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ORIGINAL PAPER

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Branchial and renal excretion of urea and urea analogues in the plainfin midshipman, *Porichthys notatus*

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Abstact This study investigated whether urea transport mechanisms were present in the gills of the ammoniotelic plainfin midshipman (*Porichthys notatus*), similar to those recently documented in its ureotelic relative (family Batrachoididae), the gulf toadfish (*Opsanus beta*). Midshipmen were fitted with internal urinary and caudal artery catheters for repetitive sampling of urine and blood in experiments and radiolabeled urea analogues ([^{14}C]-thiourea and [^{14}C]-acetamide) were used to evaluate the handling of these substances. Isosmotically balanced infusions of urea were used to raise plasma and urine urea concentrations to levels surpassing physiological levels by 8.5-fold and 6.4-fold, respectively. Despite these high urea levels, there was no observable transport maximum in either renal or branchial urea excretion rate, a result mirrored by the total uptake of fish exposed to a range of environmental urea concentrations. Permeability to urea appeared to be symmetrical in the two directions. At comparable plasma concentrations the branchial clearance rate of acetamide was 74% that of urea while branchial clearance rate of thiourea was 55% that of urea. For influx, the comparable values were 60% and 36%, indicating the same pattern. In contrast, the secretion clearance rate of acetamide by the kidney was 56% that of urea while the rate of thiourea secretion clearance was 137% greater than that of urea, with both urea and thiourea being more concentrated in the urine than in the plasma. In addition, the secretion clearance rates of thiourea and urea were significantly greater than those of water and Cl^-, whereas acetamide, water and Cl^- were found equally in the plasma and urine, appearing to passively equilibrate between the two fluids. Based on our findings, there appear to be two distinct transport mechanisms involved in urea excretion in the plainfin midshipmen, one in the gill (a facilitated diffusion type transporter) and one in the kidney (an active transport mechanism), each of which does not saturate even at plasma urea concentrations that greatly exceed physiological levels. These transporters appear to be similar to those in the midshipman's ureotelic relative, the gulf toadfish.

Keywords Urea · Gills · Kidney · Urea analogues · Plainfin midshipman

Abbreviations *GFR* glomerular filtration rate · *O-UC* ornithine-urea cycle · *tUT* toadfish urea transport protein · *UFR* urine flow rate · *UT* facilitated diffusion urea transporter

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Introduction

In aqueous environments, fish are able to eliminate nitrogenous wastes directly as ammonia as it diffuses readily through the gills and is subsequently diluted by the surrounding water. In this way, fish bypass the energy consuming detoxification steps that are necessary for land-dwelling animals. However, there are a handful of adult teleost (bony) fish species that have a fully functional ornithine-urea cycle (O-UC) giving them the ability to detoxify ammonia into urea when environmental conditions are unfavorable for ammonia

excretion (Saha and Ratha 1987; Mommsen and Walsh 1989; Randall et al. 1989; Wood et al. 1989). One of the best studied teleosts in this regard is the gulf toadfish, *Opsanus beta*, which has the unprecedented ability to switch from ammoniotelism to ureotelism when in a stressful environment (crowding, confinement, air or ammonia exposure; Walsh et al. 1990 , 1994; Walsh and Milligan 1995).

In the gulf toadfish the primary route of urea excretion is via the gills (>90% total urea excretion), where the cDNA for a urea transport protein (tUT) has been cloned that shows 62% homology at the amino acid level to mammalian hormonally controlled facilitated diffusion urea transporters (Smith et al. 1998; Walsh et al. 2000). This facilitated transport mechanism in toadfish allows for urea excretion that is not continuous but occurs in distinct 0.5–3 h pulses, on average once or twice every 24 h (Wood et al. 1995, 1997). During pulsing periods, the activation of tUT gives rise to an average 36-fold increase in urea permeability and at the same time a corresponding increase in the permeability of the urea analogues acetamide and thiourea of only 17-fold and 6-fold, respectively (Wood et al. 1998; McDonald et al. 2000). During non-pulsing periods the permeability of all three of these substances are not significantly different (McDonald et al. 2000). The aglomerular kidney of the toadfish, which forms urine primarily by secretion, is the secondary route of nitrogenous waste excretion (<10% total urea excretion). Toadfish urine urea concentrations generally exceed plasma levels by at least 30%, suggesting the presence of an active urea transport mechanism (McDonald et al. 2000). Similar to the gill, the toadfish kidney demonstrates differential handling of urea and analogues; urea and thiourea are more concentrated in the urine than in the plasma while acetamide appears to passively equilibrate between the two (McDonald et al. 2000).

The plainfin midshipman, *Porichthys notatus*, found in the northern Pacific, is a close relative of the warm-water gulf toadfish, both being members of the family Batrachoididae. To date, much of the research on the midshipman has focused on its vocal-acoustic signaling ability and the several hundred dermal luminescent photophores present on its ventral surface (for reviews see Baguet 1975; Bass et al. 1994). With respect to nitrogen metabolism and excretion, the midshipman shows a remarkable insensitivity to ammonia, being 2.5-times more tolerant than most teleosts, although not as tolerant as the gulf toadfish (Wang and Walsh 2000). Unlike the toadfish, where ammonia tolerance is for the most part due to the conversion of ammonia into urea, the midshipman does not express the O-UC and does not have the ability to switch to ureotelism during stressful conditions (Wang and Walsh 2000; Walsh et al. 2001a). Nonetheless, the midshipman does excrete some (<10%) of its nitrogenous waste as urea, but unlike the toadfish, branchial urea excretion is continuous (reviewed by Walsh 1997). The distinct vesicular trafficking evident in the gill epithelium of the pulsing toadfish, thought to play a role in pulsatile urea excretion, is not present in the gills of the midshipman (Wang and Walsh 2000; Laurent et al. 2001; Walsh et al. 2001a). Despite these differences, when a [^{32}P]-labeled cDNA probe based on the toadfish branchial tUT was used to examine gill mRNA by Northern blot analysis, the relative signal strength was strong for the plainfin midshipman (Walsh et al. 2001a). Although mRNA expression does not necessarily reflect protein expression, this result suggests the possibility of a branchial urea transport mechanism in the midshipman.

The objectives of this study were twofold. Firstly, we set out to characterize the branchial excretion of urea in the plainfin midshipman by examining the handling of urea in the face of urea loading, the handling of urea compared to the urea analogues acetamide and thiourea, and the uptake of urea from the water. At the same time, the aglomerular kidney of the midshipman was examined for potential urea transport mechanisms. Our results suggest the presence of two distinct transport mechanisms involved in urea excretion in the plainfin midshipman, found in the gill and the kidney, each of which does not saturate, even at plasma urea concentrations that greatly exceed physiological levels. In addition, both the gill and the kidney differentially handle urea, acetamide and thiourea, the gill moving urea preferentially over both acetamide and thiourea and the kidney moving urea over solely acetamide. Even though the midshipman is ammoniotelic, the characteristics of its transporters appear to be similar to those found in its ureotelic relative, the toadfish.

Materials and methods

Experimental animals

Plainfin midshipman (*P. notatus*) were obtained by chartered trawl in Berkley Sound, British Columbia, Canada, in July 1999, August 1999 and August 2000. At Bamfield Marine Station, the fish were held for 1 week prior to experimentation in large outdoor tanks served with running seawater at the experimental temperature (12 ± 1 °C), salinity (30 ± 2‰) and pH (7.90 ± 0.15). Fish were not fed throughout the experimental period.

Experimental protocol

Caudal artery and ureteral catheterization were performed simultaneously on male fish anaesthetized with MS-222 (0.07 g·l^{-1}; Sigma) and artificially ventilated on an operating table. Caudal artery catheters were inserted as described by Wood et al. (1997). The technique for inserting indwelling ureteral catheters is described in detail by McDonald et al. (2000) with our goal being to examine the function of the kidney alone by bypassing the urinary bladder and eliminating any reabsorptive/secretory roles of the bladder. Females were not used because their anatomy made it difficult to avoid the oviducts when entering the urinary sinus. During recovery and experimentation, urine was collected continuously with the catheter emptying into a vial approximately 3.0 cm below the water level of the box. The urine flow rate (UFR) was determined gravimetrically. Following surgery, fish were kept in darkened individual containers (minimum volume = 1.5 l) that were continually aerated and supplied with flowing water (200 ml·min^{-1}).

Following the procedure outlined by McDonald and Wood (1998), [^{3}H]-polyethylene glycol (PEG, Mr4000) was used as a marker for glomerular filtration rate (GFR) to detect if there were any functional glomeruli. PEG 4000 was chosen because it is considered a more accurate and conservative indicator of GFR in fish than other commonly used markers (e.g., inulin derivatives), as it undergoes minimal radioautolysis, metabolic breakdown, or post-filtration reabsorption across tubules and bladder (Beyenbach and Kirschner 1976; Erickson and Gingrich 1986; Curtis and Wood 1991). Prior to injection of PEG 4000, the fish were allowed to recover from surgery for at least 12 h, a period during which the patency of the arterial and urinary bladder catheters was confirmed. A dose of 9 μCi·100 g^{-1} [^{3}H]-PEG 4000 (New England Nuclear) was injected via the caudal arterial catheter followed by an additional 0.3 ml saline in fish where both catheters were deemed successful. The [^{3}H]-PEG 4000 was then allowed to equilibrate throughout the extracellular space for 12 h before sampling commenced.

In order to determine the normal composition of bladder urine, separate urine samples were taken directly from the bladder of six midshipmen and analyzed for Na^+, Cl^-, Mg^{++} and urea concentration.

Experimental series

Series i: urea handling during urea loading

This series examined the response of the fish to infusions of urea. The specific goal was to examine the pattern of branchial and renal handling of urea in the face of elevated plasma urea levels. In addition to branchial and renal handling of urea, we also monitored UFR, GFR, urine composition, plasma composition and the handling of Na^+, Cl^-, Mg^{++} and PEG 4000 by the kidney.

The protocol used in this series is described in detail by McDonald and Wood (1998). Following the [^{3}H] PEG 4000 injection and recovery period, two trials consisting of successive 36 h infusions via the caudal artery catheter were performed. In Trial A, eight fish were infused with isosmotic solutions (300 mosmol·kg^{-1}) containing 0, 5 mmol·l^{-1} and 20 mmol·l^{-1} urea, respectively, balanced with NaCl. Mean weights of fish in Trial A were 0.123 ± 0.006 kg ranging from 0.105 kg to 0.146 kg. In Trial B, six fish were infused with isosmotic solutions containing 0, 80 mmol·l^{-1} and 140 mmol·l^{-1} urea, respectively, balanced with NaCl. Mean weights of fish in Trial B were 0.100 ± 0.004 kg ranging from 0.082 kg to 0.109 kg. The 0 mmol·l^{-1} urea infusion served as a control for the effect of volume loading alone. In each case, one channel of a Gilson-8 channel peristaltic pump was used at an infusion rate of 3 ml·kg^{-1}·h^{-1}; the rate was checked by periodic measurements of the weight of the infusion reservoir. Branchial urea excretion was measured by closing the flux box and recording the appearance of urea in the external water. At the start of each infusion period, a blood sample was taken (200 µl, with saline plus red blood cell replacement), a fresh urine collection was started, water flow to the fish box was stopped and the water level was set to an exact volume mark of 1 l. An initial water sample was taken for measurement of urea concentration. Vigorous aeration maintained PO_2 close to air saturation. At 12 h, urine was sampled and a fresh urine collection was started, a blood sample was taken together with a second (final) water sample and the box was then rapidly flushed over a 15-min period. The box was then closed again for another 12 h, with water sampling at the beginning and water, blood and urine sampling at the end. After 36 h, the infusion solution was then changed to a higher urea concentration and the same protocol of box closure, water, urine and blood sampling repeated over the next 36 h. The final infusion for each trial was 24 h.

Thus for the first and second infusions of each trial, three blood samples were taken and three measurements of renal and branchial urea excretion were recorded. For the third infusion of each trial, only two blood samples were taken and two measurements of renal and branchial urea excretion were recorded. Blood samples were centrifuged at 10,000 *g* for 1 min and the plasma decanted. The red blood cells were resuspended in saline and re-infused. Plasma and urine were frozen and stored at –20 °C for later analysis of Na^+, Cl^-, Mg^{++}, urea and [^{3}H] PEG 4000. Water samples were analyzed immediately for urea and ammonia.

Two fish from each of Trial A and Trial B of this series were chosen to continue on with the successive 36-h infusions. The two fish from Trial A continued to be infused with isosmotic solutions containing 140, 200 mmol·l^{-1} and 300 mmol·l^{-1} urea, respectively, balanced with NaCl. In Trial B, the two fish continued to be infused with 200 mmol·l^{-1} and 300 mmol·l^{-1} urea, balanced with NaCl. Thus for these fish, additional blood samples were taken and measurements of renal and branchial urea excretion were made.

Series ii: urea handling compared to analogues

In this series, the patterns of branchial and renal urea excretion were compared to the handling of analogues, acetamide and thiourea, similar in size and structure to urea. Two trials were performed: (A) ten fish treated with acetamide and (B) ten fish treated with thiourea. Mean weights of fish were 0.089 ± 0.013 kg in the acetamide trial and 0.071 ± 0.008 kg in the thiourea trial. Following the [^{3}H]-PEG 4000 injection and 12 h equilibration period, urine collection was started and blood and water samples were taken. Water flow to the box was then stopped and the volume was set to at least 1 l. Thereafter, blood, urine and water samples were taken every 12 h, the water being changed at 12 h intervals.

Twenty-four hours after the [^{3}H]-PEG 4000 injection (i.e., after two control blood, water and urine samples) the fish were injected with a dose of 5 μCi·100 g body weight^{-1} [^{14}C]-labeled thiourea or acetamide in 160 µmol·100 g body weight^{-1} isosmotic cold analogue (concentration = 300 mmol·l^{-1}) to render internal analogue concentrations approximately equal to internal urea concentrations (2 mmol·l^{-1}). Samples were taken for an additional four 12 h periods. As described above, blood samples were immediately centrifuged. Plasma and urine were stored at –20 °C for later analysis of Na^+, Cl^-, Mg^{++}, urea, [^{14}C]-analogue and [^{3}H]-PEG 4000 concentrations. Water samples were analyzed for urea and [^{14}C]-analogue concentrations only.

Series iii: total uptake of urea from the surrounding water

Four different trials were performed using a total of 77 fish. Similar to the protocol of Wright et al. (1995b), smaller midshipmen (average weight = 0.035 ± 0.008 kg) in groups of five to seven were placed into aerated flux chambers with flow-through seawater and left to acclimate for 24 h. After the acclimation period, water flow was stopped, the volume of the chamber was set to 2 l and the fish became a part of one of the following trials. Trial A investigated the saturability of total urea uptake by placing individual groups of midshipmen in 0.5, 1, 2, 5, or 10 mmol·l^{-1} urea + 100 μCi [^{14}C]-urea in the surrounding seawater. In Trial B, a direct comparison was made between the uptake rates of urea and two urea analogues, acetamide and thiourea, using concentrations that were comparable to urea concentrations found in blood plasma in vivo. In this trial, one group of fish was exposed to 2 mmol·l^{-1} urea + 100 μCi [^{14}C]-urea in the surrounding seawater, a second group was exposed to 2 mmol·l^{-1} acetamide + 100 μCi [^{14}C]-acetamide and a third group was exposed to 2 mmol·l^{-1} thiourea + 100 μCi [^{14}C]-thiourea in the surrounding seawater. Trial C investigated analogue competition with urea using urea concentrations slightly greater than internal concentrations and analogue concentrations three times greater than urea concentrations. The first group of fish was exposed to 5 mmol·l^{-1} urea + 100 μCi [^{14}C]-urea, the second set to 5 mmol·l^{-1} urea, 100 μCi [^{14}C]-urea plus 15 mmol·l^{-1} acetamide and the third to 5 mmol·l^{-1} urea, 100 μCi [^{14}C]-urea plus 15 mmol·l^{-1} thiourea in the external water. Trial D examined the effect of phloretin, a urea transport blocker, on urea uptake. In this trial the first group of fish was exposed to 2 mmol·l^{-1} urea + 100 μCi [^{14}C]-urea + 0.250 mmol·l^{-1} phloretin in 0.04%

ethanol. A second group was exposed to 2 mmol·l^{-1} urea + 100 μCi [^{14}C]-urea + 0.04% ethanol in order to test the effect of the vehicle alone.

In all four trials, after a 12-h flux period, the fish were killed by a blow to the head, weighed, placed in 50 mmol·l^{-1} "cold" urea or analogue solution (to remove any surface binding of [^{14}C]-label), blotted dry, and then homogenized in 8% perchloric acid (2:1 parts acid:fish) using a Proctor-Silex Blend Master blender. A sample of this solution was centrifuged and the supernatant was analyzed for [^{14}C] counts.

Analytical techniques and calculations

Urea concentrations in blood, urine and water were measured using the diacetyl monoxime method of Rahmatullah and Boyde (1980), with correction as described by McDonald et al. (2000) for the presence of thiourea and acetamide, both of which tended to depress color development in a linear fashion. Ammonia concentrations in the water were measured by the method of Ivancic and Degobbis (1984). Na^+ and Mg^{++} concentrations in plasma and urine were measured using a Varian 1275 Atomic Absorption Spectrophotometer. Cl^- was measured using a Radiometer CMT10 chloridometer. For measurements of [^{3}H]-PEG 4000, [^{14}C]-urea and/or [^{14}C]-analogue, blood and urine samples (25 μl plus 5 ml seawater), seawater samples (5 ml) or whole body extracts (200 μl plus 5 ml seawater) were added to 10 ml ACS fluor (Amersham) and analyzed by scintillation counting on an LKB Rackbeta 1217 counter using an onboard quench correction program to separate [^{3}H] and [^{14}C] counts when necessary.

The concentration of analogue [A] in the plasma, urine, water or whole body was determined from the original specific activity (S; cpm·μmol^{-1}) of the injected solution by converting the radioactivity found in the samples into concentration (μmol·ml^{-1}):

$$S = \frac{cpm_{in}}{[A]_{in}} \tag{1}$$

$$[A]_s = \frac{cpm_s}{S} \tag{2}$$

where cpm_{in} (cpm·ml^{-1}) indicates the radioactivity of the injected solution, $[A]_{in}$ indicates the total analogue concentration of the injected solution, cpm_s is the radioactivity in the sample and $[A]_s$ is the analogue concentration in the sample. These analogues are not known to occur endogenously in fish.

The branchial clearance rate (CB) of any substance (X) was calculated by dividing the concentration of the substance appearing in the water $[X]_w$ by fish body weight (wt), plasma concentration $[X]_p$ and time (t):

$$CB_X = \frac{[X]_w \times V_f}{wt \times [X]_p \times t} \tag{3}$$

where V_f is the volume of water surrounding the fish. Equation 3 assumes an equilibrium between plasma and tissue urea concentrations.

The amount of any substance X taken up from the water was determined from the specific activity of the surrounding water (S_w; cpm·μmol^{-1}) by converting the radioactivity measured in fish (cpm_f) into concentration (μmol·kg^{-1} body weight). The relative uptake (RU) of a substance X was then determined by dividing the amount of X taken up from the water by $[X]_w$ and by time (t).

$$X = \frac{cpm_f}{S_w}, \tag{4}$$

$$RU_X = \frac{X}{[X]_w \times t}. \tag{5}$$

All the following renal rates were related to fish body weight by expressing UFR in milliliters per kilogram per hour. Urinary excretion rates (U) of any substance (X) were calculated as:

$$U_X = [X]_u \times UFR, \tag{6}$$

using measured values of UFR and urine concentrations $[X]_u$.

GFRs were calculated as the clearance of [^{3}H]-PEG 4000 – i.e., the excretion of radioactivity in the urine (cpm_u) relative to its concentration in the blood plasma (cpm_p):

$$GFR = \frac{cpm_u \times UFR}{cpm_p}. \tag{7}$$

The filtration rate (FR) of a substance X at the glomeruli was calculated as:

$$FR_X = [X]_p \times GFR, \tag{8}$$

and consequently the tubular secretion rate (TS) of X was calculated as:

$$TS_X = U_X - FR_X. \tag{9}$$

The renal clearance rate by tubular secretion (CR; ml·kg^{-1}·h^{-1}) of X was calculated as:

$$CR_X = \frac{TS_X}{[X]_p}, \tag{10}$$

where the secretion clearance rate (CR_X) is the volume of plasma required for X to be completely cleared by tubular secretion in a given amount of time.

Statistics

Data are reported as means ± 1 SEM (n = number of fish). Regression lines were fitted by the method of least squares, and the significance ($P < 0.05$) of the Pearson's correlation coefficient, r, was assessed. The significance of differences between means was evaluated using Student's paired, unpaired or one-sample two-tailed t-test ($P < 0.05$) as appropriate (Nemenyi et al. 1977). An ANOVA with time as the main factor was followed by a comparison of individual means using the Bonferroni correction for multiple sample comparisons.

Results

Resting values

Under resting conditions, concentrations of urea in the ureteral urine [3.83 ± 0.25(20) mmol·l^{-1}] were significantly greater than those in the plasma [2.68 ± 0.19(20) mmol·l^{-1}] but were not significantly changed further by the urinary bladder [4.77 ± 0.58(6) mmol·l^{-1}; Fig. 1A]. Na^+ concentrations were approximately 8-fold greater in the plasma [159.3 ± 5.9(20) mmol·l^{-1}] than in the ureteral urine [20.3 ± 3.4(20) mmol·l^{-1}] and were unmodified by the urinary bladder [16.5 ± 6.1(6) mmol·l^{-1}; Fig. 1B]. There was no significant difference between Cl^- concentrations in the ureteral urine [159.8 ± 7.7(20) mmol·l^{-1}], plasma [162.1 ± 4.9(20) mmol·l^{-1}] or bladder urine [142.2 ± 8.2(6) mmol·l^{-1}; Fig. 1C]. Mg^{++} concentrations in the ureteral urine [125.7 ± 8.56 (13) mmol·l^{-1}] were approximately 70-fold greater than concentrations in the plasma [1.8 ± 0.3(14) mmol·l^{-1}] with no further modification by the urinary bladder [117.2 ± 5.1(6) mmol·l^{-1}; Fig. 1D].

The midshipmen used in this study were ammoniotelic, excreting 80% ammonia-N [111.7 ± 13.4(19) μmol-

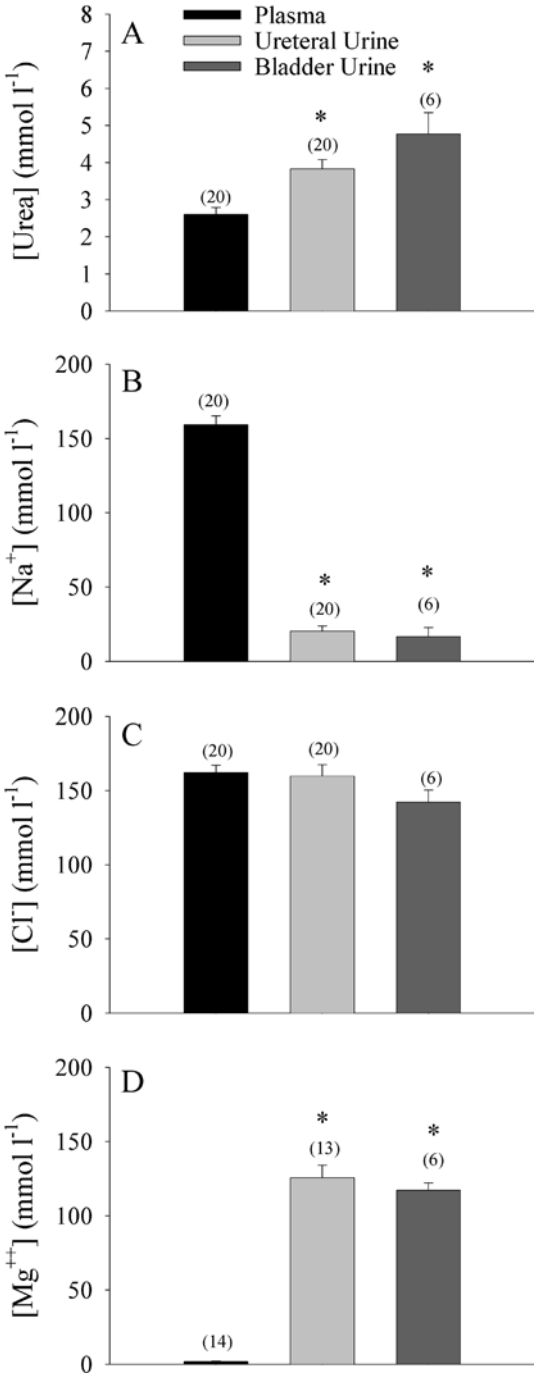

Fig. 1 Concentrations of **A** urea, **B** Na^+, **C** Cl^- and **D** Mg^{++} in the plasma, ureteral urine and bladder urine of the plainfin midshipman under resting conditions. Values are means ± 1 SEM (*n*); the *asterisk* indicates $P < 0.05$ significantly different from plasma

$N \cdot kg^{-1} \cdot h^{-1}$] and 20% urea-N [24.1 ± 5.2(20) μmol-N·kg^{-1}·h^{-1}] when averaged over a resting period. The gills were the major route of urea excretion relative to a urinary excretion rate of only 2.6 ± 0.3(20) μmol-N·kg^{-1}·h^{-1}.

Based on the renal clearance of the glomerular filtration rate marker [^{3}H]-PEG 4000, the midshipmen had a glomerular filtration rate [0.040 ± 0.003(20) ml·kg^{-1}·h^{-1}] that was significantly greater than zero, suggesting the presence of a small number of functional glomeruli. However, the urine flow rate [0.63 ± 0.12(20) ml·kg^{-1}·h^{-1}] was substantially greater (16-fold) than the glomerular filtration rate indicating that the urine was largely formed by secretion.

Urea handling during urea loading

Urea infusions were performed in Series i with the goal of raising the plasma urea concentration and therefore both the excretion rate of urea at the gill and the secretion rate of urea at the kidney tubule, to determine whether either of these processes could be saturated. Mean data for plasma and urine composition, UFR and GFR during Trial A (low dose infusion) and Trial B (high dose infusion) of this series are reported in Tables 1, 2. Although there was no significant change in most of these variables over the 96-h infusion protocol, GFR decreased significantly during the low dose infusion only (Table 1).

On average, in both trials, initial urine urea concentrations [3.04 ± 0.17(14) mmol·l^{-1}] were significantly greater than plasma urea concentrations [2.14 ± 0.14(14) mmol·l^{-1}]. Plasma urea concentrations during Trial A remained fairly stable through the infusion with 0 mmol·l^{-1} urea but rose significantly by the end of the 5 mmol·l^{-1} urea infusion (loading rate = 15 μmol·kg^{-1}·h^{-1}; Fig. 2A). Plasma urea concentrations continued to significantly rise when the infusate concentration was raised to 20 mmol·l^{-1} (loading rate = 60 μmol·kg^{-1}·h^{-1}) reaching a maximum concentration of 3.78 ± 0.19(8) mmol·l^{-1}. Urine urea concentrations stayed relatively constant throughout the experiment, rising only in the final 24 h, reaching a maximum concentration of 5.53 ± 0.29(8) mmol·l^{-1} (Fig. 2A).

Plasma urea concentrations during Trial B were relatively stable during the 0 mmol·l^{-1} urea infusion but concentrations increased significantly during the 80 mmol·l^{-1} (loading rate = 240 μmol·kg^{-1}·h^{-1}) and 140 mmol·l^{-1} (loading rate = 420 μmol·kg^{-1}·h^{-1}) infusions, reaching a maximum concentration of 18.09 ± 1.14(6) mmol·l^{-1} (Fig. 2B). Urine urea concentrations showed the same trend reaching a high of 19.35 ± 0.38(6) mmol·l^{-1} (Fig. 2B). Urine and plasma urea concentrations reached maxima that were 6.5- and 9.5- times higher, respectively, than the resting levels reported above.

The branchial excretion rate of urea (μmol·kg^{-1}·h^{-1}) exhibited a linear relationship ($r = 0.77$, $P < 0.0001$) to plasma urea concentration (mmol·l^{-1}) with a slope of 3.00 μmol·kg^{-1}·h^{-1} per mmol·l^{-1} (Fig. 3A). There was no evident limit in transport rate even though many plasma urea concentrations were well beyond physiological levels. In the opposite direction, in the experiment of Series iii-A, total uptake rate (μmol·kg^{-1}·h^{-1}) of urea from the external water exhibited a similar relationship

Table 1 Average values for plasma and urinary ion composition, urine flow rate and glomerular filtration rate during Trial A from fish of Series i. Data are shown as means ± SEM (*n*). (*GFR* glomerular filtration rate, *p* plasma, *u* urine, *UFR* urine flow rate)

Time (h)	UFR ($ml \cdot kg^{-1} \cdot h^{-1}$)	GFR ($ml \cdot kg^{-1} \cdot h^{-1}$)	$[Na^+]_p$ ($mmol \cdot l^{-1}$)	$[Na^+]_u$ ($mmol \cdot l^{-1}$)	$[Cl^-]_p$ ($mmol \cdot l^{-1}$)	$[Cl^-]_u$ ($mmol \cdot l^{-1}$)	$[Mg^{++}]_p$ ($mmol \cdot l^{-1}$)	$[Mg^{++}]_u$ ($mmol \cdot l^{-1}$)
12	0.24 ± 0.05(7)	0.044 ± 0.006(7)	164.4 ± 4.5(8)	18.3 ± 7.7(8)	173.7 ± 4.3(8)	184.0 ± 12.1(8)	1.2 ± 0.1(7)	129.6 ± 7.1(7)
24	0.27 ± 0.04(8)	0.047 ± 0.004(8)	159.0 ± 7.0(8)	6.9 ± 1.3(8)	177.4 ± 5.4(8)	164.0 ± 11.6(8)	1.2 ± 0.2(8)	128.9 ± 6.5(8)
36	0.22 ± 0.04(8)	0.031 ± 0.005(8)	153.7 ± 5.5(8)	8.1 ± 1.8(8)	170.7 ± 5.2(8)	162.8 ± 11.0(8)	1.1 ± 0.2(8)	121.6 ± 5.4(7)
48	0.30 ± 0.06(8)	0.027 ± 0.004(8)	151.7 ± 4.3(8)	14.3 ± 6.9(8)	165.9 ± 5.9(8)	171.8 ± 11.7(8)	1.1 ± 0.1(7)	123.3 ± 6.1(8)
60	0.33 ± 0.10(8)	0.026 ± 0.005(8)	153.4 ± 5.7(8)	13.6 ± 4.1(8)	172.9 ± 5.1(8)	150.3 ± 17.3(8)	1.2 ± 0.2(8)	116.6 ± 7.3(7)
72	0.34 ± 0.13(8)	0.022 ± 0.004*(8)	151.7 ± 4.5(8)	17.6 ± 6.4(8)	165.9 ± 5.7(8)	145.6 ± 18.5(8)	1.3 ± 0.2(8)	122.9 ± 11.8(7)
84	0.45 ± 0.15(8)	0.022 ± 0.004*(8)	152.5 ± 6.0(8)	22.9 ± 8.0(7)	170.0 ± 4.5(7)	135.9 ± 21.6(7)	1.4 ± 0.3(8)	103.0 ± 12.8(7)
96	0.37 ± 0.10(7)	0.018 ± 0.004*(8)	154.9 ± 5.6(8)	19.2 ± 7.7(6)	174.4 ± 6.6(8)	154.1 ± 20.5(6)	1.9 ± 0.5(8)	113.0 ± 14.7(6)

*Significantly different from first value; $P < 0.05$

Table 2 Average values for plasma and urinary ion composition, urine flow rate and glomerular filtration rate during Trial B from fish of Series i. Data are shown as mean ± SEM (*n*)

Time (h)	UFR ($ml \cdot kg^{-1} \cdot h^{-1}$)	GFR ($ml \cdot kg^{-1} \cdot h^{-1}$)	$[Na^+]_p$ ($mmol \cdot l^{-1}$)	$[Na^+]_u$ ($mmol \cdot l^{-1}$)	$[Cl^-]_p$ ($mmol \cdot l^{-1}$)	$[Cl^-]_u$ ($mmol \cdot l^{-1}$)	$[Mg^{++}]_p$ ($mmol \cdot l^{-1}$)	$[Mg^{++}]_u$ ($mmol \cdot l^{-1}$)
12	0.20 ± 0.06(6)	0.046 ± 0.005(5)	157.2 ± 7.9(6)	13.2 ± 3.2(6)	193.3 ± 6.3(6)	134.3 ± 8.5(5)	1.0 ± 0.2(6)	113.5 ± 4.2(5)
24	0.21 ± 0.06(6)	0.039 ± 0.009(6)	151.4 ± 4.5(6)	10.6 ± 1.3(6)	192.3 ± 2.9(6)	124.8 ± 15.7(6)	1.0 ± 0.2(6)	102.5 ± 6.4(6)
36	0.28 ± 0.04(6)	0.045 ± 0.010(6)	150.0 ± 4.8(6)	15.3 ± 4.2(6)	186.7 ± 2.8(6)	116.4 ± 22.7(6)	0.9 ± 0.1(6)	90.1 ± 12.0(6)
48	0.25 ± 0.04(6)	0.027 ± 0.006(6)	148.0 ± 3.2(6)	16.2 ± 7.0(6)	191.1 ± 23.0(6)	102.7 ± 21.4(6)	1.0 ± 0.1(6)	88.1 ± 16.0(6)
60	0.20 ± 0.06(6)	0.018 ± 0.007(6)	141.4 ± 2.1(6)	9.6 ± 2.7(5)	186.5 ± 2.5(6)	123.8 ± 26.6(5)	1.0 ± 0.1(6)	102.2 ± 14.4(5)
72	0.22 ± 0.05(5)	0.022 ± 0.005(5)	140.9 ± 3.5(6)	9.8 ± 2.9(5)	186.0 ± 2.7(6)	109.3 ± 22.2(5)	1.1 ± 0.1(6)	96.6 ± 13.8(5)
84	0.28 ± 0.04(4)	0.031 ± 0.011(3)	143.9 ± 4.4(6)	21.5 ± 9.9(4)	185.2 ± 2.5(6)	127.3 ± 44.6(4)	1.2 ± 0.2(6)	117.2 ± 33.7(4)
96	0.25 ± 0.03(4)	0.022 ± 0.004(4)	141.6 + 4.3(6)	6.1 ± 2.1(3)	184.6 ± 3.7(6)	92.9 ± 28.5(4)	1.2 ± 0.3(6)	98.1 ± 20.6(4)

($r = 0.96$, $P < 0.007$) to water urea concentration (m $mol \cdot l^{-1}$) with a comparable slope of 3.82, indicating that urea is capable of moving effectively in both directions (Fig. 3B). Similar to branchial excretion, there was no observable transport maximum in the uptake of urea at the gills. In addition, in Series iii-D, the rate of urea uptake was found to be insensitive to phloretin, a blocker of UT-type transport mechanisms, placed on the apical side of the gill (data not shown).

Urinary excretion rate of urea also showed a linear relationship ($r = 0.72$, $P < 0.0001$) to plasma urea concentration with a slope of 0.38 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ per $mmol \cdot l^{-1}$ (Fig. 4). This slope was approximately one order of magnitude lower than the slope observed between branchial excretion rate and plasma urea concentration, a difference that was also reflected in the mean resting values of branchial and urinary excretion rate. Again, similar to branchial urea handling, there was no observable transport maximum in the urinary excretion rate of urea.

Urea handling compared to analogues

In Series ii, branchial and renal handling of urea was compared to the handling of analogues based on the premise that if a carrier is involved it would differentially transport urea versus analogues. However, if movement is passive, urea would not be differentially transported. In the acetamide series (ii-A), the branchial clearance rate ($ml \cdot kg^{-1} \cdot h^{-1}$) of urea [3.62 ± 0.90(10) $ml \cdot kg^{-1} \cdot h^{-1}$] was not different to that of acetamide [2.40 ± 0.48(10) $ml \cdot kg^{-1} \cdot h^{-1}$; Fig. 5A]. In contrast, in the thiourea series (ii-B), the branchial clearance rate of urea [7.45 ± 1.41(10) $ml \cdot kg^{-1} \cdot h^{-1}$] was approximately three times greater than the clearance rate of thiourea [2.52 ± 0.34(10) $ml \cdot kg^{-1} \cdot h^{-1}$; Fig. 5B]. When expressed as a clearance ratio (analogue/urea) for all collection periods where simultaneous measurements were made, the ratios were 0.74 ± 0.10(9) for acetamide and 0.55 ± 0.13(10) for thiourea, both showing a significant difference from unity. The same pattern (acetamide/urea ratio = 0.60; thiourea/urea ratio = 0.36) was observed in Series iii-B when measuring the total uptake rate of these substances, thereby supporting bidirectional transport [urea = 8.15 ± 1.94(12) $ml \cdot kg^{-1} \cdot h^{-1}$, acetamide = 4.93 ± 0.67(6) $ml \cdot kg^{-1} \cdot h^{-1}$, thiourea = 2.95 ± 0.67(6) $ml \cdot kg^{-1} \cdot h^{-1}$; Fig. 5C, D] in independent experiments.

Interestingly, the total uptake rate of urea in Series iii-B (measured in the absence of analogue) and the branchial clearance rate of urea in the thiourea series (ii-B; measured in the presence of thiourea) were quite similar. However, both were significantly greater than the branchial clearance rate of urea measured in the presence of acetamide (ii-A), suggesting a possible competitive interaction between acetamide and urea. In investigating this possibility further using the uptake protocol in Series iii-C, urea movement was found to be unaffected by the presence of analogues, even at analogue concentrations in the external water that were three fold greater than urea concentrations (data not shown). Thus, there appears to be no competitive effect.

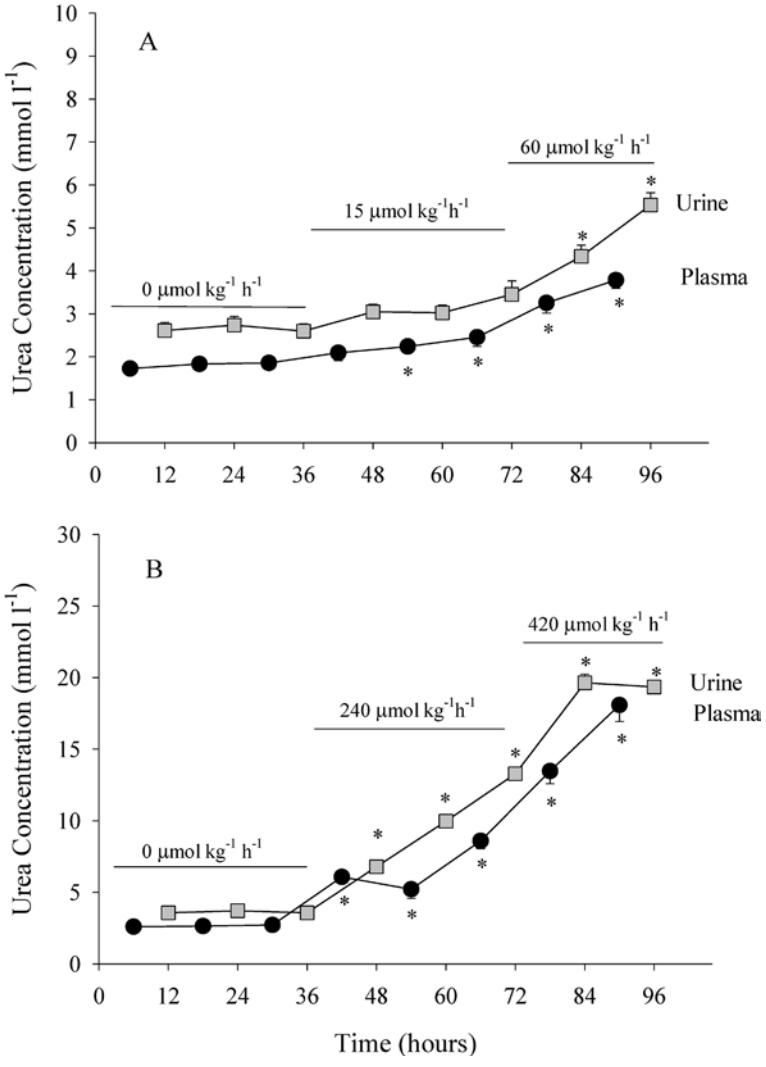

Fig. 2 Urea concentrations in the urine and plasma in **A** fish of Series i Trial A infused with urea at consecutive rates of 0, 15 and 60 μmol·kg^{-1}·h^{-1} and **B** fish of Series i Trial B infused with urea at consecutive rates of 0, 240 μmol·kg^{-1} and 420 μmol·kg^{-1}·h^{-1}. Values are means ± 1 SEM ($n = 8$ for Trial A, $n = 6$ for Trial B); the *asterisk* indicates $P < 0.05$ significantly different from the first value

Fish treated with internal acetamide loading (Series ii-A) demonstrated urine urea concentrations [4.04 ± 0.40(9) mmol·l^{-1}] that were significantly greater than plasma concentrations [2.92 ± 0.33(9) mmol·l^{-1}] throughout most of the experimental period, i.e., before and after the acetamide injection (Fig. 6A). Both plasma and urine urea concentrations showed a tendency to increase during the experimental period, although this increase was not significant. Plasma and urine acetamide concentrations were not significantly different from one another [1.65 ± 0.12(9) mmol·l^{-1} and 1.98 ± 0.14(9) mmol·l^{-1}, respectively; Fig. 6B] and by the end of the experiment, acetamide levels were actually slightly greater in the plasma than in the urine.

As in the acetamide series, urea concentrations in the urine of fish treated with internal thiourea loading [Series ii-B; 4.44 ± 0.38(10) mmol·l^{-1}] were significantly greater than plasma levels [2.85 ± 0.23(10) mmol·l^{-1}] both before and after injection of the analogue (Fig. 6C). Thiourea concentrations in the urine and plasma showed the same trend as urea concentrations, in that the thiourea was much greater in the urine [3.37 ± 0.45(10) mmol·l^{-1}] than in the plasma [1.29 ± 0.17(10) mmol·l^{-1}]. These data suggest that the kidney handles thiourea in the same way that it handles urea (Fig. 6D) and very differently from the way it handles acetamide (Fig. 6B).

In simultaneous secretion clearance measurements, urea secretion clearance [0.54 ± 0.13(9) ml·kg^{-1}·h^{-1}] was 1.8-times greater than acetamide secretion clearance [0.30 ± 0.05(9) ml·kg^{-1}·h^{-1}; Fig. 7A]. In contrast, in the presence of thiourea, the secretion clearance of urea [0.45 ± 0.07(10) ml·kg^{-1}·h^{-1}] was less than that of thiourea [0.71 ± 0.12(10) ml·kg^{-1}·h^{-1}; Fig. 7B]. When expressed as clearance ratios (analogue/urea) for all collection periods where simultaneous measurements were made, the ratios were 2.43 ± 0.66(10) for thiourea and only 0.59 ± 0.08(9) for acetamide, a highly significant difference showing that thiourea was cleared more effectively than urea, whereas acetamide was cleared less effectively.

As mentioned earlier, urine Na^+ concentrations were on average eight fold lower than plasma concentrations (Fig. 1B). Conversely, in accordance with active

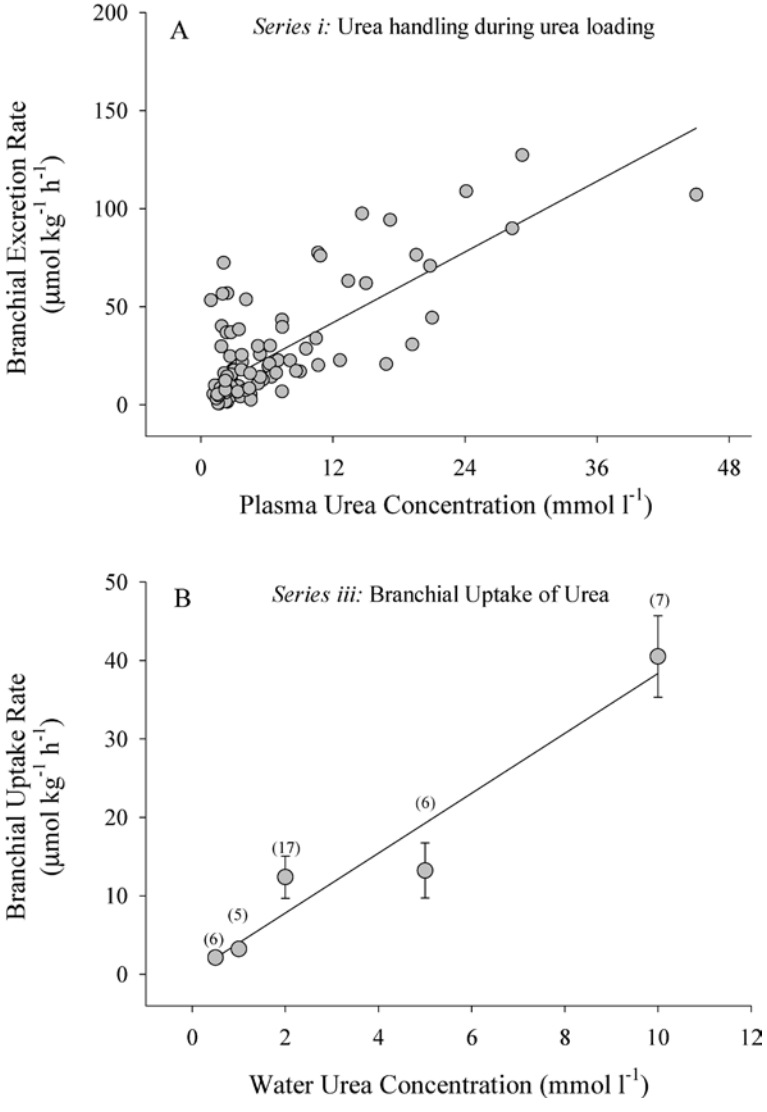

Fig. 3 **A** Linear regression of the branchial excretion rate of urea (y-axis) versus the plasma urea concentration (x-axis) indicating a linear, proportional relationship with no apparent transport maximum. The equation of the line and the significance of the correlation are $y = 3.00x + 5.83$, $r = 0.77$ ($n = 118$ points from 14 fish; $P < 0.0001$). **B** A linear regression of the branchial uptake rate (y-axis) versus water urea concentration (x-axis) showing the same trend in the reverse direction. The equation of the line and the significance of the correlation are $y = 3.82x + 0.181$, $r = 0.96$. Values are means ± 1 SEM (n) $P < 0.007$

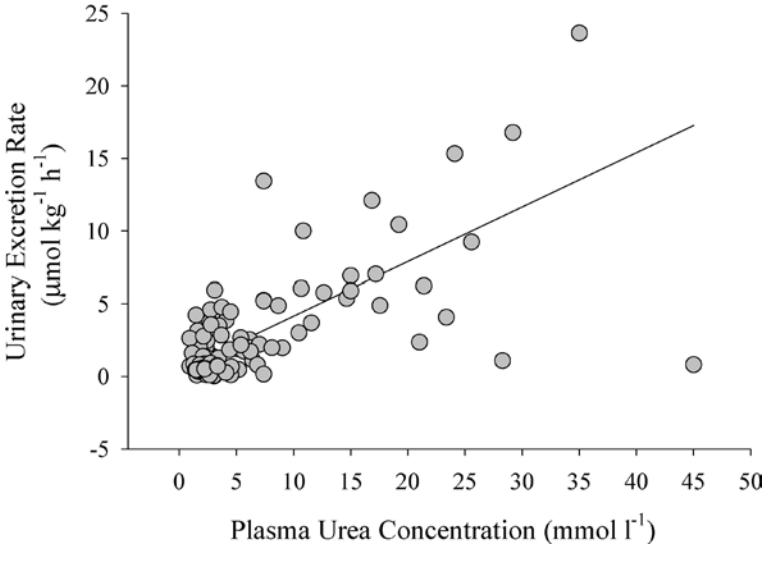

Fig. 4 Linear regression of the urinary excretion rate of urea (y-axis) versus the plasma urea concentration (x-axis) demonstrating a linear, proportional relationship with no observable transport maximum. The equation of the line and the significance of the correlation are $y = 0.37x + 0.41$, $r = 0.72$ ($n = 130$ points from 14 fish; $P < 0.0001$)

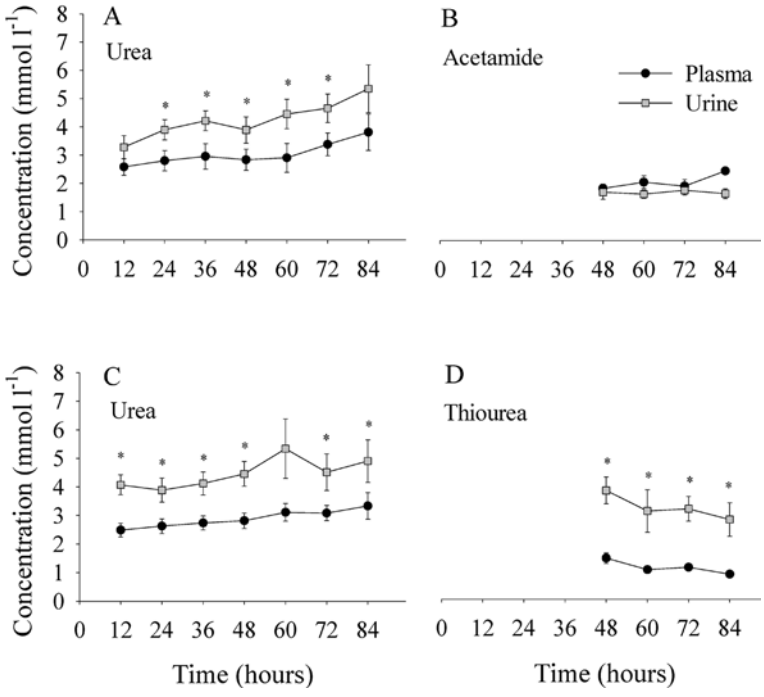

Fig. 5 The branchial clearance rates ($ml \cdot kg^{-1} \cdot h^{-1}$) of fish in the **A** acetamide series demonstrating similar handling of urea and acetamide. Fish in the **B** thiourea series have a significantly lower branchial clearance of thiourea compared to urea. A similar trend is illustrated by measured branchial uptake rates of **C** urea and acetamide and **D** urea and thiourea. Values are means ± 1 SEM (n); the *asterisk* indicates $P < 0.05$ significantly different from analogue

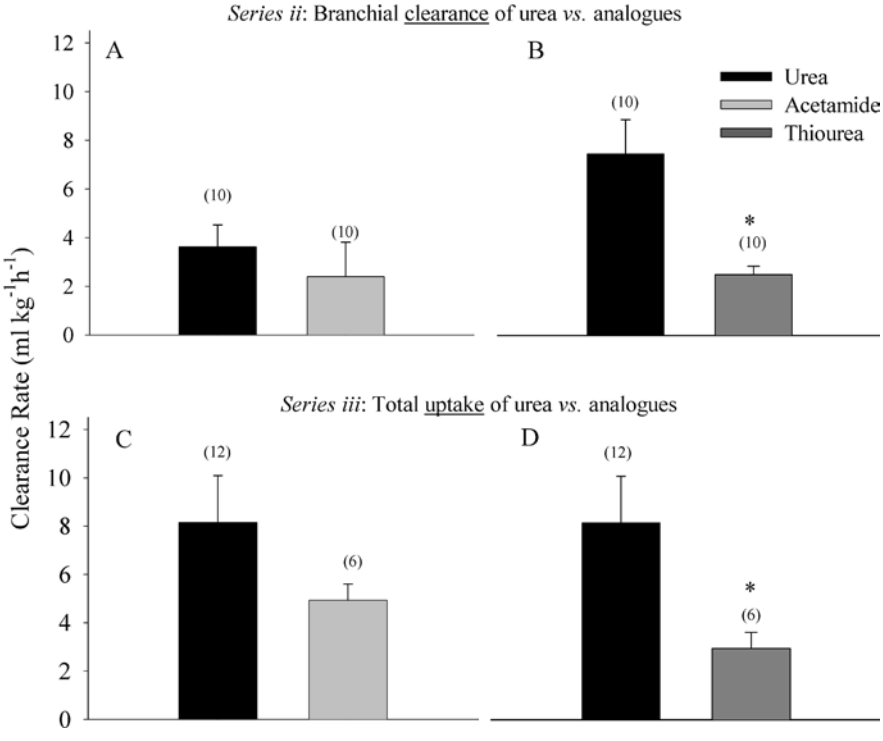

Fig. 6 Concentrations of **A** urea and **B** acetamide, **C** urea and **D** thiourea in the urine and plasma demonstrating differences in the renal handling of urea and acetamide but similar handling between urea and thiourea. Values are means ± 1 SEM ($n = 9$ for acetamide series, $n = 10$ for thiourea series); the *asterisk* indicates $P < 0.05$ significantly different from plasma concentrations

secretion, is the observation that urine Mg^{++} concentrations were 70-fold greater than plasma concentrations (Fig. 1D). However, plasma and urine Cl^- concentrations were not significantly different from one another, suggesting that Cl^- is passively distributed between the plasma and the urine (Fig. 1C). When expressed as secretion clearance rates, the rate of Mg^{++} secretion clearance [$38.22 \pm 7.05(14)$ $ml \cdot kg^{-1} \cdot h^{-1}$] was approximately 100-times greater than that of urea secretion clearance rate [$0.48 \pm 0.07(20)$ $ml \cdot kg^{-1} \cdot h^{-1}$; Fig. 8A]. However, urea secretion clearance rate was significantly greater than the secretion of both Cl^- [0.27 ± 0.04 (20) $ml \cdot kg^{-1} \cdot h^{-1}$] and water [$0.26 \pm 0.03(20)$ $ml \cdot kg^{-1} \cdot h^{-1}$], which were not significantly different from each other. Thus, on a relative basis, 1.8-times more urea was secreted by the kidney than Cl^- or water movement. Not

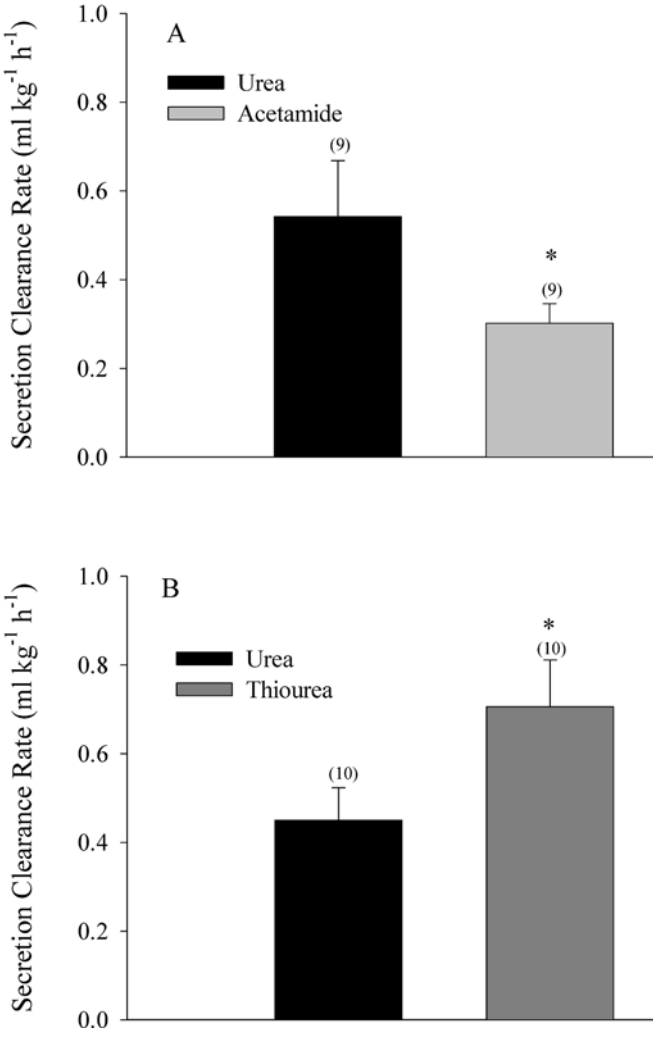

Fig. 7 The secretion clearance rate ($ml \cdot kg^{-1} \cdot h^{-1}$) of fish in the **A** acetamide series showing a urea clearance rate that is significantly greater than the clearance rate of acetamide. In contrast, fish in the **B** thiourea series demonstrated a clearance rate of urea that was significantly less than the clearance rate of thiourea. Values are means ± 1 SEM ($n = 9$ for acetamide series, $n = 10$ for thiourea series); the *asterisk* indicates $P < 0.05$ significantly different from analogue

surprisingly, urea secretion clearance rate was significantly greater than the secretion clearance rate of Na^+. Overall there was no net secretion of Na^+.

As observed for urea, the secretion clearance rate of thiourea [$0.71 \pm 0.12(10)$ $ml \cdot kg^{-1} \cdot h^{-1}$] was significantly less than Mg^{++} [$28.5 \pm 9.2(10)$ $ml \cdot kg^{-1} \cdot h^{-1}$] but significantly greater than Cl^- [$0.26 \pm 0.11(10)$ $ml \cdot kg^{-1} \cdot h^{-1}$] and water [$0.25 \pm 0.09(10)$ $ml \cdot kg^{-1} \cdot h^{-1}$; Fig. 8B]. Thus, the renal secretion of thiourea was 2.7–2.8-times more than either Cl^- or water movement.

The secretion clearance rate of acetamide [$0.31 \pm 0.05(9)$ $ml \cdot kg^{-1} \cdot h^{-1}$], like thiourea and urea, was significantly less than Mg^{++} [$59.8 \pm 17.6(3)$ $ml \cdot kg^{-1} \cdot h^{-1}$; Fig. 8C]. However, the clearance of acetamide was not significantly different than Cl^- [$0.23 \pm 0.07(9)$ $ml \cdot kg^{-1} \cdot h^{-1}$] or water [$0.22 \pm 0.05(9)$ $ml \cdot kg^{-1} \cdot h^{-1}$]. Therefore, on a relative basis, acetamide moved into the kidney tubule at the same rate as Cl^- and water.

Discussion

Prior results suggested that there is mRNA for a UT-type urea transporter in the gills of the plainfin midshipman (Walsh et al. 2001a). However, this does not necessarily mean that the protein is expressed and/or functional. Hence, the objectives of this study were to examine and characterize the movement of urea through the gills of the plainfin midshipman and to determine whether the branchial excretion of urea is in fact carrier-mediated. In addition, the kidney was examined for urea transport mechanisms. Our results suggested that despite a lack of saturation kinetics, there is evidence for two distinct transport mechanisms involved in the excretion of urea, one in the gill and one in the kidney, both of which demonstrate differential handling of urea and analogues. However, the pattern of urea versus analogue handling in the two organs is not the same.

When plasma urea concentrations of midshipman were elevated by infusion of exogenous urea loads, there was no observable transport maximum in the branchial excretion of urea, even at plasma urea concentrations well exceeding physiological levels. Although the lack of a transport maximum is usually indicative of passive diffusion through cell membranes, UT-type urea transport mechanisms are notably difficult to saturate (Chou et al. 1990). However, saturation kinetics have been described for urea transport mechanisms of lower vertebrates (Levine et al.1973; Shpun and Katz 1989, 1990; Pilley and Wright 2000; Fines et al. 2001). Similar to observations in the present study, a linear relationship between branchial excretion rate and plasma urea concentration was observed in the freshwater rainbow trout, *Oncorhynchus mykiss*, upon exogenous urea loading at the same rates (McDonald and Wood 1998). However, using the in vitro membrane vesicle preparation of Perry and Flik (1988), saturation kinetics for urea transport across the basolateral membrane of the trout gill ($K_m = 1.17$ $mmol \cdot l^{-1}$ and $V_{max} = 0.42$ $\mu mol \cdot mg$ $protein^{-1} \cdot h^{-1}$) has recently been demonstrated (M.D. McDonald and C.M. Wood, unpublished data). Thus, by using an in vitro approach to study the midshipman gill, it may be possible to determine saturation kinetics for urea movement.

In addition to the linear relationship between branchial excretion rate and concentration, there was a very similar relationship between total uptake rate and concentration; the two functions exhibited similar slopes, indicating that urea passes symmetrically in both directions. This same symmetry of transport in two directions has been found in the gill of the gulf toadfish (Wood et al. 1998) as well as the tidepool sculpin (*Oligocottus maculosus*; Wright et al. 1995b) and is characteristic of facilitated diffusion transporters for urea (Chou et al. 1990).

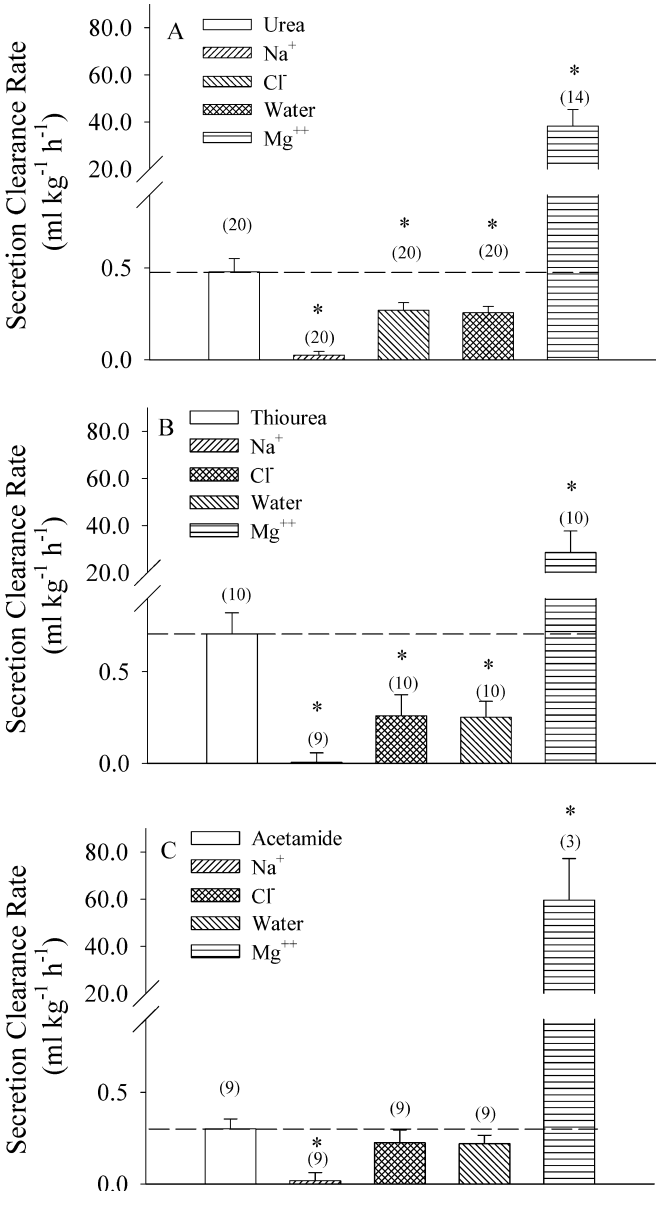

Fig. 8 The secretion clearance rates ($ml \cdot kg^{-1} \cdot h^{-1}$) of **A** urea, **B** acetamide and **C** thiourea versus Na^+, Cl^-, water and Mg^{++} demonstrating a secretion of urea and thiourea at a greater relative rate than the movements of water and Cl^-. The rate of secretion of acetamide was comparable to those of both water and Cl^-. In all cases, there was no net secretion of Na^+. Values are means ± 1 SEM; the *asterisk* indicates $P < 0.05$

The similarity between branchial excretion and total uptake of urea argues that uptake is through the gill and the contribution of the skin and gut is likely to be minor. The permeability of midshipman skin to urea may be similar to that of toadfish where the measured permeability is low [$5.07 \pm 0.56(8) \times 10^{-7}$ $cm \cdot sec^{-1}$] compared to the permeability of tritiated water [$72.0 \pm 15.0(8) \times 10^{-7}$ $cm \cdot sec^{-1}$; Part et al. 1999]. With respect to the contribution of the gut, toadfish drink only 0.75 ± 0.06 (24) $ml \cdot kg^{-1} \cdot h^{-1}$ seawater (M. Grosell, C.M. Wood, M.D. McDonald and P.J. Walsh, unpublished data), hence the gut could account for only a small component urea uptake from the water if drinking rate in midshipmen is similar. Conceivably, the slightly greater slope observed in uptake versus concentration could be due to the small contribution of the gut.

Further evidence for the presence of a branchial urea transport mechanism in the midshipman is the preferential clearance of urea over both acetamide and thiourea. The ratio of analogue/urea clearance revealed a clearance of acetamide that was 74% of urea clearance while thiourea clearance was only 55% that of urea. In the influx direction, these ratios were 60% and 36%, respectively. This pattern is similar to that in the gills of the gulf toadfish where there were increases in permeability in the order urea > acetamide > thiourea of toadfish gills observed during pulses when the facilitated diffusion transporter (tUT) was activated (McDonald et al. 2000). At this time, the branchial clearance of acetamide was 35–50% that of urea clearance and the relative thiourea clearance was at most only 16% of urea clearance during pulsing events. However, all three substances had similar permeabilities during non-pulsing periods, suggesting that the gill handled urea, acetamide and thiourea identically when tUT was not activated, despite small differences in calculated oil/water partition coefficients and lipid permeabilities of the three substances (Goldstein and Solomon 1960; Lippe 1969; Galluci et al. 1971). Similar to the toadfish during a pulse event, the UT-type transporter in the Lake Magadi tilapia (mtUT with 75% identity to tUT) demonstrated a permeability to thiourea that was 19% that of urea permeability (Walsh et al. 2001b). Therefore, it seems probable that a facilitated diffusion transporter for urea, similar to those in the ureotelic species, is continually expressed at low levels in the gills of the plainfin midshipman, in accord with recent molecular evidence (Walsh et al. 2001a). Indeed this type of transporter may be present in the gills of many other ammoniotelic teleosts (Walsh et al. 2001a) as well as in teleost embryos (Pilley and Wright 2000). It would be interesting to know whether the transporter is subject to physiological regulation in ammoniotelic fish.

Differentiation between urea and analogues by urea transport mechanisms is not an uncommon observation, as studies characterizing urea transport mechanisms in other organisms have shown that urea transporters customarily move analogues less effectively than urea and can often distinguish between analogues. Classic work on the kidney of the spiny dogfish (*Squalus acanthias*) showed that acetamide and methylurea were reabsorbed nearly as well as urea but thiourea was not (Schmidt-Nielsen and Rabinowitz 1964). Since then, Smith and Wright (1999) have cloned a renal urea transporter (ShUT) of the dogfish that appears related to the mammalian UT-A family of facilitated diffusion transporters. Additionally, work on frog kidney (Schmidt-Nielsen and Shrauger 1963), rat kidney (UT-A1: Chou et al. 1990) and human erythrocytes (UT-B; Naccache and Sha'fi 1973) have

demonstrated differential handling of urea and urea analogues.

Competitive interactions between urea and analogues are often observed in cases where UT-type transport mechanisms are involved including the gills of both the gulf toadfish (tUT; Wood et al. 1998; Walsh et al. 2000) and the Lake Magadi tilapia (mtUT; Walsh et al. 2001b) and even in the rainbow trout (*Oncorhynchus mykiss*) embryo (Pilley and Wright 2000), where a facilitated diffusion transport mechanism for urea is believed to be present. In the present study, there was no competitive inhibition of analogues on urea transport when analogues were injected into the fish or when looking at the uptake of [^{14}C]-urea in the presence an excess of cold thiourea or acetamide. Possibly, acetamide and thiourea may not be moving through the transport mechanism at all, but rather passively diffusing through the membrane and thus not interfering with urea movement.

Phloretin, a potent inhibitor of UT-type transport mechanisms (Chou and Knepper 1989), had no effect when placed in the water (having access to the apical membrane), suggesting that a UT-type transport mechanism is not present at least on the apical membrane of the gill. However, phloretin-insensitivity on the apical side of the cell does not rule out the possibility that a phloretin-sensitive mechanism could be present on the basolateral membrane. In the gulf toadfish, phloretin introduced systematically via a caudal artery catheter (having access to the basolateral membrane) significantly inhibited pulse frequency and urea output as pulses, causing a significant internal accumulation of urea-N, though interpretation was confounded because of the toxicity of the drug when administered internally (Wood et al. 1998).

To maintain osmotic equilibrium marine teleosts drink seawater, consequently ingesting monovalent and divalent ions that, if not excreted, would increase in the extracellular fluid to physiologically intolerable concentrations. Reflecting this, the dominant osmolytes in the urine of seawater-acclimated toadfish are Mg^{++}, Cl^{-} and SO_4^{-}; Mg^{++} and SO_4^{-} are found in significantly higher concentrations in the urine than in the plasma while Cl^{-} is found equally in both media (Howe and Gutknecht 1978; Baustain et al. 1997; McDonald et al. 2000). It was therefore not surprising that Mg^{++} concentrations were low in the plasma and high in the urine of the midshipman, while Cl^{-} concentrations were in equal concentrations in plasma and urine. Although SO_4^{-} was not measured in this study, it can be postulated that it would make up the anion deficit as high levels of SO_4^{-} are found in the urine of several marine teleosts (Hickman and Trump 1969; Renfro and Dickman 1980).

In addition to drinking seawater to maintain osmotic equilibrium, marine teleosts generally exhibit a degenerated glomerulus that is smaller than that found in freshwater teleosts, as there is no need for excreting high amounts of water in marine animals (Marshall 1929; Hickman and Trump 1969). The extreme case of glomerular degeneration is the aglomerular kidney believed to be present in the goosefish (*Lophius americanus*), toadfish, and midshipman (Marshall 1929; Lahlou et al. 1969; Baustain et al. 1997). The appearance of [^{3}H]-PEG 4000 in the urine of the midshipman supports findings by McDonald et al. (2000) showing the presence of [^{3}H]-PEG 4000 in the urine of the gulf toadfish, *Opsanus beta*. In addition, an earlier study by Lahlou et al. (1969) showed the appearance of [^{14}C]-inulin in the urine of the oyster toadfish, *Opsanus tau*. PEG 4000 is thought to be a more accurate indicator of GFR than inulin since it undergoes minimal metabolic breakdown (Beyenbach and Kirschner 1976; Erickson and Gingrich 1986; Curtis and Wood 1991). Therefore, there appear to be a small number of functional glomeruli present in the kidney in the family Batrachoididae. However, in the case of the midshipman (as in the toadfish) GFR is less than 15% of UFR so despite measurable glomerular filtration, urine in the midshipman is formed mainly by secretion.

Our results suggest the presence of a urea transport mechanism in the midshipman kidney that is unlike the facilitated diffusion transporter that may be present in the gill for two reasons. Firstly, the analogues are handled differently by the kidney and gill, suggesting a different transporter in the kidney. In contrast to the gill where acetamide and urea were handled similarly (and different from thiourea), the opposite trend was observed in the kidney where it appeared that thiourea and urea were handled similarly, accumulating in the urine against a concentration gradient. Conversely, acetamide appeared to equilibrate between the plasma and urine. The secretion of thiourea was 37% greater than that of urea, whereas that of acetamide was 45% less than that of urea.

Secondly, the secretion of thiourea and urea could not occur entirely via a facilitated diffusion mechanism since they appear to move against a concentration gradient, suggesting the involvement of an active transport mechanism. In addition, both thiourea and urea appeared to be secreted at least 1.8–2.7-times more effectively than Cl^{-}, which was found equally in the urine and plasma, appearing to passively equilibrate between the two fluids. Acetamide secretion clearance, on the other hand, was not significantly different from Cl^{-} secretion clearance. Since the relative secretions of thiourea and urea were greater than the relative movements of Cl^{-} or water, their passage into the kidney tubule cannot be accounted for by passive diffusion. In addition, with acetamide found equally in both urine and plasma, the possibility that thiourea and urea were concentrated in the tubule as a consequence of water reabsorption can be eliminated.

To date, little research has been done investigating the renal mechanisms for urea handling in fish. Similar to the findings of this study, the aglomerular kidney of the gulf toadfish demonstrated the same pattern in analogue differentiation, with the secretion clearance rates of urea and thiourea being relatively greater than the

secretion of acetamide and Cl^- (McDonald et al. 2000). In that study, the authors speculated what transport mechanisms could be responsible for the secretion of urea against a concentration gradient into the kidney tubule, such as the Na^+-dependent urea transporter originally identified in the elasmobranch kidney (Schmidt-Nielsen et al. 1972) and more recently in the mammalian kidney of protein depleted animals (Isozaki et al. 1994a, 1994b). As Na^+ reabsorption is not substantial in an aglomerular kidney, perhaps a unique mechanism is involved that exploits the renal physiology of toadfish and midshipmen, such as a Mg^{++}/urea cotransport mechanism, Mg^{++} being an important component of secretory urine formation.

In summary, it appears that the ammoniotelic midshipman has urea transporters in both the gill and the kidney, similar to its close relative, the facultatively ureotelic gulf toadfish. The patterns of analogue versus urea handling by the gill and the kidney are similar between the two species, suggesting the involvement of functionally similar transporters. The gill and kidney transporters exhibit different patterns of urea versus analogue handling; the gill transporter appears to be of the facilitated diffusion type, while the kidney transporter appears to be of the active type. The significance of an ammoniotelic teleost fish possessing specific urea excretory mechanisms when urea makes up only 10% of its total nitrogenous waste is unclear. However, growing evidence suggests a role for urea in early life stage development of ammoniotelic teleosts (Wright et al. 1995a). Further investigation is required to fully understand the mechanisms involved and their physiological relevance in the plainfin midshipman.

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