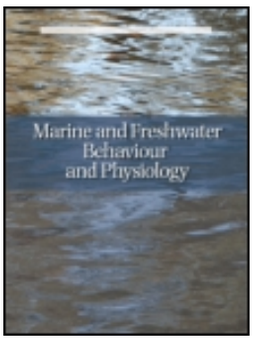

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NITROGEN METABOLISM AND EXCRETION IN AN INTERTIDAL POPULATION OF THE GULF TOADFISH (*OPSANUS BETA*)

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The present study was undertaken to assess the ability of gulf toadfish (*Opsanus beta*) from an intertidal population in northern Florida to make and excrete urea as a nitrogenous waste product. Using an electronic tide gauge and data logger, we were able to demonstrate that toadfish in the intertidal mudflats at Panacea, Florida were air-exposed approximately 9.3% of the over 2000 h monitored, and that these periods could last as long as 9 h. Toadfish were collected from this site during several seasons in 1993–1994, and were subjected to experiments which measured their nitrogenous waste production (as ammonia and urea). Samples were also analyzed for several biochemical proxies of urea excretion (including liver ornithine-urea cycle (O-UC) enzyme activities, and activities of nitrogen feeder enzymes, including the key enzyme glutamine synthetase, GSase), as well as plasma cortisol, urea and other variables. Although freshly collected toadfish from Panacea exhibited relatively high activities of the O-UC enzymes, they showed rather low activities of GSase, indicating a low potential for immediate urea production. Furthermore, urea excretion rates were extremely low during the first 24–36 h in captivity, even when toadfish were subjected to an experimental emersion period of 8 h intended to simulate air-exposure in nature. When compared to responses and values of subtidal toadfish from Biscayne Bay, Florida published in a prior report, and from a single sample of subtidal toadfish at Panacea, it is apparent that urea synthesis and excretion in the gulf toadfish are likely not a direct consequence of air-exposure during the tidal cycle. Our data are discussed in the context of an ammonia-exposure hypothesis explaining urea synthesis and excretion in this species.

Keywords: *Opsanus beta*; gulf toadfish; intertidal; ureogenesis; urea; ammonia

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INTRODUCTION

The gulf toadfish (*Opsanus beta*) is unusual amongst teleost (bony) fishes in that under a variety of circumstances its predominant nitrogen waste product is not ammonia, but urea (for review, see Walsh, 1997). For example, in the laboratory, exposure to air, or elevated water ammonia concentrations, as well as even simple confinement or crowding, will induce *O. beta* to make and excrete predominantly urea (Walsh *et al.*, 1990; 1994). Furthermore, from measurements of a suite of biochemical proxies for urea excretion (e.g., elevated glutamine synthetase (GSase) activity, plasma cortisol levels, etc.) in a *subtidal* population of *O. beta* in Biscayne Bay, Florida, we concluded that substantial urea excretion was probably occurring in nature at this site (Hopkins *et al.*, 1997). Although *O. beta* is primarily distributed in the subtidal zone over most of the species range, there are several anecdotal reports that the species can be found exposed at low tides (e.g., Rudloe, 1977; Robins and Ray, 1986). If the species is truly intertidal over parts of its range, we reasoned that it might excrete urea naturally under these circumstances, given the species' ability to excrete urea following air-exposure in the laboratory. Indeed, it might be possible that toadfish from intertidal populations are adapted to be even more ureogenic/ureotelic than their subtidal counterparts.

In this study we focus on *O. beta* from the northern Panhandle, portion of Florida where they have been reported to occur both inter- and subtidally (Rudloe, 1977). Our first aim was to document how frequently gulf toadfish were exposed at low tide in this habitat. Once the extent of air-exposure was documented, our next aim became to determine if naturally air-exposed gulf toadfish are ureotelic, and if they have the biochemical characteristics of ureogenesis seen in fish from our subtidal study population (Biscayne Bay, Florida). Lastly, we also wished to determine if both intertidal and subtidal gulf toadfish of the Florida Panhandle could be made to become ureogenic/ureotelic by laboratory treatments shown to induce ureotely in subtidal gulf toadfish, namely air-exposure and confinement.

Our main conclusions are that gulf toadfish from the Florida Panhandle do experience substantial periods of air-exposure during low tide, but that the capability of freshly collected fish from these habitats to produce urea is substantially lower than subtidal fish from Biscayne Bay. However, both subtidal and intertidal fish from the Florida Panhandle population exhibit the ability to switch to ureotely in the laboratory setting. Our results are discussed in the context of hypotheses for why this species makes urea.

MATERIALS AND METHODS

Fish Capture and Sample Collection and Storage

A total of 61 gulf toadfish were collected during low tides in October and December 1993, and February, May and July 1994, from Dickerson Bay tidal flats, near Panacea, northern Florida (under and around the "Living Dock" described by Rudloe, 1977). Toadfish were captured by either collecting them directly from exposed sediments, or by turning over rocks, oyster clumps, and other debris on the mudflat and sweeping the area underneath with a dipnet or by hand. In December 1993, high winds pushed water over the mudflats of Panacea making toadfish difficult to find and capture, so we collected 5 fish from mudflats in the lee of Turkey Point off the nearby Florida State University Marine Laboratory (approximately 12 km away). For our trawling study we collected 6 fish in July, 1994 from Apalachee Bay (immediately southwest and outside of Dickerson Bay) using a 3 m wide otter trawl towed on the bottom for 10 min in a depth of 3 m. Fish captured on tidal flats or in the trawl were immediately placed into buckets of aerated seawater and plasma samples were taken within 10 min of capture or being brought aboard ship. A 10 min period post-capture has been shown to have no effect on the variables we measured compared to values immediately post-capture or laboratory controls (Hopkins *et al.*, 1997).

Immediately prior to blood sampling, fish were placed into a 9 l bucket containing a $2\,g\,l^{-1}$ solution of MS-222 (tricaine methanesulphonate) in aerated seawater which rendered them unconscious within 30 s. A 0.2 ml blood sample was then taken by caudal puncture using a syringe containing 10 µl of $1000\,i.u.\,ml^{-1}$ sodium heparin in 0.9% NaCl solution. Blood samples were immediately centrifuged for 2 min using a hand-powered centrifuge. Plasma was aspirated and both carcasses and plasma were frozen. Samples and carcasses were stored in the laboratory at −80°C (plasma samples) or −20°C (carcasses) until analyzed (typically within < 1 month) (again, conditions which had previously been found to be suitable, Hopkins *et al.*, 1997). Additionally, at selected times of the year (October 1993, February and May 1994) toadfish livers were frozen at −80°C for later assay of a suite of enzymes of the Ornithine-Urea Cycle (O-UC).

Tide and Physical Measurements

An Omega model LV-80 liquid level switch connected to a HoboVolt logger was mounted on a pier piling of the "Living Dock" of Gulf Specimen Co. in

Dickerson Bay (Rudloe, 1977). The logger recorded whether the level switch was open (high tide: surrounding mudflats under water) or closed (low tide: surrounding mudflats exposed) every 36 min. The level switch triggered (opened or closed) at approximately 0.4 m above mean lowest low water (MLLW), a height which indicated when nearly all of the intertidal flats in the bay were aerially exposed. Water levels were monitored from 25 February to 24 May 1994. Temperature, salinity and dissolved oxygen were measured during fish collections using standard methodology, including a refractometer for salinity and a YSI Model 51B Oxygen Meter for oxygen.

Nitrogen Excretion Measurements

Toadfish (different from those used for plasma and tissue samples) less than 1 h post-capture were placed in covered plastic beakers (beaker size: 0.5–4 l) with aerators made of PE-50 cannulae tubing and various volumes (0.1–1.8 l) of seawater collected from their capture location, immediately after capture. Volumes varied as fish size ranged from 15 to 227 g body weight (9–21 cm standard length) and we attempted to keep the ratio of fish weight: water volume reasonably consistent. Water temperature during experiments was held within four degrees of the temperature at which fish were collected (see Table I for collection temperatures). Water samples were taken at various intervals using a small plastic syringe and frozen at −20°C for later analysis. Nearly complete water changes were accomplished with a minimum of disturbance to the fish using larger (e.g., 50 ml) syringes every 24 h. Some toadfish were given an 8 h emersion treatment to simulate natural air-exposure (see Results below). These fish were held in similar plastic containers, but with only about 10 ml of seawater to prevent desiccation and collect nitrogenous waste which had been excreted. Water samples for analyses were taken from this small volume of water which was removed at the end of 8 h, then a larger volume of seawater was added to the fish to re-submerge it, and water sampling and periodic changeouts continued as above, i.e., a water sample was taken at the beginning and end of each flux period, and the difference in final minus initial concentrations taken, such that the excretion rates shown in Figs. 3–5 are the average rates for each indicated time period.

Laboratory Analyses

Plasma cortisol concentrations were measured using a commercially available radioimmunoassay kit (ICN Immuno Corp., Costa Mesa, CA) as previously

TABLE I Abiotic and biotic variables measured in the Florida Panhandle and in gulf toadfish, *O. beta*, in various seasons of 1993–94 (Values are means ± SE (*N*))

	Temp (°C)	*Salinity* (ppt)	*Diss. oxygen* (mg/l)	*Std. length* (cm)	*Total length* (cm)	*Body mass* (g)	*GSase* (units/g)	*Cortisol* (ng/ml)	*Urea* (m mol/l)	*Ca* (ng/ml)	*HSI*	*GSI*
Month year exposed												
Oct '93	22.2 (8)	22.5 (8)	5.2 (8)	10.4 ± 0.9 (8)	12.3 ± 1.0 (8)	36.0 ± 14.0 (8)	1.62 ± 0.75 (8)	0.55 ± 0.28 (7)	4.0 ± 0.59 (7)			
Dec '93	12.1 ± 0.43 (9)	17.3 ± 5.8 (9)	5.8 ± 2.9 (4)	19.0 ± 0.9 (9)	21.8 ± 1.0 (9)	179.8 ± 27.5 (9)	1.00 ± 0.33 (9)	0.29 ± 0.10 (9)	3.03 ± 0.19 (9)	12.2 (2)		
Feb '94	17.2 (6)	24.0 (6)	9.2 (6)	10.3 ± 4.2 (6)	12.1 ± 5.0 (6)	44.1 ± 18.0 (6)	1.15 ± 0.47 (6)	0.27 ± 0.16 (3)	2.83 ± 1.63 (3)	0.2 (1)	1.43 ± 0.28 (6)	0.68 ± 0.28 (6)
May '94	26.1 (7)	24.0 (7)	6.4 (7)	10.7 ± 1.8 (7)	12.6 ± 2.1 (7)	28.8 ± 10.9 (7)	1.87 ± 0.71 (7)	0.30 ± 0.06 (5)	3.71 ± 1.20 (5)	12.2 ± 5.5 (5)	0.82 ± 0.31 (7)	0.51 ± 0.19 (7)
July '94	28 (11)	28 (11)		11.0 ± 2.7 (11)	12.9 ± 3.1 (11)	31.9 ± 9.6 (11)	0.89 ± 0.27 (11)	0.09 ± 0.03 (8)	2.91 ± 1.03 (8)	9.0 ± 0.52 (4)	1.21 ± 0.36 (11)	0.09 ± 0.03 (11)
Subtidal												
July '94	28 (6)	28 (6)		11.6 ± 4.7 (6)	13.6 ± 1.3 (6)	33.8 ± 8.2 (6)	3.91 ± 1.38 (6)	0.22 ± 0.09 (6)	2.39 ± 0.84 (6)	8.6 (2)	0.9 ± 0.25 (6)	0.10 ± 0.04 (6)

applied to toadfish (Hopkins *et al.*, 1995; 1997); the protein content of standards was adjusted to reflect values found in toadfish plasma, which increased the assay sensitivity at low cortisol concentrations. Plasma calcium concentration was assayed using the arsenazo colorimetric method (Sigma Kit # 588). Plasma calcium concentrations were used as an indicator of plasma vitellogenin (a phospholipoprotein with high bound calcium content) and have been shown to correlate with ovarian but not testicular maturation (Ng and Idler, 1983; Mommsen and Walsh, 1988). Water samples were analyzed for ammonia using the method of Ivancic and Deggobis (1984), and urea was assayed in plasma and water samples using the method of Price and Harrison (1987). Both the ammonia and urea assays have detection limits of 1–2 $\mu mol\,l^{-1}$, as previously applied to toadfish (Walsh *et al.*, 1994). Fish carcasses were partially thawed at room temperature (only enough to permit dissection) and the standard length (SL), total length (TL), and body mass were determined. The sex of each fish was determined and the presence or absence of food in the intestine was noted. The liver was removed, weighed, and the hepatosomatic index (HSI) was calculated as: [(liver mass/total body mass)*100]. A sample of liver tissue was taken for determination of hepatic GSase activity. Liver tissue was sonicated at 4°C in 4 volumes of homogenization buffer (20 $mmol\,l^{-1}$ K_2HPO_4, 10 $mmol\,l^{-1}$ HEPES, 0.5 $mmol\,l^{-1}$ EDTA, 1 $mmol\,l^{-1}$ dithiothreitol, 50% glycerol, adjusted to pH 7.5 at 24°C), centrifuged for 1 min at 13,000 *g* and the supernatant assayed at 24°C as previously detailed by Mommsen and Walsh (1989); GSase assay contained 60 $mmol\,l^{-1}$ glutamine, 15 $mmol\,l^{-1}$ hydroxylamine, 20 $mmol\,l^{-1}$ KH_2AsO_4, 0.4 $mmol\,l^{-1}$ ADP, 3 $mmol\,l^{-1}$ $MnCl_2$, 50 $mmol\,l^{-1}$ HEPES, pH 6.7. Freezing was found to have no effect on any of the above parameters (T. Hopkins, pers. obs.).

A subset of toadfish livers which had been stored at −80°C were assayed for the following enzyme activities according to previously described methods as applied in Hopkins *et al.* (1997) (buffer is NaHEPES, pH 7.5 unless indicated and all concentrations in $mmol\,l^{-1}$): AlaAT = alanine amino transferase, NADH 0.12, alanine 200, pyridoxyl 5′ phosphate 0.025, 2-oxoglutarate 10.5, 12 units/ml LDH; AspAT = aspartate amino transferase, NADH 0.12, aspartate 40, pyridoxyl 5′ phosphate 0.025, 2-oxoglutarate 7, 8 units/ml MDH; GDH = glutamate dehyrodrogenase, NADH 0.12, ammonium acetate 250, EDTA 0.1, ADP 1, 2-oxoglutarate 14; CPSase III = carbamoyl phosphate synthetase, $MgCl_2$ 25, ATP 20, $NaHCO_3$ 5, N-acetyl glutamate 5, DTT 2, ornithine 5, glutamine 20, Phopho(enol)pyruvate 25, UTP 1.7, 6 units/ml PK, 1 unit/ml OTC, pH 8.0; OTC = ornithine transcarbamoylase, ornithine 10, carbamoyl phosphate 10, pH 8.5; AS/AL = a combined assay

for arginino succinate synthetase and lyase, aspartate 1.4, $MgCl_2$ 25, ATP 14, EDTA 1, DTT 2; and ARG = arginase, arginine 250, $MnCl_2$ 1, pH 8.0. Livers were sampled from three different times of the year to attempt to ascertain any seasonal patterns.

General relations between the biotic variables (GSase, plasma urea, plasma cortisol, HSI, female plasma calcium) and abiotic variables (temperature, salinity, dissolved oxygen) were assessed from Pearson correlation coefficients and the strongest relationships were plotted. To reduce problems of non-normality and heteroscedasticity, the variables GSase, plasma urea, and plasma cortisol, were log transformed [ln (variable + 1)] prior to analyses. Linear regressions were used to determine the importance of sex and size for all biotic variables. The effect of sampling time on the activities of the suite of O-UC enzymes was tested with an ANOVA.

RESULTS

Correlation analysis yielded no significant relationships between biotic variables (GSase, plasma urea, plasma cortisol, HSI, female plasma calcium) and the abiotic variables (temperature, salinity, dissolved oxygen) for fish collected from the mudflats (Table I). We found no significant linear relationships between the dependent variables (plasma urea, plasma cortisol, GSase, HSI, female plasma calcium) and size. Sex was not significantly related to any of the biotic variables. These findings are identical to those of Hopkins *et al.* (1997) in Biscayne Bay. Unlike the results of Hopkins *et al.* (1997), we detected no season-specific trends in this population/study site.

Toadfish inhabiting the intertidal areas of Dickerson Bay were found to be exposed 9.3% of the time we monitored from 25 February to 24 May 1994 (Fig. 1). During this period intertidal toadfish were exposed to several 9 h long exposures (Figs. 1 and 2). Nitrogen excretion in fish taken directly from the mudflats showed a high level of ammonia excretion, with a small component of urea excretion, for the first 24 h (Fig. 3). This period was followed by a large increase in urea excretion beginning around 36 h ($p < 0.05$), after which the fish appeared to oscillate between being predominantly ammoniotelic or ureotelic (Fig. 3). Several individuals (but not all) were found in the post-mortem dissections to have significant amounts of food in their stomachs and guts at the end of the excretion measurements.

During an 8 h emersion period, fluxes of nitrogen were greatly reduced (Fig. 4), consistent with the fact that the gills are the site of ammonia and urea excretion in toadfish (Gilmour *et al.*, 1998) and these could not be

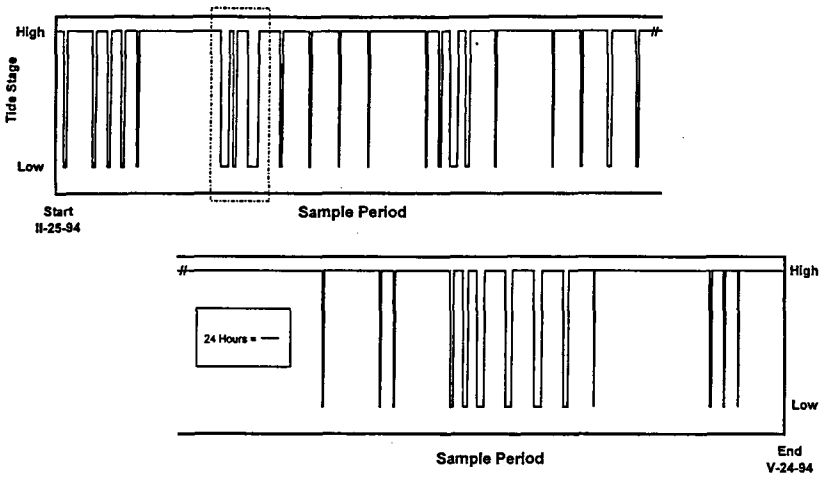

FIGURE 1 Water level data from the "Living Dock" (Rudloe, 1977) in Panacea, Florida from 1055 on 25 February to 2020 on 24 May, 1994 showing frequency of air-exposure for toadfish inhabiting the surrounding intertidal mudflats. The logger recorded whether a float switch was open (high tide: surrounding mudflats under water) or closed (low tide: surrounding mudflats exposed). The float level switch was mounted at approximately 0.4 m above mean lowest low water. Dashed box indicates data shown in Fig. 2.

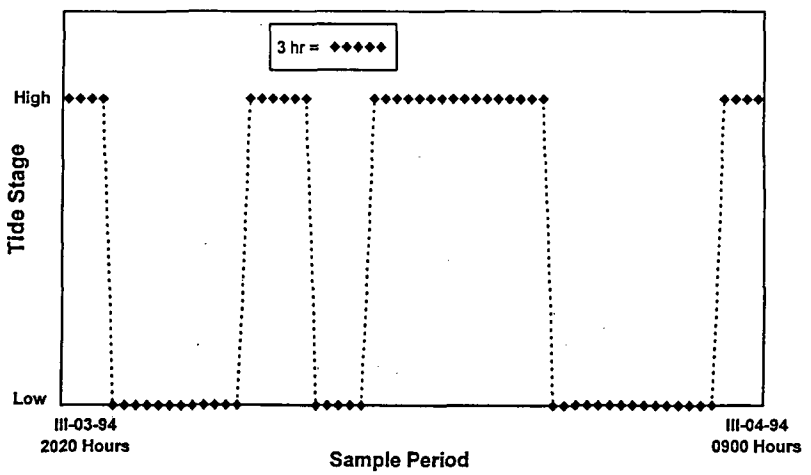

FIGURE 2 A 12.6 h selection of data from the dashed box in Fig. 1, showing water level data from a datalogger on the "Living Dock" (Rudloe, 1977) in Panacea, Florida from 2020 on 3 March to 0900 on 4 March, 1994. This plot shows three successive air-exposure events for toadfish inhabiting the local mudflats of 7.2, 2.4 and 8.4 h, respectively.

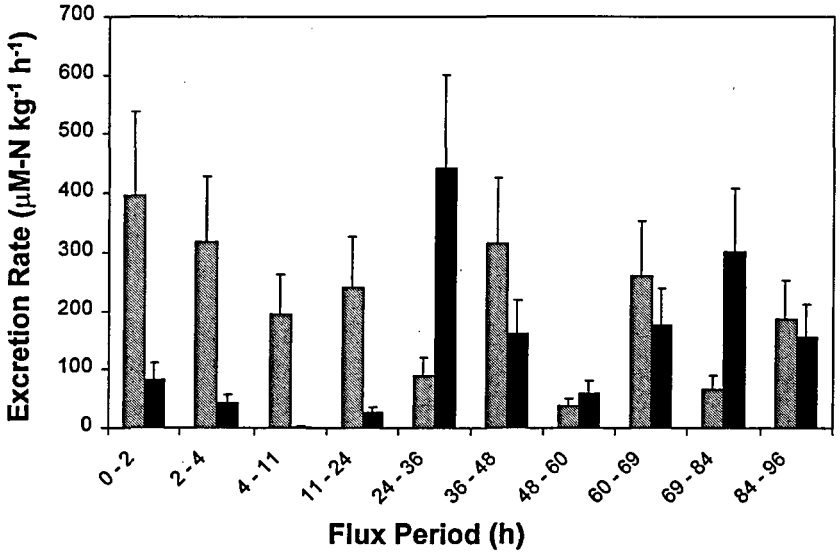

FIGURE 3 A plot of nitrogen excretion rate (μmol-N kg^{-1} h^{-1}; ammonia-N grey bars; urea-N solid bars) versus time in toadfish collected from mudflats during low tide events in Panacea, Florida. Values are means + 1 SE ($N = 8$).

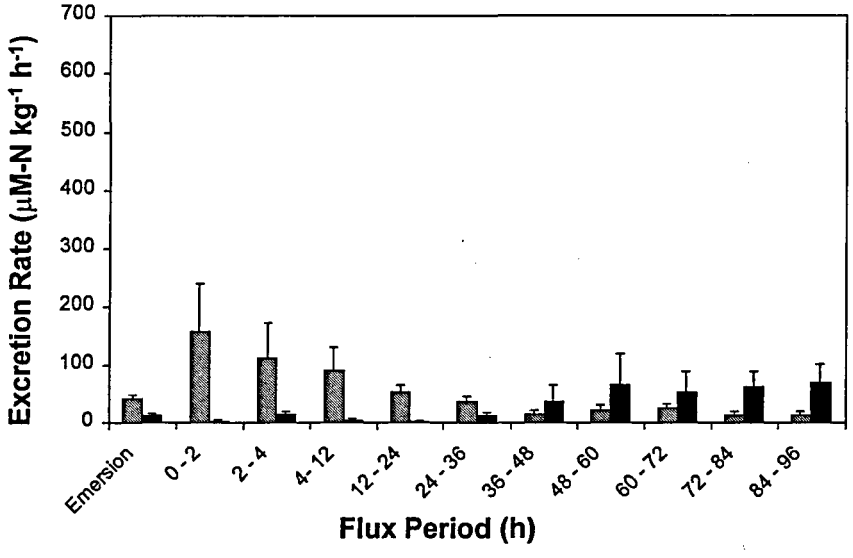

FIGURE 4 A plot of nitrogen excretion rate (μmol-N kg^{-1} h^{-1}; ammonia-N grey bars; urea-N solid bars) versus time in toadfish collected from mudflats in Panacea, Florida, and given an 8 h emersion treatment to simulate air-exposure. Values are means + 1 SE ($N = 7$).

irrigated with water under these exposure conditions. However, even following this emersion period, toadfish again took an additional 36 h to make the transition to being predominantly ureotelic (Fig. 4). The transition to ureotely was more complete and consistent in this group of fish. Toadfish captured from a subtidal population at Panacea showed a similar pattern of nitrogen excretion, namely, primarily ammoniotelic for the first 24 h, followed by a switch to ureotely (Fig. 5).

GSase activities, an important indicator of degree of ureotely in laboratory toadfish (Hopkins *et al.*, 1995) were rather low in livers of toadfish collected from the mudflats at Panacea (Table I), with a grand mean for the population of 1.60 units g^{-1} ($N=41$) and a range of 0.10–4.15 units g^{-1}. Toadfish captured by trawling at Panacea had significantly higher GSase activity 3.91 units g^{-1} (Table I) than mudflat toadfish ($p<0.05$, t-test, df = 9). There were no significant differences (t-test) between the mean values of plasma cortisol or plasma urea from trawl-caught fish and the fish taken off the mudflats (see Table I).

Toadfish collected from the mudflats at Panacea showed substantial levels of activity for all of the enzymes of the O-UC (and nitrogen feeder enzymes

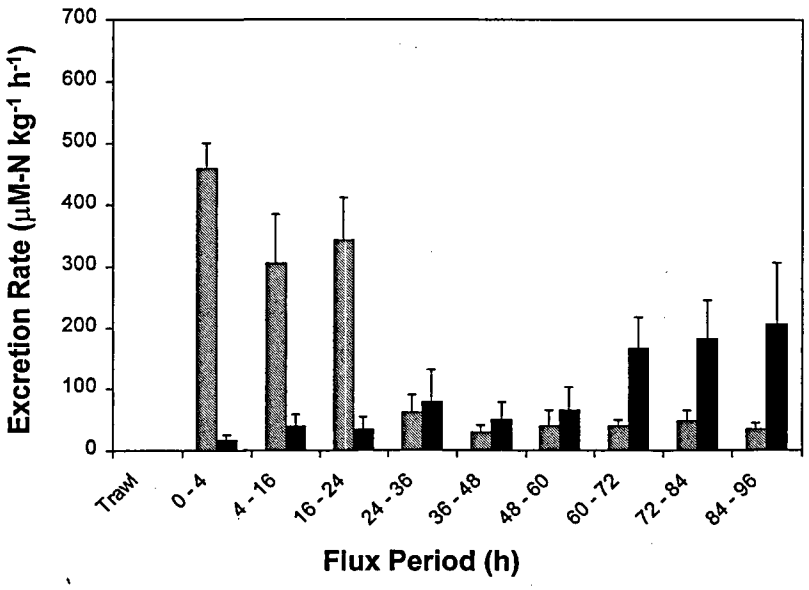

FIGURE 5 A plot of nitrogen excretion rate (μmol-N kg^{-1} h^{-1}; ammonia-N grey bars; urea-N solid bars) versus time in toadfish collected by trawling in Apalachee Bay (near Panacea), Florida. Values are means + 1 SE ($N=6$).

TABLE II Activities of O-UC and related enzymes in liver of gulf toadfish, *O. beta*, collected from the intertidal zone at Panacea, Florida in various seasons of 1993–94 (Values are means ± SE (*N*))

Enzyme	*Month* (*Year*)		
	October 1993	*February* 1994	*May* 1994
AlaAT	31.91 ± 2.36	27.01 ± 3.53	23.59 ± 2.02
AspAT	42.42 ± 1.83	49.50 ± 5.08	42.33 ± 2.47
GDH	32.11 ± 2.12	27.47 ± 1.87	23.27 ± 2.08
GSase	1.62 ± 0.28	0.98 ± 0.25	1.24 ± 0.32
CPSase	0.08 ± 0.01	0.13 ± 0.06	0.09 ± 0.01
OTC	37.08 ± 2.02	54.36 ± 5.54	30.51 ± 1.96
AS/AL	0.51 ± 0.01	0.44 ± 0.02	0.90 ± 0.01
ARG	18.95 ± 2.07	30.27 ± 7.09	65.27 ± 2.66
N	8	3	5

GDH, AlaAT, AspAT) when collected at three periods of the year (Table II). GSase activities in these fish were low compared to other studies (e.g., Hopkins *et al.*, 1997) of toadfish and not substantially different from the other subsamples of toadfish taken at this site (Table II).

DISCUSSION

The overall rationale for performing this study was to examine an air-exposed population of gulf toadfish which might potentially be synthesizing and excreting urea at high rates in comparison to subtidal populations and laboratory-acclimated toadfish studied previously. Indeed, air-exposure, and the accompanying difficulty in excreting nitrogenous waste as ammonia, is one important rationale applied to explain the transition to ureotely and uricotely in terrestrial animals (Walsh and Wright, 1995).

This study is the first to document that significant air-exposure does indeed occur in the Panacea, FL population of gulf toadfish, with toadfish being exposed for 9.3% of the more than 2000 h monitored, and air-exposures lasting as long as 9 h (Figs. 1 and 2). However, the intertidal mudflat population of gulf toadfish at Panacea does not appear to be substantially ureotelic in nature. When nitrogen excretion is measured directly in fish collected from the mudflat (Fig. 3) or during and after a laboratory air-exposure period (Fig. 4), nitrogen is excreted primarily as ammonia for the first 24–36 h. However, in previous laboratory studies of the transition to ureotely in the gulf toadfish, it has been demonstrated that the toadfish appears to possess a remarkable ability to rapidly depress nitrogen excretion, and reorganize metabolism to become ureogenic during this period (reviewed by Walsh,

1997). Thus, even periods as short as the first 24 h of laboratory confinement can affect the nitrogen excretion patterns of the organism to the point where these patterns may be different from what is occuring in nature at the time of capture. This reorganization appears to first involve an activation of GSase activity by a transient increase in cortisol (Hopkins *et al.*, 1995) followed by an activation of a specific urea facilitated diffusion transporter at the gill which appears to correlate with a return of cortisol to minimal levels (Wood *et al.*, 1998).

For the above reasons, we decided to also measure several biochemical proxies for nitrogen metabolism and excretion in freshly collected intertidal toadfish in order to provide an independent assessment of the *in situ* nitrogen excretion patterns of the fish. These proxies also indicate an overall low probability of significant urea excretion taking place in the natural habitat. First, total activities of O-UC enzymes are reasonably high (Table II), a condition which appears to be a necessary, but permissive factor in allowing urea synthesis to take place in batrachoidid fishes (Walsh, 1997). However, GSase activities are uniformly low in intertidal Panacea fish (Table I), a condition which would preclude significant feeding of the substrate glutamine to CPSase III for urea production. Indeed these values are about 50% lower than GSase activities indicative of $< 30\%$ ureotely in laboratory-acclimated toadfish (Hopkins *et al.*, 1995), indicating an even lower percent ureotely in the Panacea population. Furthermore the GSase values for intertidal Panacea toadfish are 3.5-fold lower than the grand mean ($5.6\,\text{units}\,g^{-1}$) reported for the subtidal toadfish population at Biscayne Bay, FL and interpreted to indicate a mean of about 50% ureotely in this population (Hopkins *et al.*, 1997) based on the laboratory correlation between percent ureotely and GSase activity reported by Hopkins *et al.* (1995). Additionally, some subpopulations at Biscayne Bay (e.g., Matheson Hammock) and individuals showed even higher GSase activities, e.g., 14–40 units g^{-1}, respectively, indicative of full ureotely in nature. Although there were no differences in plasma urea concentration between the present study and prior studies, the plasma cortisol levels reported here (Table I) are substantially lower than in Biscayne Bay toadfish (Hopkins *et al.*, 1997) or laboratory-acclimated toadfish (Hopkins *et al.*, 1995) which is consistent with the interpretation of no stress-related GSase activation and urea synthesis.

The responses of subtidal (trawled) toadfish at Panacea appear to present an interesting situation. These fish had a higher level of GSase activity, about 2.5-fold higher than the $1.6\,\text{units}\,g^{-1}$ mean for all seasons, and about 4.3-fold higher than the direct seasonal comparison with their intertidal counterparts in July 1994 (Table I). Additionally, these fish, although ammoniotelic

during the first 24 h, showed a much more rapid and consistent transition to ureotely (Fig. 5) than either intertidal experimental group (Figs. 3 and 4). The nitrogen metabolism and excretion patterns of the subtidal Panacea toadfish more closely resemble the patterns of the subtidal Biscayne Bay toadfish (Hopkins *et al.*, 1997). Hopkins *et al.* (1997) recently advanced the hypothesis that an important factor in triggering toadfish to excrete urea in nature is the apparently high levels of ammonia encountered by them in the rhizome environment (Fourqurean *et al.*, 1992) of the seagrasses which they inhabit in the subtidal zone. We did not specifically measure seagrass coverage in the Panacea study area, but there was virtually no seagrass coverage in the intertidal mudflat where toadfish were collected (Rudloe, 1977). Thus, these observations are consistent with the hypothesis of Hopkins *et al.* (1997), that ammonia loading by the seagrass rhizome environment might be necessary to induce or prime ureotely in the toadfish. This hypothesis deserves further, more direct, testing in future studies.

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References

Fourqurean, J.W., Zieman, J.C. and Powell, G.V.N. (1992). Relationships between porewater nutrients and seagrasses in a subtropical carbonate environment. *Mar. Biol.*, **114**, 57–65.

Gilmour, K.M., Perry, S.F., Wood, C.M., Henry, R.P., Laurent, P., Pärt, P. and Walsh, P.J. (1998). Nitrogen excretion and the cardiorespiratory physiology of the gulf toadfish. *Opsanus beta. Physiol. Zool.*, **71**, 492–505.

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

Hopkins, T.E., Serafy, J.E. and Walsh, P.J. (1997). Field studies on the ureogenic gulf toadfish, in a subtropical bay. II. Nitrogen excretion physiology. *J. Fish Biol.*, **50**, 1271–1284.

Ivancic, I. and Deggobis, D. (1984). An optimal manual procedure for ammonia analysis in natural waters by the indophenol blue method. *Water Research*, **18**, 1143–1147.

Mommsen, T.P. and Walsh, P.J. (1988). Vitellogenesis and oocyte assembly. Chapter Five In *Fish Physiology, Vol. XI, The Physiology of Developing Fish.* W.S. Hoar and D.J. Randall (Eds.) San Diego: Academic Press, pp. 347–406.

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

Ng, T.B. and Idler, D.R. (1983). Yolk formation and differentiation in teleost fishes. In *Fish Physiology. Vol. IXA. Reproduction.* W.S. Hoar, D.J. Radall and E. M. Donaldson (Eds.). New York, NY: Academic Press, pp. 373–404.

Price, N.M. and Harrison, P.J. (1987). Comparison of methods for the analysis of urea in seawater. *Mar. Biol.*, **94**, 307–313.

Robins, C.R. and Ray, G.C. (1986). *A Field Guide to Atlantic Coast Fishes.* Houghton Mifflin Co., Boston.

Rudloe, J. (1977). *The Living Dock at Panacea.* New York, NY: Alfred A. Knopf.

Walsh, P.J., Danulat, E.M. and Mommsen, T.P. (1990). Variation in urea excretion in the gulf toadfish, *Opsanus beta. Mar. Biol.*, **106**, 323–328.

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

Walsh, P.J. and Wright, P.A. (Eds.) (1995). *Nitrogen Metabolism and Excretion.* CRC Press, Boca Raton, FL., 337 pp.

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

Wood, C.M., Gilmour, K.M., Perry, S.F., Pärt, P., Laurent, P. and 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**, 805–817.