cell layers thick, was essentially the retaining wall for an immense urea concentration gradient against a virtually urea-free seawater environment. Not surprisingly, the gill epithelium contributes to >90% of the internal urea lost to the environment; in comparison, the kidney is very effective at minimizing urinary urea excretion (Wood et al., 1985). However, if the elasmobranch gill epithelium had a permeability to urea that was similar to that of teleost fish, it would result in a urea efflux across the gill that was 40 times higher than the actual measured value (Boylan, 1967; Wood, Pärt & Wright, 1995b; Pärt, Wright & Wood, 1998). Early work by Boylan (1967) was the first to establish the low permeability of the elasmobranch gill to urea by examining the movement of [^{14}C]-labeled urea and the urea analogue, thiourea, from the blood into the water. He also found that thiourea permeabilities at the gill were equal to those of urea, in contrast to the kidney where thiourea was not reabsorbed, suggesting different transporters in the two organs (Boylan, 1967).

Later work by Wood and coworkers (1995) observed that intravenous infusion of thiourea or acetamide resulted in an increase in branchial urea excretion in *Squalus acanthias*, helping them to establish the hypothesis that a gill urea "backtransporter" was responsible for returning the leak of urea into the cell back to the blood plasma. Competitive interference with this transporter by thiourea or acetamide would allow the leak to continue unabated, leading to an increase in net excretion. Led this time by Pärt, the same group elaborated on this hypothesis by supposing that the location of the backtransporter would be on the basolateral membrane (Pärt et al., 1998). Thus, the net excretion of urea across the gill epithelium would be a balance between the passive leakage of urea across the basolateral membrane into the cell and the action of a basolateral urea transporter pumping the urea back into the blood. Using the *in situ* isolated perfused head preparation from *Squalus acanthias*, Pärt and coworkers (1998) were able to demonstrate that the basolateral membrane was approximately 14 times more permeable to urea than the apical membrane and that phloretin addition to the perfusate nearly doubled urea excretion rates. They concluded that the apical membrane was extremely impermeable to urea and the low permeability of the basolateral membrane was in part due to the active backward pumping of urea into the blood.

It is now known that the low permeability of the gill epithelium to urea, especially, and to other solutes (i.e., NaCl, water), in general, is due to an unusual basolateral membrane lipid composition (Fines, Ballantyne & Wright, 2001). To elaborate, cholesterol content within a lipid bilayer is directly correlated with the permeability of a membrane to urea. Lipid analysis of the basolateral membrane of the spiny dogfish, *Squalus acanthias*, revealed high levels of cholesterol contributing to the highest cholesterol to

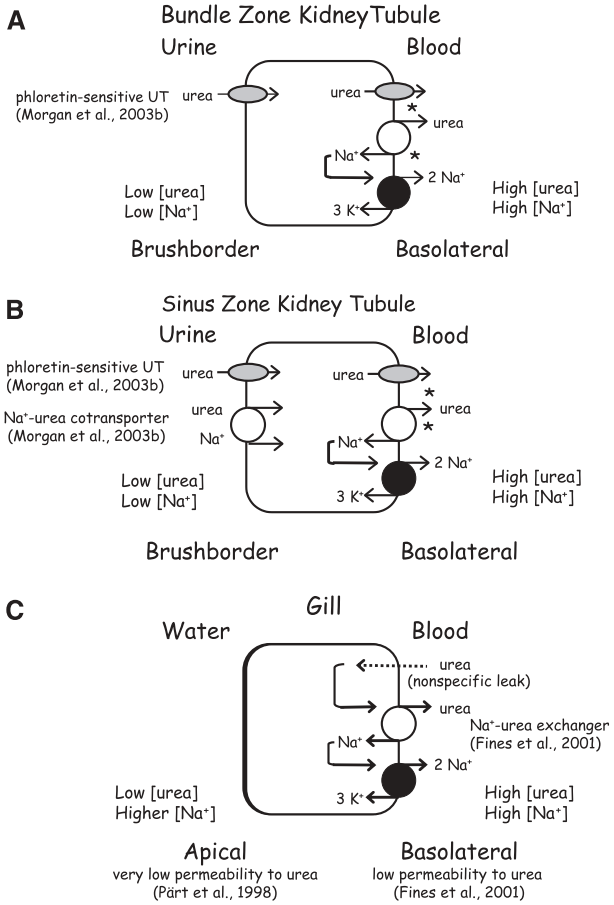

Fig. 1. Hypothetical urea transport model of the elasmobranch kidney tubule bundle zone (*A*), sinus zone (*B*), and gill (*C*). Urea would cross the brushborder membrane of a sinus zone tubular cell via a Na^+-urea cotransporter and a phloretin-sensitive UT and of a bundle zone tubular cell via a phloretin-sensitive UT, as shown in the little skate (Morgan et al., 2003b). In the gill, urea would leak across the basolateral membrane and enter the gill cell (Fines et al., 2001). Urea would be pumped out basolaterally in exchange for Na^+, driven by the Na^+, K^+ ATPase (Fines et al., 2001). The apical membrane has a very low permeability to urea, as suggested by Pärt et al. (1998). Asterisks denote hypothesized transporters in the basolateral membrane that have not been resolved experimentally.

phospholipid molar ratio (3.68) ever reported (Fines et al., 2001). In addition, there is now clear evidence for a basolateral gill urea "backtransporter" (Fines et al., 2001), as originally hypothesized by Wood et al. (1995) and Pärt et al. (1998). Analysis of urea uptake by gill basolateral membrane vesicles (BLMV) revealed the presence of phloretin-sensitive, Na^+-dependent, secondary active urea transport that demonstrated Michaelis Menton kinetics (K_m = 10.1 mmol·l^{-1} and V_{max} = 0.34 μmol·mg protein^{-1}·h^{-1}) suggestive of a high-affinity transporter (Fines et al., 2001). This transporter would act to scavenge intracellular urea and pump it from the cell back into the blood in exchange for Na^+ (i.e., counter transport), the Na^+ would then get pumped out of the cell via the Na^+, K^+ ATPase (Fig. 1*C*). This would act to decrease the intracellular urea concentration of the gill epithelial cells, reducing the concentration gradient for urea across the apical surface of the cells and thus the rate of urea diffusion or loss across the gill.

Phloretin sensitivity is a characteristic of both facilitated (UT) and secondary active Na^+-urea countertransport systems (Chou & Knepper, 1989; Isozaki et al., 1993, 1994a, b; Sands et al., 1996; Kato & Sands, 1998), but not Na^+-urea cotransporters. Thus, the phloretin sensitivity shown by both Pärt et al. (1998) and Fines et al. (2001) is consistent with the presence of a Na^+-urea countertransporter. Interestingly, under low stringency conditions, Smith and Wright (1999) did find evidence for a UT homologue in *Squalus acanthias* gill tissue using Northern analysis. The location of this UT has never been pursued (i.e., basolateral or apical membrane, branchial blood vessels) and its existence has essentially been ignored. However, if this transporter does exist in the gill epithelium, it is likely not present in the apical membrane, as it is much less permeable to urea than the basolateral membrane (Pärt et al., 1998).

To date, there are no studies that have focused on the regulation of branchial urea transport in elasmobranchs. During acclimation to a dilute environment, when there are major changes in the renal handling of urea, the permeability of the gill to urea stays constant (Goldstein & Forster, 1971a, b; Payan et al., 1973).

Teleost Fish

Unlike terrestrial animals and elasmobranchs, most teleosts (bony fishes) do not produce urea as their primary nitrogenous waste. Instead, being surrounded by water allows teleosts to excrete the more toxic but less metabolically expensive ammonia, which passively moves across their gills and is diluted by the environment. This is in contrast to land-dwelling animals that essentially cannot excrete enough water to dilute ammonia to a nontoxic level without becoming dehydrated. However, most teleost fish do produce some urea and generally these fish can be split into three distinct groups with respect to urea metabolism and excretion: *i.* those that always produce and excrete primarily urea (obligately ureotelic), *ii.* those that occasionally produce and excrete primarily urea (facultatively ureotelic) and *iii.* those that produce or excrete primarily ammonia and very little urea (ammoniotelic), with the majority of teleost fish falling under this category. Based on these differences, one can imagine that the need for urea transporters would vary depending on the level of urea production, with fish producing very little urea requiring little or no urea transport. In fact, evidence

suggests that both ammoniotelic and ureotelic fish have urea transporters regardless of the apparent necessity of these proteins in their daily survival. Since the two main routes of nitrogen excretion in teleosts are the gill and kidney, it is not surprising that research has focused on these two organs. The following will describe the types of teleost fish on the basis of ureotely and urea transporters found to date.

Obligately Ureotelic Teleosts

With respect to urea transport, obligately and facultatively ureotelic teleost fish are the exception to most other teleost fish in that, similar to terrestrial animals and elasmobranchs, they have a fully functional ornithine urea cycle (O-UC) as adults that allows them to effectively produce copious amounts of urea. The Lake Magadi tilapia (*Alcolapia grahami*) is the only known obligately ureotelic fish and is so due to its unique and rather extreme environment (Randall et al., 1989; Wood et al., 1989; Wright et al., 1990 Wood et al., 1994, Walsh et al., 2001a). Specifically, this fish lives in the hot (up to 40°C), strongly alkaline (pH = 10), highly buffered (total CO_2 = 180 mmol·l^{-1}) Lake Magadi in the Rift valley of Kenya (Coe, 1966) under conditions that would kill most teleost fish. The alkaline pH and elevated buffering capacity of the water makes diffusive ammonia excretion virtually impossible, mainly because the blood-to-water diffusion gradients for NH_3 are extremely low when the environmental pH is above the pK (9.0–9.5) of the NH_3/NH_4^+ reaction (Randall & Wright, 1989). In addition, because of the high buffering capacity of the water, acidification of the gill water boundary layer cannot occur and the conversion of NH_3 to NH_4^+ in water passing over the gill, which facilitates NH_3 excretion, does not happen (Wright, Randall & Perry, 1989; Wood et al., 1989). Thus, the tilapia excretes only urea and does so at rates of 5000–10000 μmol-N·kg^{-1}·h^{-1} (Wood et al., 1989), easily the highest values for nitrogen excretion ever recorded for resting fish and approaching mammalian values (Fig. 2).

In 2001, Walsh and coworkers gave an in depth analysis of the mechanism of urea excretion in the Lake Magadi tilapia. In brief, the tilapia excretes urea continuously and 80% of urea excretion occurs across the anterior end of the fish (Wood et al., 1994, 2001a). Gill permeability to urea is 10-fold greater than that for standard lipid bilayers (Galluci, Micell & Lippe, 1971) and is 5-fold higher than that of the gulf toadfish (Wood et al., 1998; McDonald et al., 2000), in which tUT is present (*see* below; Fig. 2). The permeability of the gill to the urea analogue, thiourea, is significantly lower than that of urea, a similar handling pattern to the elasmobranch kidney and the gulf toadfish, which have a ShUT and tUT, respectively. Most convincingly, molecular analysis of the Magadi tilapia gill resulted in a 1699 bp sequence

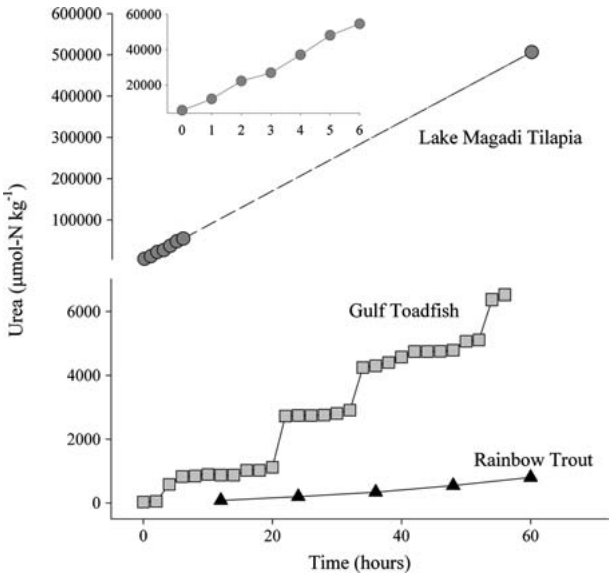

Fig. 2. An illustration of the different patterns of branchial urea excretion, measured by the appearance of urea in the water, of an obligate ammoniotele (rainbow trout, *Oncorhynhus mykiss*); a facultative ureotele (gulf toadfish, *Opsanus beta*) that only periodically excretes urea and an obligate ureotele (Lake Magadi tilapia, *Alcolapia grahami*). Approximation for urea appearance at 60 h for Lake Magadi tilapia was extrapolated from 6 h data set shown in inset. Data from McDonald and Wood (2003), McDonald et al. (2000) and Walsh et al. (2001).

(mtUT) with high homology to UT-A2 transporters in other species of fish (i.e., ShUT, tUT) and mammals (rabbit and rat). Lastly, Northern analysis revealed a single band of 1.9 kb in the gill but not the liver, muscle, red blood cells or brain. To date, immunolocalization of mtUT has not been completed; however, morphological analysis of gill pavement cells revealed a great density of Golgi stacks and numerous electron-dense vesicles scattered around the Golgi apparatus compared to an ammoniotelic tilapia (Walsh et al., 2001a). Walsh and coworkers (Walsh et al., 2001a) hypothesize that the intense vesicular trafficking is the structural manifestation of the facilitated diffusion urea transport process, representing either the insertion of the transporter into the apical membrane and/or the packaging of urea into vesicles for transport.

Facultatively Ureotelic Teleosts

Facultatively ureotelic fish such as the Indian air-breathing Singhi catfish, *Heteropneustes fossilis*, and the gulf toadfish, *Opsanus beta*, have the ability to produce urea due to their fully functional O-UC. Unlike the Lake Magadi tilapia, under normal conditions these fish are ammoniotelic. However, they will switch to ureotelism under stressful circumstances, in the case of the catfish, high ambient ammonia or semidry habitat conditions (Saha &

Ratha, 1986, 1987, 1998) and in the case of the toadfish, high ammonia, air exposure, crowding or confinement (Walsh, Tucker & Hopkins, 1994a; Walsh & Milligan, 1995; Hopkins, Wood & Walsh, 1995).

The Gill. The gulf toadfish, *Opsanus beta* is one of the best-described teleost fish with respect to urea transport and is also one of the most unusual. When ureotelic, the excretion of urea is not continuous, as is the excretion of ammonia. Instead, unlike any other fish described to date, urea is stored internally, resulting in a gradual elevation in blood urea concentrations (Wood et al., 1995a, 1997). The urea is then excreted once or twice daily in a distinct pulse of urea that lasts up to 3 hours and lowers stored levels throughout the whole body (Wood et al., 1995a, 1997; Fig. 2). The major route of excretion is the gill; intestinal and urinary excretion accounts for less than 10% of whole-body urea excretion (Wood et al., 1995a).

Wood and coworkers (1998) came up with three general hypotheses regarding the pulsatile excretion of urea in toadfish of which one, the insertion/activation of a urea-specific facilitated diffusion urea transport system, was the most convincingly supported by physiological evidence. Specifically, the permeability of the gill to urea increased during pulsing periods, but the permeabilities of ^3H_2O and the paracellular marker PEG 4000, did not (Wood et al., 1998). Furthermore, there was differential handling of urea, acetamide and thiourea. In brief, the permeability of the gill to urea and acetamide increased by 36- and 17-fold, respectively, during pulsing periods compared to non-pulsing periods, while the permeability to thiourea increased only 6-fold during pulsing periods (Wood et al., 1998; McDonald et al., 2000), similar to the pattern observed in mtUT of the Lake Magadi Tilapia (Walsh et al., 2001a). Interestingly, the permeability of the gill to all three substances was not significantly different during non-pulsing periods when the transporter is believed to be inactive (McDonald et al., 2000). Bidirectionality, a characteristic of facilitated diffusion, was demonstrated by placing high levels of urea in the water, i.e., reversing the urea gradient, which resulted in the movement of urea into the fish when a urea pulse occurred, as measured by the appearance of [^{14}C]-urea in the water (Wood et al., 1998). This physiological evidence was ultimately supported by molecular analysis of toadfish gill that uncovered a facilitated diffusion urea transporter (tUT; Smith et al., 1999; Walsh et al., 2001a), similar to mammalian UT-A2 on the amino acid level and cementing the role of at least one UT in pulsatile urea excretion. However, Northern analysis suggested the possibility of a second, larger urea transporter in the gill (3.5 kb), potentially the UT-A1 isoform as of yet only described in mammals. The cellular localization of tUT is still being pursued. However, during a urea pulse, morphological changes similar to those observed in the Lake Magadi tilapia, occur within gill pavement cells but not chloride cells, namely, alterations in the apical membrane and elevations in the number of cytosolic vesicles, suggesting a potential role of these cells in pulsatile urea excretion (Laurent et al., 2001).

Over the past few years the research focus has turned to determining the regulatory pathway involved in stimulating a urea pulse. Early work suggested a role for the hormone, arginine vasotocin (AVT; the teleost homologue of arginine vasopressin) in regulating pulsatile excretion, much like arginine vasopressin is involved in the regulation of mammalian UT-A1. Intravenous injection of AVT does cause a urea pulse event to occur (Perry et al., 1998; Wood et al., 2001), however, these urea pulses are at most 10% the size of natural pulses, and occur only at supraphysiological levels of AVT (10^{-10}–10^{-9} M). In addition, circulating AVT levels are low (10^{-12} to 10^{-11} M) in toadfish and show no relationship to the occurrence of natural urea pulses (Wood et al., 2001), suggesting that the involvement of AVT is minor at very best.

In contrast, there is a very strong link between pulsatile urea excretion and the glucocorticoid, cortisol, which acts as a stress hormone in teleost fish. As described above, ureotelism in toadfish is strongly correlated with stress, thus, ureotelic toadfish maintain plasma cortisol concentrations that are typical for chronically (but moderately) stressed teleosts (Hopkins et al., 1985; reviewed by Mommsen, Vijayan & Moon, 1999; Wood, Hopkins & Walsh, 1997, Wood et al., 2001; McDonald et al., 2004). However, 2–4 h before a natural urea pulse event, plasma cortisol levels fall steadily, a urea pulse occurs and cortisol levels return to pre-pulse levels rapidly thereafter (Wood et al., 2001). Plasma cortisol levels will also decrease without the occurrence of a natural pulse and so the decline in cortisol concentrations is not believed to be the direct trigger of pulsatile urea excretion (Wood et al., 2001). Wood and coworkers (2001) suggested that the decline in cortisol may be permissive to pulsatile excretion, however, more recently it was shown that the drop in cortisol does not have to take place in order for pulses to occur (McDonald et al., 2004). Indeed, continuous infusion with cortisol in an attempt to prevent a pre-pulse decline in levels had no effect on the frequency of urea pulses, although the infusion did cause a significant reduction in pulse size. These results suggest a role for cortisol in the regulation of the number of transporters, i.e., through the regulation of transcription as described in mammalian UT-A1 transporters (Knepper et al., 1975, Naruse et al., 1997; Peng, Sands & Bagnasco, 2002) or perhaps through non-

genomic pathways. Notably, tUT shows high homology to mammalian UT-A2, which in mammals are insensitive to cortisol (Peng et al., 2002), lending further support to the presence of a cortisol-sensitive UT-A1-like transporter in the toadfish gill that may be affected on the transcriptional level. Alternatively, cortisol may directly interfere with the urea pulse "triggering" mechanism. Perhaps during chronic cortisol loading or during the natural pulse cycle when cortisol levels are elevated more acutely, fewer urea transporters get activated, resulting in a smaller pulse size.

Interestingly, interactions between cortisol and the monoamine, serotonin (5-hydroxytryptamine; 5-HT), are well-established in mammals (reviewed by Chaouloff, 1993; Carrasco & Van De Kar, 2003) and to a lesser extent, in fish (Winberg et al., 1997; Øverli, Harris & Winberg, 1999; Höglund, Balm &Winberg, 2002). In 2003, we found that arterial injections of 5-HT resulted in natural size urea pulses, suggesting 5-HT as the trigger for the pulsatile mechanism (Wood et al., 2003). McDonald and Walsh (2004) went on to show that intravenous injection of the 5-HT_2 receptor agonist, α-methyl 5-HT, also elicited a natural size urea pulse while the 5-HT_1 agonist, 8-OH-DPAT, did not. The α-methyl 5-HT-induced pulse was blocked in a dose-dependent manner by the 5-HT_2 receptor antagonist, ketanserin, which at low doses caused a significant inhibition of pulse size, and at high doses significantly inhibited the occurrence of pulsatile excretion altogether. Taken together these data suggest the involvement of a 5-HT_2-like receptor in mediating the activation of a urea pulse. Possibly, the co-localization of tUT and 5-HT_2 could potentially result in the direct activation of tUT through 5-HT_2 receptor-mediated phosphorylation. Sequence analysis of tUT shows two potential phophorylation sites within a much longer C-terminal sequence that is unique to tUT and has been suggested to be related to the rapid upregulation of urea transport during a urea pulse (Walsh et al., 2000). Ketanserin could then be inhibiting the 5-HT_2–mediated phosphorylation of proximal tUTs, reducing the number of activated transporters and subsequently causing a reduction in pulse size. Perhaps through the sensitization/desensitization of 5-HT_2 receptors, fluctuating plasma cortisol concentrations during the natural pulse cycle are involved in mediating pulsatile urea excretion. More research is necessary to determine the interaction between cortisol and 5-HT; however, it is clear that the mechanism of regulation of urea excretion in the toadfish is different from that in mammals.

The Kidney. In most teleost fish studied to date, the renal route of urea excretion is minor compared to that of the gill (Sayer & Davenport, 1987; Wright , Pärt & Wood, 1995b; Wood, 1993, 1994, 1995a). However, physiological evidence has accumulated that suggests that the renal handling of urea, albeit minor, is carrier-mediated. The gulf toadfish, *Opsanus beta*, is interesting with respect to kidney physiology and renal urea handling because it has an aglomerular kidney and thus urine is formed primarily by secretion. Interestingly, toadfish urine urea concentrations generally exceed plasma levels by at least 30% and both urine and plasma concentrations increase when the fish are ureotelic (McDonald et al., 2000, 2003; Wood et al., 1995a). McDonald and coworkers (2000) suggested that the movement of urea into the kidney tubule might occur against a concentration gradient and by active transport. To investigate this hypothesis, the renal handling of urea was compared to the handling of acetamide and thiourea *in vivo* as well as the movement of Na^+, Cl^- and water (McDonald et al., 2000). In contrast to the gill, urea and thiourea were handled similarly (i.e., were more concentrated in ureteral urine unmodified by the urinary bladder) by the kidney, whereas acetamide was found equally in plasma and ureteral urine. Furthermore, the rate of urea secretion was correlated with the movement of water and Cl^- but was almost 3 times faster than the movement of either substance. There was no correlation between the movement of urea or Na^+, making a Na^+-linked mechanism, as seen in elasmobranch kidney and gill, unlikely. Upon exogenous urea loading *in vivo*, renal urea secretion rate maintained a strong linear relationship to plasma urea levels with no observable transport maximum (McDonald et al., 2003).

Northern analysis using a tUT cDNA probe was negative for the kidney, supporting the pattern of urea and analogue handling that suggests a renal urea transporter unique from that in the gill (Walsh et al., 2000). However, the recent finding of eUT-C (Mistry et al., 2005; see below) has brought a new perspective to the original work on the ureotelic gulf toadfish and its close relative, the plainfin midshipman. Based on physiological and pharmacological characteristics, the renal urea transporters in both these species were hypothesized to be *(a)* different than that found in the gill and *(b)* active, due to the uphill movement of urea. However, differential expression across the segments of the fish nephron (eUT-C is expressed in the proximal tubule but not in the distal tubule) makes it possible for a facilitated diffusion transporter to exist in the nephrons of other teleosts, even if urea appears to be moving against a concentration gradient. If this were the case, the tubule segments of lower urea permeability (i.e., no UT-C expression) would be responsible for concentrating the urea.

Ammoniotelic Teleosts

Despite the lack of a fully functional O-UC, even adult ammonioteles retain low levels (1–2 $mmol \cdot l^{-1}$) of circulating urea that is produced by uricolysis and/or argininolysis and is excreted continuously (Fig. 2).

While the reasons may not be as clear, like ureotelic teleosts, many ammonioteles have UTs in their gill and less well described urea transporters in their kidney to effectively excrete urea (McDonald & Wood, 1998; Pilley & Wright, 2000; McDonald & Wood, 2002, 2003, 2004; Mistry et al., 2001; Walsh et al., 2001b; Mistry et al., 2005).

The Gill. In 2001, Walsh and colleagues completed an intensive survey of 14 ammoniotelic teleosts that excreted less than 25% of their total nitrogen waste as urea. Despite the relatively low level of urea excretion, expression of UT-like mRNA using tUT cDNA as a probe was discovered in the gill of most of the species surveyed, suggesting that a large proportion of ammoniotelic fish have UT expression in their gill. Whether this translates to UT protein function in many of them is yet to be determined, as not all teleosts may have UT function. A physiological study on the tidepool sculpin, *Oligocottus maculosus*, suggested that urea excretion was likely by passive diffusion and not carrier-mediated (Wright et al., 1995b) and several teleosts in the Walsh et al. (2001) survey showed low to negative signal strength for tUT.

However, there is clear evidence for functional gill UT in many ammoniotelic fish. When characterized on the basis of the handling of urea, acetamide and thiourea, the rainbow trout (*Oncorhynchus mykiss*) and the plainfin midshipman (*Porichthys notatus*) gill showed a similar handling pattern as the activated tUT in toadfish and the mtUT in Magadi tilapia (McDonald, Walsh & Wood, 2002; McDonald & Wood, 2003). Specifically, the permeability of the gill to urea was greater than that of acetamide and both substances were more permeable than thiourea, cementing the idea that this pattern of urea, acetamide and thiourea handling is characteristic for UTs in fish. In both trout and midshipman, urea transport was bidirectional and there was no evidence of saturation of the mechanism during exogenous urea loading in vivo (McDonald et al., 2002; McDonald & Wood, 1998). However, urea uptake by trout isolated BLMV (basolateral membrane vesicles) from gill displayed a sensitivity to phloretin and saturation kinetics at physiological concentrations (K_m = 1.17 mmol·l^{-1} and V_{max} = 0.42 μmol·mg protein·h^{-1}), suggesting the presence of a UT on the basolateral membrane of the gill (McDonald & Wood, 2004). Similarly, in rainbow trout embryos, exposure to phloretin and urea analogues significantly inhibited urea excretion from the embryos to the external water (Pilley & Wright, 2000). Influx of urea from the water to the embryo tended to saturate at elevated external urea concentrations with a K_m that was similar to adult trout (K_m = 2 mmol·l^{-1} and V_{max} = 10.50 nmol·g^{-1}·h^{-1}). In both adult trout gill BLMV and trout embryos, there was also a non-saturable component of urea movement that could be simple diffusion or movement through non-specific pathways (Pilley & Wright, 2000; McDonald & Wood, 2004). This physiological and pharmacological evidence was supported by molecular analysis of adult rainbow trout gill (P.A. Wright, C. Rexroad, M.D. McDonald, P.J. Walsh unpublished) in which a small (1000 bp) cDNA fragment was amplified that showed high homology to other UTs on the amino acid level. Midshipman gill showed strong signal strength with Northern analysis using a tUT cDNA probe (Walsh et al., 2001b).

Mistry and coworkers (2001) reported the first cellular localization and expression analysis of a fish UT, specifically eUT in the seawater-acclimated, ammoniotelic Japanese eel, *Anguilla japonica*. Expressed only in the gill, a comparison of the eUT sequence (~1.9 kb) with others revealed high homology with mtUT (Magadi tilapia), tUT (toadfish) and shUT (shark). A significant increase in eUT mRNA expression was measured in eels during adaptation to seawater. Lastly, immunohistochemistry localized eUT to the basolateral membrane of gill chloride cells (Mistry et al., 2001), supporting BLMV data from rainbow trout gill of a UT located on that membrane (McDonald & Wood, 2004). Interestingly, it is the pavement cells that are hypothesized to be involved in urea excretion in the Magadi tilapia (Walsh et al., 2001a) and the gulf toadfish (Laurent et al., 2001) due to morphological changes in these cells.

The Kidney. Similar to the toadfish, renal urea transporters have been described on a physiological and pharmacological level in both the glomerular rainbow trout and the aglomerular midshipman, a close relative of the gulf toadfish, which has a urea secretory mechanism with pharmacological characteristics similar to that of toadfish (McDonald et al., 2002). With respect to the glomerular rainbow trout, renal urea transport is complicated (McDonald & Wood, 1998, 2003; Bucking & Wood, 2004). Put simply, it appears that at least two different urea transporters may be present, one that is responsible for urea reabsorption that is strongly correlated with Na^+ reabsorption but independent of glucose transport (Wood, 1993; McDonald & Wood, 1998, 2003; Bucking & Wood, 2004), perhaps similar to the Na^+-coupled urea cotransporter in elasmobranch kidney. The second appears to move urea in the secretory direction independently of Na^+ (McDonald & Wood, 2003). The influence of either transporter in the trout varies as urea has been shown to move in both the net reabsorptive (Wood, 1993; McDonald & Wood, 1998; Bucking & Wood, 2004) and net secretory directions (McDonald & Wood, 2003). Interestingly, the trout kidney handles urea and acetamide identically, whereas there was no net renal transport of thiourea, surprisingly similar to the handling pattern characteristic of UTs, suggesting that the Na^+-

independent transporter in trout kidney may be a UT, similar to what is hypothesized in the elasmobranch kidney (*see* above). Furthermore, a small (< 500 bp) cDNA fragment has been isolated from trout kidney that shows high homology with mtUT (Magadi tilapia) and tUT (toadfish) (M.D. McDonald, P.A. Wright, P.J. Walsh, unpublished). The presence of a trout renal UT suggests that changes in the direction of net urea transport may be regulated at the level of the possible Na^+-linked reabsorptive mechanism. Consistent with a secretory kidney and a lack of glomerular filtration, there is no evidence for Na^+-linked urea reabsorption in aglomerular fish (McDonald & Wood, 1998; McDonald et al., 2000, 2002).

From a molecular standpoint, the best-described renal urea transporter is the unique eUT-C that is expressed in the renal proximal tubules of the seawater-acclimated Japanese eel, with weak expression in the stomach (Mistry et al., 2005). Originally found in the pufferfish (*Takifugu rubrigpes;* fgUT-C), using a database mining approach, eUT-C is a facilitated diffusion transporter that is sensitive to phloretin and not dependent on the presence of Na^+. However, it is distantly related to other UTs, sharing only 35% identity with eUT found in the gill of the same species and even less sequence homology to other known UTs. Similar to the branchial eUT, expression of eUT-C is upregulated upon acclimation to seawater, suggesting a potential role of urea transporters in ammoniotelic fish (*see* below).

Why Urea Transport in Ammoniotelic Fish? While it is not difficult to understand the reason for urea transport proteins in fish that effectively produce and predominantly excrete urea, it is more difficult to explain why an ammoniotelic teleost fish would possess specific urea excretory mechanisms when urea makes up only 10% of their total nitrogenous waste. Only a very few teleost fish have a fully functional O-UC as adults, but during early life stages all teleost species studied to date, including *Zoarces viviparous* (Korsgaard, 1994), the Atlantic cod (*Gadus morhus*, Chadwick & Wright, 1999), the rainbow trout (*Oncorhynchus mykiss*, Wright, Felskie & Anderson, 1995a; Wright & Land, 1998; Pilley & Wright, 2000), the African catfish (*Clarias gariepinus*, Terjesen, Verreth & Fhyn, 1997, Terjesen et al., 2001), the guppy (*Poecilia reticulata*, Dépêche et al., 1979) and Atlantic halibut (*Hippoglossus hippoglossus*, Terjesen et al., 2000), express a full complement of O-UC enzymes (*see* review by Wright & Fyhn, 2001). The capacity to produce urea effectively at this life stage has been hypothesized to assist in detoxification of ammonia accumulating from protein catabolism (Griffith, 1991; Wright et al., 1995a) and urea transporters present in embryos then facilitate urea removal (Pilley & Wright, 2000). However, when ammonia toxicity is not a daily problem in adult life, O-UC function is lost and the fish becomes ammoniotelic. Yet, adult ammonioteles retain urea transporters, despite a lowered capacity to produce urea, suggesting that these proteins may be useful under the right circumstances.

For instance, an elevation in internal cortisol causes a rise in endogenous urea concentrations and in urea excretion in the ammoniotelic marine sea raven, *Hemitripterus americanus* and the adult rainbow trout (Vijayan et al., 1996; McDonald et al., 2004). The increase in endogenous urea appears to be through the uricolytic pathway, as there are reported increases in glutamine synthetase, arginase and allantoicase in response to exogenous cortisol loading (Wilkie et al., 1993; Vijayan et al., 1996). Since cortisol has been documented to stimulate gluconeogenesis and proteolysis in fish resulting in elevated plasma ammonia levels, the glucocorticoid-mediated increase of urea synthesis may somehow be a mechanism of ammonia detoxification (McDonald et al., 2004). Regardless, the availability of urea transporters to facilitate the excretion of excess urea produced during times of stress would be beneficial.

Another reason for retaining urea transporters is salinity acclimation. As mentioned above, the expression of eUT and eUT-C is stimulated by seawater acclimation (Mistry et al., 2005). Unlike elasmobranchs, the role of urea and urea transporters in marine teleost osmoregulation is not quite clear. However, it is interesting that cortisol, which stimulates urea production in both ureotelic and ammoniotelic fish, is also secreted upon seawater exposure to stimulate chloride cell proliferation and changes in gill permeability.

Feeding (Beamish & Thomas, 1984), temperature (Alsop, Kieffer & Wood, 1999) and exercise (Lauff & Wood, 1996; Alsop & Wood, 1997, Alsop et al., 1999) also have implications on nitrogen metabolism, stress and cortisol secretion and could be reasons for which ammoniotelic teleosts have retained urea transporters in adulthood long after they have lost the capacity to effectively produce urea through the O-UC (reviewed by Wood, 2001).

Piscine Perspectives on Evolution of Urea Transporters

The urea transporter gene family appears to have undergone some rather interesting evolutionary changes, no doubt associated with its critical functional role in the evolution of terrestriality in the vertebrates (Fig. 3). Indeed, special kidney isoforms of the "A" group are critical to the processing of concentrated urine in mammals to prevent water loss. Furthermore, it is likely that one selective pressure for evolution of the "B" group (expressed in red blood cells, erythropoetic tissue, some blood vessels,

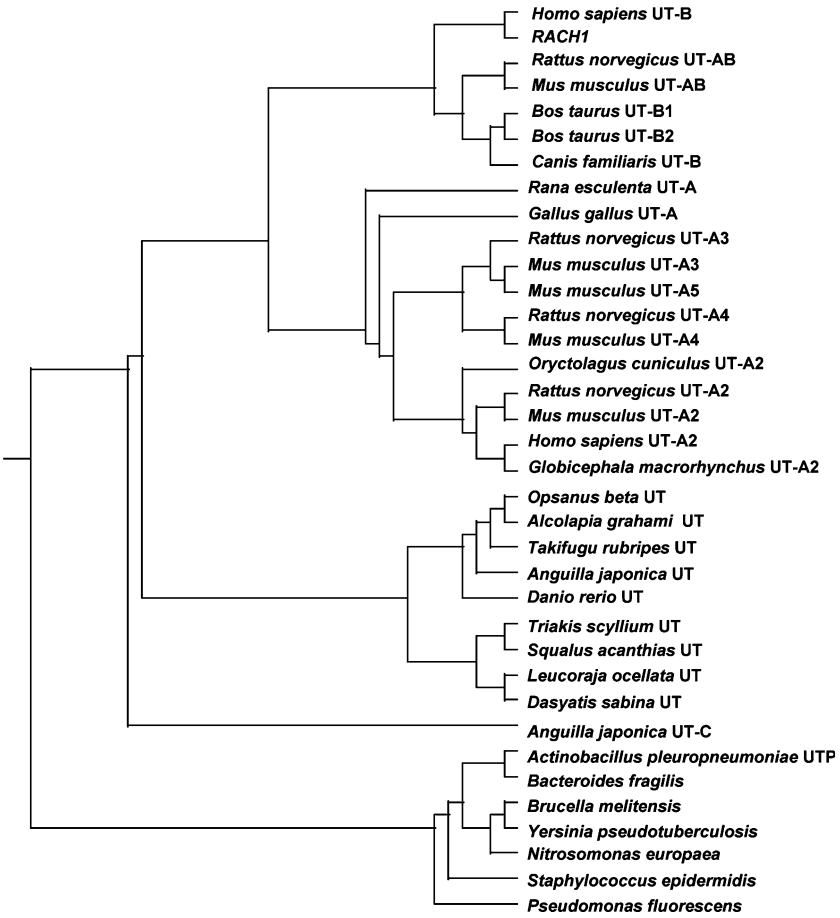

Fig. 3. Tree of the urea transporter family was created using Clustal W software at: http://clustalw.genome.jp/ We used the SLOW/ACCURATE Multiple Alignment, with Multiple Alignment Parameters as follows: Gap Open Penalty:10; Gap Extension Penalty: 0.05; Weight Transition: NO; Hydrophilic Residues for Proteins: GPSNDQERK; Hydrophilic Gaps: YES NO; Select Weight Matrix:BLOSUM (for protein); Dendogram parameters were the default settings.

etc.) also relates to kidney architecture and the need to maintain urea gradients within the kidney (*see* 'Hepatocytes and Red Blood Cells', above). Thus far, the model of UT evolution within the mammals strongly suggests two duplications of the ancestral UT gene (Fenton et al., 1999), which to date in fish and amphibians encodes a protein of 380 to 475 amino acids with 8–10 transmembrane segments. A first duplication is believed to have led to a similar-sized UT-B that then evolved a relatively different amino acid sequence consistent with its function in erythrocytes and related tissues; in mammals, identities between UT-A and UT-B fall in the range of 50 to 70%. A second duplication yielded a double-sized A gene with very similar halves (and a connector motif to yield a 929 AA protein). When UT-A is fully transcribed and translated it yields UT-A1, but the gene can be alternatively processed to yield a total of 5 other isoforms (UT-A2, 3, 4, 5 and 6) with a very diverse tissue-specific expression pattern. The tree in Fig. 3 and those previously published (e.g., Minocha, Studley & Saier, 2003) suggests that the duplication and divergence yielding UT-B took place before the internal UT-A duplication. It would be very interesting to know if UT-B occurs in non-mammalian vertebrates, especially fishes. Walsh and Smith (2001) have presented arguments as to why a specialized blood UT might not be present in erythrocytes and other fishes, and we have reiterated these above. However, continuing database searches and more B-specific primer designs for PCR cloning would be useful in searching for fish analogs of B.

Another interesting facet of the UT family is the absence so far of identified UT's in the invertebrates and other eukaryotes (e.g., plants, fungi, etc.). This is surprising given that there are many bacterial urea transporters and the physiological and biochemical evidence that at least some invertebrates make and excrete urea (Cohen & Lewis, 1949; Bishop & Campbell, 1965). If these transporters are truly absent, a potential explanation is that urea passage through other transporters (e.g., promiscuous versions of AQPs, like AQP-9) may be rapid enough to support the relatively noncomplex invertebrate nephridial tubule systems (e.g., Tillinghast, 1967). Minocha et al. (2003) conducted a very thorough examination of databases at the time and first pointed out this gap in eukaryotic UT expression. They speculated that vertebrate UTs could have arisen either from: (1) vertical transmission of UTs from

prokaryotes to very early eukaryotes, and subsequent gene loss in groups like invertebrates, plants and fungi; or (2) horizontal transmission directly from a bacterium to an early vertebrate. The parsimony of the second hypothesis is that no selective loss from major groups of organisms needs to be invoked.

We suggest a third possibility, namely that it could simply be that other eukaryotic urea transporters have diverged significantly enough from bacterial transporters and fish/early vertebrate UTs (which until very recently appeared to be only exclusively UT-A homologs) to remain undetected so far. This third possibility becomes a more viable alternative if there are more primitive chordate UTs yet to be discovered. In this regard, very recently, a UT has been cloned from Japanese eel kidney (and a homologue found in *Fugu*, pufferfish) that appears to be more basal to UT-A (of even elasmobranchs) and UT-B or mammals, and has been termed UT-C (Mistry et al., 2005). Notably, UT-C shares only 35% amino acid similarity with its closest relatives. It may be that as we work *backwards* within the chordate lineage to more primitive forms (e.g., hagfish, lamprey, amphioxus, etc.), using primers that are more specific to UT-C and perhaps other more primitive UTs yet to be discovered, we may find more UT-like genes, but with rather different sequences, in earlier eukaryotes. We are clearly only at the beginning of discovery of UT types, and it will be exciting to discover which of the above (or potentially other) scenarios are most likely.

M.D.M. is supported by NSF (#IOB0455904) and P.J.W. by NSERC Discovery Grants and the Canada Research Chair Program.

References

Alsop, D.H., Kieffer, J.D., Wood, C.M. 1999. The effects of temperature and swimming speed on instantaneous fuel use and nitrogenous waste excretion of the Nile tilapia. *Physiol. Biochem. Zool.* **72:**474–483

Alsop, D.H., Wood, C.M. 1997. The interactive effects of feeding and exercise on oxygen consumption, swimming performance and protein usage in juvenile rainbow trout (*Oncorhynchus mykiss*). *J. Exp. Biol.* **200:**2337–2346

Anderson, P.M. 2001. Uera and glutamine synthesis: environmental influences on nitrogen excretion. *In:* Wright, P.A., Anderson, P.M., (eds) Nitrogen Excretion. pp 239–278, Academic Press, San Diego

Anderson, P.M., Walsh, P.J. 1995. Subcellular localization and biochemical properties of the enzymes of carbamoyl phophate and urea synthesis in the batrachoidid fishes *Opsanus beta, Opsanus tau* and *Porichthys notatus*. *J. Exp. Biol.* **198:**755–766

Beamish, F.W.H., Thomas, E. 1984. Effects of dietary protein and lipid on nitrogen losses in rainbow trout (*Salmo gairdneri*). *Aquaculture*. **41:**359–371

Bishop, S.H., Campbell, J.W. 1965. Arginine and urea biosynthesis in the earthworm *Lumbricus terrestris*. *Comp. Biochem. Physiol.* **15:**51–71

Boylan, J. 1967. Gill permeability in *Squalus acanthias*. *In:* Gilbert, P.W., Mathewson, R.F., Rall, D.P., (eds) Sharks, skates and rays. pp 197–206, Johns Hopkins University Press, Baltimore

Boylan, J.W. 1972. A model for passive urea reabsorption in the elasmobranch kidney. *Comp. Biochem. Physiol.* **42A:**27–30

Bucking, C., Wood, C.M. 2004. Does urea reabsorption occur via the glucose pathway in the kidney of the freshwater rainbow trout? *Fish Physiol. Biochem.* **30:**1–12

Carby, J.M.N., Gorelich-Feldman, D.A., Kozono, D., Praetorius, J., Nielsen, S., Agre, P. 2003. Aquaglyceroporin AQP9: solute permeation and metabolic control of expression in liver. *Proc. Nat. Acad. Sci.* **100:**2945–2950

Carlson, S.R., Goldstein, L. 1997. Urea transport across the cell membrane of skate erythrocytes. *J. Exp. Zool.* **277:**275–282

Carrasco, G.A., Van De Kar, L.D. 2003. Neuroendocrine pharmacology of stress. *European J. Pharmacol.* **463:**235–272

Chadwick, T.D., Wright, P.A. 1999. Nitrogen excretion and expression of urea cycle enzymes in the Atlantic cod (*Gadus morhus* L.): a comparison of early life stages with adults. *J. Exp. Biol.* **202:**2653–2662

Chan, D.K.O., Wong, T.M. 1977. Physiological adjustements to dilution of the external medium in the lip-shark *Hamiscyllium plagiosum* (Bennett). I. Size of body compartments and osmolute composition. *J. Exp. Zool.* **200:**71–84

Chaouloff, F. 1993. Physiolpharmacological interactions between stress hormones and central serotonergic systems. *Brain Research Reviews*. **18:**1–32

Chou, C.L., Knepper, M.A. 1989. Inhibition of urea transport in inner medullary collecting duct by phloretin and urea analogues. *Am. J. Physiol.* **257:**F359–F365

Coe, M.J.. 1966. The biology of *Tilapia grahami* Boulenger in Lake Magadi, Kenya. *Acta. Trop.* **23:**146–177

Cohen, S., Lewis, H.B. 1949. The nitrogenous metabolism of the earthworm (*Lumbricus terrestris*). *J. Biol. Chem.* **180:**79–91

Cooper, R.A., Morris, S. 1998. Osmotic and haemotological response of the Port Jackson shark, *Heterodontus portusjacksoni* and the common stingaree *Trygonoptera testacea* upon exposure to diluted sea water. *Mar. Biol.* **132:**29–42

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

Denis, W.. 1913. Metabolism studies on cold-blooded animals. II The blood and urine of fish. *J. Biol. Chem.* **16:**389–393

Dépêche, J., Gilles, R., Daufresne, S., Chiapello, H. 1979. Urea content and urea production via the ornithine-urea cycle pathway during the ontogenic development of two teleost fishes. *Comp. Biochem. Physiol.* **63A:**51–56

Fenton, R., Hewitt, J.E., Howorth, A., Cottingham, C.A., Smith, C.P. 1999. The murine urea transporter genes S1c14a1 and S1c14a2 occur in tandem on chromosome 18. *Cytogen. Cellular. Genet.* **87:**95–96

Fines, G.A., Ballantyne, J.S., Wright, P.A. 2001. Active urea transport and an unusual basolateral membrane composition in the gills of a marine elasmobranch. *Am. J. Physiol.* **280:**R16–R24

Forster, R.P., Goldstein, L. 1976. Intracellular osmoregulatory role of amino acids and urea in marine elasmobranchs. *Am. J. Physiol.* **230:**925–931

Friedman, P.A., Hebert, S.C. 1990. Diluting segment in kidney of dogfish shark. I. Localization and characterization of chloride absorption. *Am. J. Physiol.* **258:**R398–R408

Galluci, E., Micell, S., Lippe, C. 1971. Non-electrolyte permeability across thin lipid membranes. *Arch. Int. Physiol. Biochim.* **79:**881–887

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

Goldstein, L., Forster, R.P. 1971b. Urea biosynthesis and excretion in freshwater and marine elasmobranchs. *Comp. Biochem. Physiol.* **39B:**415–421

Griffith, R.W. 1991. Guppies, toadfish, lungfish, coelacanths and frogs - a scenario for the evolution of urea retention in fishes. *Environ. Biol. Fish.* **32:**199–218

Hays, R.M., Levine, S.D., Myers, J.D., Heinemann, H.O., Kaplan, M.A., Franki, N., Berliner, H. 1977. Urea transport in the dogfish kidney. *J. Exp. Zool.* **199:**309–316

Hickman, C.P., Trump, B.F. 1969. The kidney. *In:* Hoar, W.S., Randall, D.J., (eds) Fish Physiology. pp 91–239, Academic Press, New York

Höglund, E., Balm, P.H.M., Winberg, S. 2002. Stimulatory and inhibitory effects of 5-HT_{1A} receptors on adrenocorticotropic hormone and cortisol secretion in an teleost fish, the Arctic charr (*Salvelinus alpinus*). *Neurosci. Let.* **324:**193–196

Hopkins, T.E., Wood, C.M., Walsh, P.J. 1995. Interactions of cortisol and nitrogen metabolism in the ureogenic gulf toadfish *Opsanus beta*. *J. Exp. Biol.* **198:**2229–2235

Hyodo, S., Katoh, F., Kaneko, T., Takei, Y. 2004. A facilitative urea transporter is localized in the renal collecting tubule of the dogfish *Triakis scyllia*. *J. Exp. Biol.* **207:**347–356

Isozaki, T., Gillin, A.G., Swanson, C.E., Sands, J.M. 1994a. Protein restriction sequentially induces new urea transport processes in rat initial IMCD. *Am. J. Physiol.* **266:**F756–F761

Isozaki, T., Lea, J.P., Tumlin, J.A., Sands, J.M. 1994b. Sodium-dependent net urea transport in rat initial inner medullary collecting ducts. *J. Clin. Invest.* **94:**1513–1517

Isozaki, T., Verlander, J.W., Sands, J.M. 1993. Low protein diet alters urea transport and cell structure in rat initial inner medullary collecting duct. *J. Clin. Invest.* **92:**2448–2457

Jenkinson, C.P., Grody, W.W., Cederbaum, S.D. 1996. Comparative properties of arginases. *Comp. Biochem. Physiol.* **114B:**107–132

Kajimura, M., Walsh, P.J., Mommsen, T.P., Wood, C.M. 2006. The dogfish shark (*Squalus acanthias*) increases both hepatic and extrahepatic ornithine urea cycle enzyme activities for nitrogen conservation after feeding. *Physiol. Biochem. Zool.* **79:**603–613

Kato, A., Sands, J.M. 1998. Evidence for sodium-dependent active urea secretion in the deepest subsegment of the rat inner medullary collecting duct. *J. Clin. Invest.* **101:**423–428

Kempton, R.T. 1953. Studies of the elasmobranch kidney II. Reabsorption of urea by the smooth muscle of dogfish, *Mustelis canis*. *Biol. Bull.* **104:**45–56

Klein, J.D., Timmer, R.T., Rouillard, P., Bailey, J.L., Sands, J.M. 1999. UT-A urea transporter protein expressed in liver: upregulation by uremia. *J. Am. Soc. Nephrol.* **10:**2076–2083

Knepper, M.A., Danielson, R.A., Saidel, G.M., Johnston, K.H. 1975. Effects of dietary protein restriction and glucocorticoid administration on urea excretion in rats. *Kidney Int.* **8:**303–315

Korsgaard, B.. 1994. Nitrogen distribution and excretion during embryonic postyolk sac development in Zoarces viviparus. *J. Comp. Physiol.* **164B:**42–46

Lacy, E.R., Reale, E., Schlusselberg, D.S., Smith, W.K., Woodward, D.J. 1985. A renal countercurrent system in marine elasmobranch fish: a computer aided reconstruction. *Science* **227:**1351–1354

Lauff, R.F., Wood, C.M. 1996. Respiratory gas exchange, nitrogenous waste excretion, and fuel usage during aerobic swimming in juvenile rainbow trout. *J. Comp Physiol.* **166B:**501–509

Laurent, P., Wood, C.M., Wang, Y., Perry, S.F., Gilmour, K.M., Part, P., Chevalier, C., West, M., Walsh, P.J. 2001. Intracellular vesicular trafficking in the gill epithelium of urea-excreting fish. *Cell Tissue Res.* **303:**197–210

Macey, R.I., Yousef, L.W. 1988. Osmotic stability of red cells in renal circulation requires rapid urea transport. *Am. J. Physiol.* **254:**C669–C674

McDonald, M.D., Grosell, M., Wood, C.M., Walsh, P.J. 2003. Branchial and renal handling of urea in the gulf toadfish, *Opsanus beta*: the effect of exogenous urea loading. *Comp. Biochem. Physiol.* **134A:**763–776

McDonald, M.D., Walsh, P.J. 2004. Dogmas and controversies in the handling of nitrogenous wastes: 5-HT_{2A}-like receptors are involved in triggering pulsatile urea excretion in the gulf toadfish, *Opsanus beta*. *J. Exp. Biol.* **207:**2003–2010

McDonald, M.D., Walsh, P.J., Wood, C.M. 2002. Branchial and renal excretion of urea and urea analogues in the plainfin midshipman, *Porichthys notatus*. *J. Comp. Physiol.* **172B:**699–712

McDonald, M.D., Wood, C.M. 1998. Reabsorption of urea by the kidney of the freshwater rainbow trout. *Fish Physiol. Biochem.* **18:**375–386

McDonald, M.D., Wood, C.M. 2003. Differential handling of urea and its analogues suggests carrier-mediated urea excretion in the freshwater rainbow trout. *Physiol. Biochem. Zool.* **76:**791–802

McDonald, M.D., Wood, C.M. 2004. Evidence for facilitated diffusion of urea across the gill basolateral membrane of the rainbow trout (*Oncorhynchus mykiss*). *Biochim. Biophys. Acta.* **1663:**89–96

McDonald, M.D., Wood, C.M., Grosell, M., Walsh, P.J. 2004. Glucocorticoid receptors are involved in the regulation of pulsatile urea excretion in toadfish. *J. Comp. Physiol.* **174B:**649–658

McDonald, M.D., Wood, C.M., Wang, Y., Walsh, P.J. 2000. Differential branchial and renal handling of urea, acetamide and thiourea in the gulf toadfish, *Opsanus beta*: evidence for two transporters. *J. Exp. Biol.* **203:**1027–1037

Minocha, R., Studley, K., Saier, M.H. 2003. The urea transporter (UT) family: bioinformatics analuses leading to structural, functional and evolutionary predictions. *Receptor Channels* **9:**345–352

Mistry, A.C., Chen, G., Kato, A., Nag, K., Sands, J.M. 2005. A novel type of urea transporter, UT-C, highly expressed in proximal tubule of seawater eel kidney. *Am. J. Physiol.* **288:**F455–F465

Mistry, A.C., Honda, S., Hirata, T., Kato, A., Hirose, S. 2001. Eel urea transporter is localized to chloride cells and is salinity dependent. *Am. J. Physiol. Regul.* **281:**R1594–R1604

Mommsen, T.P., Vijayan, M.M., Moon, T.W. 1999. Cortisol in teleosts: dynamics, mechanisms of action and metabolic regulation. *Rev. Fish Biol. Fisheries* **9:**211–268

Mommsen, T.P., Walsh, P.J.. 1989. Evolution of urea synthesis in vertebrates: the piscine connection. *Science* **243:**72–75

Morgan, R.L., Ballantyne, J.S., Wright, P.A. 2003a. Regulation of a renal urea transporter with reduces salinity in a mrine elasmobranch, *Raja erinacea*. *J. Exp. Biol.* **206:**3285–3292

Morgan, R.L., Wright, P.A., Ballantyne, J.S. 2003b. Urea transport in kideny brush-border membrane vesicles from an elasmobranch, *Raja erinacea*. *J. Exp. Biol.* **206:**3293–3302

Murdaugh, H.V., Robin, E.D., Hearn, C.D. 1964. Urea: apparent carrier-mediated transport by facilitated diffusion in dogfish erythrocytes. *Science* **144:**52–53

Naruse, M., Klein, J.D., Ashkar, Z.M., Jacobs, J.D., Sands, J.M. 1997. Glucocorticoids downregulate the vasopressin-regulated urea transporter in rat terminal inner medullary collecting ducts. *J. Am. Soc. Nephrol.* **8:**517–523

Øverli, O., Harris, C.A., Winberg, S. 1999. Short-term effects of fights for social dominance and the establishment of dominant-

subordinate relationships on brain monoamines and cortisol in rainbow trout. *Brain Behav. Evol.* **54:**263–275

Pärt, P., Wright, P.A., Wood, C.M. 1998. Urea and water permeability in dogfish (*Squalus acanthias*) gills. *Comp Biochem. Physiol.* **119A:**117–123

Payan, P., Goldstein, L., Forster, R.P. 1973. Gills and kidneys in ureosmotic regulation in euryhaline skates. *Am. J. Physiol.* **224:**367–372

Peng, T., Sands, J.M., Bagnasco, S.M. 2002. Glucocorticoids inhibit transcription and expression of the UT-A urea transporter gene. *Am. J. Physiol.* **282:**F853–F858

Perry, S.F., Gilmour, K.M., Wood, C.M., Part, P., Laurent, P., Walsh, P.J. 1998. The effects of arginine vasotocin and catechlamines on nitrogen excretion and the cardio-respiratory physiology of the gulf toadfish, *Opsanus beta*. *J. Comp. Physiol.* **168B:**461–472

Piermarini, P.M., Evans, D.H. 1998. Osmoregulation of the Atlantic stingray (*Dasyatis sabina)* from the freshwater Lake Jesup of the St. Johns River, Florida. *Physiol. Zool.* **71:**553–560

Pilley, C.M., Wright, P.A. 2000. The mechanisms of urea transport by early life stages of rainbow trout (*Oncorhynchus mykiss*). *J. Exp. Biol.* **203:**3199–3207

Price, K.S., Creaser, E.P. 1967. Fluctuation in two osmoregulatory componenets, urea and sodium chloride, of the clearnose skate, *Raja eglanteria* Bosc 1802. I. Upon laboratory modification of external salinities. *Comp. Biochem. Physiol.* **23:**77–82

Rabinowitz, L., Gunther, R.A. 1973. Urea transport in elasmobranch erythrocytes. *Am. J. Physiol.* **224:**1109–1115

Randall, D.J., Wood, C.M., Perry, S.F., Bergman, H., Maloiy, G.M., Mommsen, T.P., Wright, P.A. 1989. Urea excretion as a strategy for survival in a fish living in a very alkaline environment. *Nature* **337:**165–166

Randall, D.J., Wright, P.A. 1989. The interaction between carbon dioxide and ammonia excretion and water pH in fish. *Can. J. Zool.* **67:**2936–2942

Saha, N., Ratha, B.K. 1986. Effect of ammonia stress on ureogenesis in a freshwater air-breathing teleost, *Heteropneustes fossilis*. In: Contemporary Themes in Biochemistry. pp. 342–343. Cambridge University Press, London

Saha, N., Ratha, B.K. 1987. Active ureogenesis in a freashwater air-breathing teleost, *Heteropneustes fossilis*. *J. Exp. Zool.* **52:**1–8

Saha, N., Ratha, B.K. 1998. Ureogenesis in Indian air-breathing teleosts: adaptation to environmental constraints. *Comp. Biochem. Physiol.* **120A:**195–208

Sands, J.M., Gargus, J.J., Fröhlich, O., Gunn, R.B., Kokko, J.P. 1992. Urinary concentrating ability in patients with Jk(a-b-) blood type who lack carrier-mediated urea transport. *J. Am. Soc. Nephrol.* **2:**1689–1696

Sands, J.M., Martial, S., Isozaki, T. 1996. Active urea transport in the rat inner medullary collecting duct: functional characterization and initial expression cloning. *Kidney Int.* **49:**1611–1614

Sayer, M.D.J., Davenport, J. 1987. The relative importance of the gills to ammonia and urea excretion in five seawater and one freshwater teleost species. *J. Fish Biol.* **31:**561–570

Schmidt-Nielsen, B., Rabinowitz, L. 1964. Methylurea and acetamide:active reabsorption by elasmobranch kidney tubules. *Science*. **146:**1587–1588

Schmidt-Nielsen, B., Truniger, B., Rabinowitz, L. 1972. Sodium-linked urea transport by the renal tubule of the spiny dogfish *Squalus acanthias*. *Comp Biochem. Physiol.* **42A:**13–25

Smith, C.P., Wright, P.A. 1999. Molecular characterization of an elasmobranch urea transporter. *Am. J. Physiol.* **276:**R622–R626

Smith, H.W. 1931a. The absorption and excretion of water and salts by elasmobranch fishes. II. Marine elasmobranchs. *Am. J. Physiol.* **98:**296–310

Smith, H.W. 1931b. The absorption and excretion of water and salts by the elasmobranch fishes. I. Fresh water elasmobranchs. *Am. J. Physiol.* **98:**279–295

Smith, H.W.. 1936. The retention and physiological role of urea in the elasmobranch. *Biol. Rev.* **11:**49–73

Städeler, G., Frérichs, F.T. 1858. über das Vorkommen von Harnstoff, Taurin und Scyllit in den Organen der Plagiostomen. *J. Prakt. Chem.* **73:**48–55

Steele, S.L., Yancey, P.H., Wright, P.A. 2005. The little skate *Raja erinacea* exhibits an extrahepatic ornithine urea cycle in the muscle and modulates nitrogen metabolism during low-salinity challenge. *Physiol. Biochem. Zool.* **78:**216–226

Sulikowski, J.A., Maginniss, L.A. 2001. Effects of environmental dilution on body fluid regulation in the yellow stingray, *Urolophus jamaicensis*. *Comp. Biochem. Physiol.* **128A:**223–232

Terjesen, B.F., Chadwick, T.D., Verreth, J.A., Ronnestad, I., Wright, P.A. 2001. Pathways for urea production during early life of an air-breathing teleost, the African catfish *Clarias gariepinus* Burchell. *J. Exp. Biol.* **204:**2155–2165

Terjesen, B.F., Ronnestad, I., Norberg, B., Anderson, P.M. 2000. Detection and basic properties of carbamoyl phosphate synthetase III during teleost ontogeny: a case study in the Atlantic halibut (*Hippoglossus hippoglossus* L.). *Comp. Biochem. Physiol.* **126B:**521–535

Terjesen, B.F., Verreth, J., Fyhn, H.J. 1997. Urea and ammonia excretion by embryos and larvae of the African catfish *Claria gariepinus* (Burchell 1822). *Fish. Physiol. Biochem.* **16:**311–321

Thorson, T.B 1967. Osmoregulatiom in freshwater elasmobranchs. *In:* Gilber, P.W., Mathewson, R.F., Rall, D.P., (eds) Sharks, skates and rays. pp 265–270, Johns Hopkins University Press, Baltimore

Tillinghast, E.K. 1967. Excretory pathways of ammonia and urea in the earthworm *Lumbricus terrestris* L. *J. Exp. Zool.* **166:**295–300

Urist, M.R. 1962. Calcium and other ions in the blodd and skeleton of the Nicaraguan freshwater shark. *Science* **137:**985–986

Vijayan, M.M., Mommsen, T.P., Glemet, H.C., Moon, T.W. 1996. Metabolic effects of cortisol treatment in a marine teleost, the sea raven. *J. Exp. Biol.* **199:**1509–1514

Walsh, P., Milligan, C. 1995. Effects of feeding and confinement on nitrogen metabolism and excretion in the gulf toadfish *Opsanus beta*. *J. Exp. Biol.* **198:**1559–1566

Walsh, P., Tucker, B., Hopkins, T. 1994a. Effects of confinement/crowding on ureogenesis in the gulf toadfish, *Opsanus beta*. *J. Exp. Biol.* **191:**195–206

Walsh, P., Wood, C., Perry, S., Thomas, S. 1994b. Urea transport by hepatocytes and red blood cells of seleced elasmobranch and teleost fishes. *J. Exp. Biol.* **193:**321–335

Walsh, P.J. 1997. Evolution and regulation of urea synthesis and ureotely in (batrachoidid) fishes. *Annu. Rev. Physiol.* **59:**299–323

Walsh, P.J., Grosell, M., Goss, G.G., Bergman, H.L., Bergman, A.N., Wilson, P., Laurent, P., Alper, S.L., Smith, C.P., Kamunde, C., Wood, C.M. 2001a. Physiological and molecular characterization of urea transport by the gills of the Lake Magadi tilapia (*Alcolapia grahami*). *J. Exp. Biol.* **204:**509–520

Walsh, P.J., Heitz, M.J., Campbell, C.E., Cooper, G.J., Medina, M., Wang, Y.S., Goss, G.G., Vincek, V., Wood, C.M., Smith, C.P. 2000. Molecular characterization of a urea transporter in the gill of the gulf toadfish (*Opsanus beta*). *J. Exp. Biol.* **203:**2357–2364

Walsh, P.J., Smith, C.P 2001. Urea Transport. *In:* Wright, P.A., Anderson, P.M.., (eds) Nitrogen Excretion. pp 279–307, Academic Press, New York

Walsh, P.J., Wang, Y., Campbell, C.E., De Boeck, G., Wood, C.M. 2001b. Patterns of nitrogen waste excretion and gill urea

transporter mRNA expression in several species of marine fish. *Mar. Biol.* **139:**839–844

Walsh, P.J., Wood, C.M. 1996. Interactions of urea transport and synthesis in hepatocytes of the gulf toadfish, *Opsanus beta*. *Comp. Biochem. Physiol.* **113B:**411–416

Wilkie, M.P., Wright, P.A., Iwama, G.K., Wood, C.M. 1993. The physiological responses of the Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*), a resident of highly alkaline pyramid lake (pH 9.4), to challenge at pH 10. *J. Exp. Biol.* **175:**173–194

Winberg, S., Nilsson, A., Hylland, P., Soderstom, V., Nilsson, G.E. 1997. Serotonin as a regulator of hypothalamic-pituitary-interrenal activity in teleost fish. *Neurosci. Lett.* **230:**113–116

Wood, C., Bergman, H., Laurent, P., Maina, J., Narahara, A., Walsh, P. 1994. Urea production, acid-base regulation and their interactions in the lake Magadi tilapia, a unique teleost adapted to a highly alkaline environment. *J. Exp. Biol.* **189:**13–36

Wood, C., Hopkins, T., Hogstrand, C., Walsh, P. 1995a. Pulsatile urea excretion in the ureagenic toadfish *Opsanus beta*: an analysis of rates and routes. *J. Exp. Biol.* **198:**1729–1741

Wood, C.M. 1993. Ammonia and urea metabolism and excretion. *In:* Evans, D.H., (eds) The Physiology of Fishes. pp 379–425, CRC Press Inc, Baton Rouge

Wood, C.M. 2001. Influence of feeding, exercise and temperature on nitrogen metabolism and excretion. *In:* Wright, P.A., Anderson, P.M., (eds) Nitrogen Excretion. pp 201–221, Academic Press, New York

Wood C.M., Gilmour K.M., Perry S.F., Part P., Walsh P.J. 1998. Pulsatile urea excretion in gulf toadfish (*Opsanus beta*): evidence for activation of a specific facilitated diffusion transport system. J. Exp. Biol. **201** (Pt 6): 805–817

Wood, C.M., Hopkins, T.E., Walsh, P.J. 1997. Pulsatile urea excretion in the toadfish (*Opsanus beta*) is due to a pulsatile excretion mechanism, not a pulsatile production mechanism. *J. exp. Biol.* **200:**1039–1046

Wood, C.M., McDonald, M.D., Sundin, L., Laurent, P., Walsh, P.J. 2003. Pulsatile urea excretion in the gulf toadfish: mechanisms and controls. *Comp. Biochem. Physiol.* **136A:**667–684

Wood, C.M., Part, P., Wright, P.A. 1995b. Ammonia and urea metabolism in relation to gill function and acid-base balance in a marine elasmobranch, the spiny dogfish (*Squalus acanthias*). *J. Exp. Biol.* **198:**1545–1558

Wood, C.M., Perry, S.F., Wright, P.A., Bergman, H.L., Randall, D.J. 1989. Ammonia and urea dynamics in the Lake Magadi tilapia, a ureotelic teleost fish adapted to an extremely alkaline environment. *Respir. Physiol.* **77:**1–20

Wood, C.M., Warne, J.M., Wang, Y., McDonald, M.D., Balment, R.J., Laurent, P., Walsh, P.J. 2001. Do circulating plasma AVT and/or cortisol levels control pulsatile urea excretion in the gulf toadfish (*Opsanus beta*)? *Comp. Biochem. Physiol.* **129A:**859–872

Wright, P., Felskie, A., Anderson, P. 1995a. Induction of ornithine-urea cycle enzymes and nitrogen metabolism and excretion in rainbow trout (*Oncorhynchus mykiss*) during early life stages. *J. Exp. Biol.* **198:**127–135

Wright, P.A., Campbell, A., Morgan, R.L., Rosenberger, A.G., Murray, B.W. 2004. Dogmas and controversies in the handling of nitrogenous wastes: expression of arginase Type I and II genes in rainbow trout: influences of fasting on liver enzyme activity and mRNA levels in juveniles. *J. Exp. Biol.* **207:**2033–2042

Wright, P.A., Fyhn, H.J. 2001. Ontogeny of nitrogen metabolism and excretion. *In:* Wright, P.A., Anderson, P., (eds) Nitrogen Excretion. pp 149–200, Academic Press, San Diego

Wright, P.A., Land, M.D. 1998. Urea production and transport in teleost fishes. *Comp. Biochem. Physiol.* **119A:**47–54

Wright, P.A., Pärt, P., Wood, C.M. 1995b. Ammonia and urea excretion in the tidepool sculpin (*Oligocottus maculosus*): sites of excretion, effects of reduced salinity and mechanisms of urea transport. *Fish Physiol. Biochem.* **14:**111–123

Wright, P.A., Perry, S.F., Randall, D.J., Wood, C.M., Bergman, H. 1990. The effects of reducing water pH and total CO2 on a teleost fish adapted to an extremely alkaline environment. *J. Exp. Biol.* **151:**361–369

Wright, P.A., Randall, D.J., Perry, S.F. 1989. Fish gill water boundary layer: a site of linkage between carbon dioxide and ammonia excretion. *J. Comp. Physiol.* **158B:**627–635