The Journal of Experimental Biology 209, 4254-4261
Published by The Company of Biologists 2006
doi:10.1242/jeb.02533

Use of urea as a chemosensory cloaking molecule by a bony fish

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Accepted 7 September 2006

Summary

Because urea is bioenergetically expensive to synthesize, few aquatic teleostean (bony) fish make or excrete much urea beyond early development and excrete the majority of nitrogenous waste as the readily diffusible ammonia. The gulf toadfish is one of a few adult teleostean fish that excretes predominately urea. Most studies of chemosensing by fish predators have focused on amino acids as odorants, but we tested the chemo-attractiveness of both urea and ammonia. We report that characteristic 'prey-attack' behaviors by a key toadfish predator, gray snapper, were elicited by low ammonia concentrations (<100 nmol N l^{-1}) and similar urea concentrations blunted the ammonia-induced component of attacks. Thus, urea functions as a cloaking molecule, explaining why toadfish co-excrete urea with ammonia. Furthermore, ammonia waste is an important chemical attractant for piscine predators.

Supplementary material available online at
http://jeb.biologists.org/cgi/content/full/209/21/4254/DC1

Key words: urea, ammonia, chemosensory, predator-prey interaction, toadfish, snapper.

Introduction

Waste urea synthesis *via* the ornthine–urea cycle is bioenergetically expensive, requiring four to five moles of ATP per mole of urea in vertebrates (Walsh and Mommsen, 2001). Therefore, ureogenesis is used sparingly as a solution to selected evolutionary pressures (Withers, 1998) such as in water conservation during terrestrial adaptation where non-toxic urea can be accumulated to higher concentrations than the highly toxic ammonia (Walsh and Mommsen, 2001). Because aquatic gill breathers can directly excrete ammonia, there has been considerable selective pressure to ontogenetically silence their genes for urea production and excretion (Mommsen and Walsh, 1989). Indeed, few teleostean (bony) fish synthesize much urea beyond a brief window in early embryonic development when low permeability membranes limit ammonia excretion at physiological pH (Wright and Fyhn, 2001). One of several important exceptions to this rule, the gulf toadfish *Opsanus beta* which continues to facultatively excrete urea as adults, led us to consider the chemo-attractiveness of both urea and ammonia (Walsh, 1997).

Surprisingly, few aquatic chemoreception studies have focused on either ammonia or urea as odorants. Studies of prey detection by chemoreception in teleosts have generally focused on amino acids as odorants (Hara, 1992). Although threshold sensitivities for amino acids are often in the nano-molar range, gill and renal membranes are thought to be 'effectively impermeable' to amino acids (Heinz, 1972) especially compared with lower molecular mass compounds such as ammonia or urea. Indeed, it was recently demonstrated in unfed and relatively unstressed rainbow trout that amino acid nitrogen (amino acid-N) accounted for ~4% of excreted N while ammonia-N and urea-N accounted for ~66% (Kajimura et al., 2004); the proportion of amino acid-N increased to ~10% in fed individuals experiencing surgical stress (Kajimura et al., 2004). Additionally, protein-N, creatine-N and creatinine-N, and unknown N sources constituted 3–11%, ~1.4% and 12–20% of total waste-N, respectively (Kajimura et al., 2004). Considering the tendency for organisms to conserve amino acids and their need to excrete nitrogenous waste, we hypothesized that ammonia and/or urea are detectable as odorants by aquatic predators.

Crypsis in fish by chemical masking agents was previously hypothesized to camouflage metabolic waste (Atema, 1995) whereas anthropogenic contaminants such as metals mask biologically important chemical signals (Hara, 1992; Sutterlin and Gray, 1973). Furthermore, teleostean predators exhibit behavioral adaptations to achieve chemical crypsis. For instance, the northern pike (*Esox lucius*) defecate away from foraging areas since prey can detect conspecific alarm pheromones in pike feces (Brown et al., 1996).

We hypothesized that ammonia as an odorant is detectable by teleostean predators and that urea excretion (ureotely) will mask other chemosensory stimuli detectable by predators. We tested this hypothesis with behavioral assays using the responsiveness and attraction of a key toadfish predator, the gray snapper to ecologically relevant concentrations of: (1) waste-N in the form of urea and/or ammonia and (2) an amino acid mixture with and without waste-N (ammonia and/or urea).

Materials and methods

Experimental animals

Adult gray snapper *Lutjanus griseus* L. (230–281 mm total length) were collected by light tackle. We selected only individuals hooked in the lower jaw to guard against traumatization of the nares or olfactory epithelium. Snappers were directly transferred to outdoor 2×2 m mesocosm tanks (~8000 l) with flow through seawater (50 l min^{-1}) and aeration (Fig. 1). These tanks were designed after the mesocosm concept (Odum, 1984) to simulate the natural South Florida seagrass ecosystems. The substrate of each mesocosm consisted of local carbonate sediment planted with the seagrass *Thalassia testudinum* to simulate natural habitat. Snappers were fed live shrimp (*Penaeus duorarum*) *ad libitum* and minimally allowed 10 days to recover from capture stress before utilization in odorant trials. Naïve snappers were transferred to an experimental mesocosm 48 h prior to odorant trials to enforce a uniform fasting period.

Experimental setup

Experimental shelters for odorant delivery were fabricated from 20 cm lengths of 10 cm diameter polyvinyl chloride (PVC) pipe with one opening sealed by a PVC end-cap. Each PVC end-cap was fitted with a model MVC2000 Micro Video Products submersible infrared video camera cabled to a remote Panasonic model RT650 video recorder. Cameras were positioned to view the shelter's inner chamber as well as an arena extending outward 50 cm from the shelter's open end (Fig. 1). A clay toadfish model (7.5 cm total length), with correct form and coloration pattern, was positioned at the shelter entrance (Fig. S1 and Fig. S2 in supplementary material).

A dilution experiment was conducted without animals present and mesocosm flow patterns were mapped during constant pumping of rhodamine dye (Kingscote Chemicals, Miamisburg, OH, USA) for 30 min into each shelter *via* odorant delivery ports (Fig. S3 in supplementary material) at a concentration of 7.2 mg l^{-1} to visualize propagation of cohesive dye plumes. The plume structure was also evaluated with four water samplers positioned either directly inside the shelter, at 50 cm from the shelter opening along the shelter centerline (0°) and at ± 45° from the 0° centerline. Samplers were 2.5 mm polyethylene (PE) tubing positioned either 10 cm above sediments (at 50 cm) or connected to the shelter sampling port (Fig. S1 in supplementary material). Samplers continually siphoned water (~5 ml min^{-1}), which was collected at 10 min intervals for 90 min. The rhodamine dye, visibly bright red, has excitation and fluorescence wavelengths of 550 and 588 nm, respectively. Samples were processed using a Perkin-Elmer model LS-3B fluorescence spectrometer. We calculated time delay in plume propagation, turbulent mixing, and diffusive losses from plume spreading, by comparing values of 0 and 50 cm samples. Thus, we could predict odorant concentration at 50 cm relative to the concentration inside the shelter.

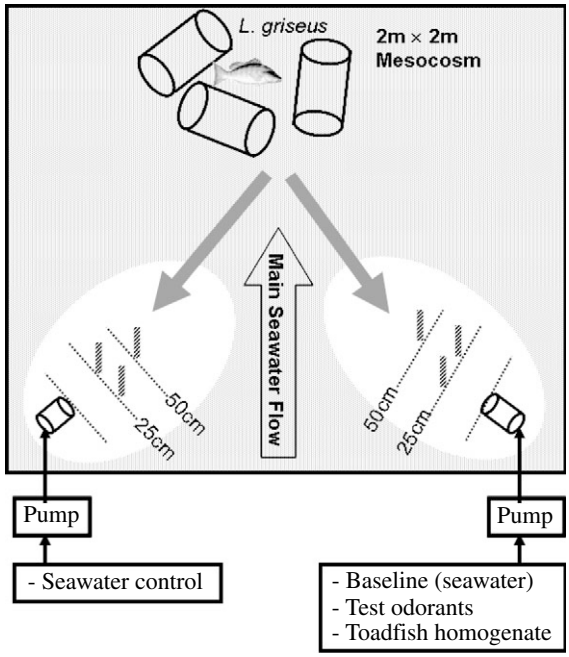

Fig. 1. Top view of experimental setup. Two behavioral arenas were cleared in the seagrass canopy where PVC stakes marked set distances from experimental shelter openings (25 and 50 cm). Two experimental shelters provided simultaneous delivery points for either an odorant or a negative (seawater) control in a modified Y-choice configuration with corresponding shelter assignments randomized. A submersible infrared camera was mounted on each shelter's rear end-cap and directed outward. Polyethylene tubing was connected to the odorant injection and water-sampling ports, with odorants and controls injected *via* peristaltic pumps to respective shelters. Baseline snapper activity was established prior to any odorant delivery. The order of odorants was randomized for each individual gray snapper to prevent treatment order effects, and the positive control was always delivered last. Overall tank flow was directed away from shelters with inflow pipes facing a reef-like structure, behind which was the tank drain pipe. Gray snappers generally hovered within the reef structure.

Similar experimental shelters were previously deployed in field studies, and the mean waste-N concentration (i.e. ammonia-N+urea-N) inside toadfish-inhabited shelters was 23.0±2.1 μmol N l^{-1} (Barimo et al., 2004). Using the rhodamine shelter dilution factors and manual calibration, we determined that 50 ml of 33 mmol N l^{-1} concentrated ammonia-N and/or urea-N delivered at a constant rate by a

VWR model 54856-070 peristaltic pump over 30 min would result in an internal shelter concentration of 23.8± 6.4 μmol N l^{-1} after 30 min. The pump reservoir was then switched from odorant to seawater and the internal shelter ammonia-N and urea-N concentrations fell below our assay detection threshold (<2 μmol N l^{-1}) after an additional 30 min. We delivered odorants as detailed above and documented snapper behaviors for 60 min intervals with waste-N concentrations gradually ramped-up to match field values and then returned to background levels.

Odorant challenges

Experimental mesocosms were equipped with two shelters positioned in adjacent corners each of which served as either the odorant point source or the seawater control (Fig. 1), and alternated with each trial to control for position preference. The seawater control guarded against the potential attraction of snappers to pump sounds, low-frequency vibrations, or water flow. The sequence of odorant delivery in each trial was randomized to avoid treatment order effects, and positive controls of clarified toadfish homogenate were conducted last. The positive control was prepared by homogenizing whole toadfish carcasses in seawater (1:10 ratio) in a Waring laboratory blender (model 38BL54) for 2 min at maximum speed. (No discernable heating of homogenate was observed.) The homogenate was then centrifuged (Jouan model CR412) at 4000 ***g*** at 2°C for 10 min, after which the supernatant was decanted into 50 ml samples and frozen at –20°C. The positive control was presented to gray snapper during pilot trials to observe behavioral patterns associated with tracking an odorant plume. The internal shelter water was sampled after 30 min to assure that the target concentration of 24 μmol N l^{-1} ammonia-N and/or urea-N was achieved. Ammonia and urea concentrations were determined by standard chemical techniques (Ivancic and Deggobis, 1984; Price and Harrison, 1987). Baseline activity of each gray snapper was monitored for 60 min before the delivery of each odorants sequence and to assure snapper fidelity to the reef structure. There was a 60 min interval between each odorant delivery/observation period to allow odorant plumes to disperse. One trial was conducted per day which commenced between 07:00 h and 08:00 h and all trials were conducted with one näive gray snapper per each trial replicate.

The first odorant trial was designed to determine if individual untethered gray snapper responded with a preference toward ammonia, urea, or a 1:1 mix of ammonia and urea with a 30 min target concentration of ~24 μmol N l^{-1}. In the ammonia/urea mix the concentration for each constituent was 12 μmol N l^{-1}. The second odorant trial was a comparison of 12 and 24 μmol N l^{-1} ammonia to determine if snapper were more responsive to the higher concentration.

The third trial examined snapper responsiveness to the amino acids including L-proline (P), L-alanine (A), and L-glycine (G) which were administered individually or in mixes of A and G; or P, A and G. The 2.5 μmol N l^{-1} value for total amino acid-N was consistent with a previous study of rainbow trout (*Oncorhynchus mykiss*) in which the highest estimate of total amino acid-N was 10% of the total waste-N (Kajimura et al., 2004). We chose amino acids with the lowest known olfactory thresholds consistently reported in teleost predators, namely L-alanine and L-glycine, and L-proline for gustation (Sorensen, 1992; Brand and Bruch, 1992). We believe that this selection process was conservative since individual amino acids would be excreted at such low concentrations (Kajimura et al., 2004) and amino acids with higher threshold sensitivities might go undetected by snappers in our experimental setup. The protocol for this and subsequent odorant trials followed those detailed in the first trial. Odorant trials 4 and 5 examined snapper responsiveness to amino acids using a 2.5 μmol N l^{-1} mixture of L-proline, L-alanine, and L-glycine in a 1:1:1 ratio (i.e. 0.83 μmol N l^{-1} of each amino acid) with or without 25 μmol N l^{-1} waste-N (i.e. ammonia-N and/or urea-N).

We employed behavioral assays based on stereotypical attack responses (see Movie 1, in supplementary material), thereby considering both the arousal and search phases of predation. Experimental setup and odorant challenges followed previously published protocols and recommendations (Atema et al., 1980; Hay et al., 1998). Total ammonia was not detected in the inflowing seawater or ambient tank water, nor was it detectable in the water column at toadfish habitat in Florida Bay (Barimo et al., 2004).

Data analysis

Time stamped video tapes of each odorant trial were labeled with a serial number and subsequently reviewed without knowledge of odorant delivery sequences or times. Snapper were previously noted to make several close passes after which they may choose to strike targets (Starck and Schroeder, 1971), and methodologies for scoring snapper behavior reflect previous field observations. Hence, we created a behavioral index which gave higher weighting to snappers entering shelters and striking the clay toadfish model which required snappers to alter their swimming trajectory and velocity to enter a confined space. Snappers entering shelters and attacking clay toadfish models were awarded 5 index points; snapper approaches 0–25 cm from the shelter opening 2 points; and snapper approaches 25–50 cm from the shelter opening 1 point. Additionally, if snappers hovered in front of the shelter opening from more than 1 alternation of pectoral fin sweeps and their eyes were noted to visually scan the shelter's interior (slow motion review of tape), they were awarded 1 additional point. Scoring index was summed over the 60 min observation period.

Sigma Stat software version 3.0 was used for statistical analyses. The paired *t*-test was used to examine each treatment and its alternate seawater control. Differences between treatment groups for each odorant trial were examined with one way repeated measures ANOVA with a Holm–Sidak pair-wise comparison test (Zar, 1996). Data were $\log(x+1)$ transformed since variance increased with increased mean values. Values are presented as means ± 1 s.e.m., P=0.05.

Results

Mesocosm dilution experiment

The rhodamine dye clearly propagated from each experimental shelter (*N*=10) as a cohesive bright red plume with a well-defined frontal boundary (Fig. S3 in supplementary material). Plumes tended to drift from the shelter's 0° centerline and toward the tank's overriding flow field (Fig. 1) where they were steered toward the artificial reef and tank drain. After 40 min the steep red color gradient dissipated and by 60 min, tank water was a uniform but faint pink color, which faded with fresh seawater inflow.

Water samples were collected from four rhodamine trials and their corresponding fluorescence values for samplers within shelters (0 cm) were compared with those positioned 50 cm from the shelter opening. At 10 min, fluorescence was 71.8±43.1 times more concentrated inside the shelter than at 50 cm. This ratio peaked at 40 min (102.0±32.3), declined to 27.2±16.4 and 2.8±0.8 after 60 and 90 min, respectively.

Odorant challenges

During pilot trials, we observed the response of individual gray snapper to the clarified toadfish homogenate. When the homogenate was pumped into an experimental shelter, snapper emerged from the reef structure with initial approaches in a circular pattern >50 cm from the shelter and then returned to the reef structure. Snapper came closer to the shelter with successive circular approaches, eventually pausing directly outside the shelter for visual scans. Eventually, snapper might enter the shelter on a more direct or linear track from the reef structure and nip at the clay toadfish model or substrate.

Snapper were generally more responsive to 24 μmol N l^{-1} treatments of ammonia than either urea or the ammonia/urea mixture (Table 1). The greatest values were observed for the toadfish homogenate (positive control) and similar behavior patterns were noted among treatments. Values in Table 1 were used to calculate the behavioral index (Fig. 2), which for ammonia was 29.5±3.4 and that was significantly different from either urea alone (14.6±3.2) or the ammonia/urea mix (13.6±2.5). Each constituent of the ammonia/urea mixture had a concentration of 12 μmol N l^{-1} (24 μmol N l^{-1} total waste-N); however, there was no statistical difference in behavioral responses between 12 and 24 μmol N l^{-1} ammonia treatments (Table 1).

Data from this first trial were separated into 10-min intervals expressed as the total number of all snapper approaches <50 cm (Fig. 3A). These data indicate that snappers responded to both ammonia and urea at the lowest levels during the initial 10 min of odorant delivery (Fig. 3B). The mean threshold sensitivity to ammonia was approximately 55 nmol N l^{-1}, based on snapper behavioral responses to ammonia concentrations of 3.97±0.97 μmol N l^{-1} occurring at the end of the 0–10 min (Fig. 3A,B) and the aforementioned 71.8 ratio after 10 min.

There were no statistical differences in response to L-proline (P), L-alanine (A), L-glycine (G), the AG mix and the PAG mix, except for duration within shelter (Table 2 and Fig. S4 in supplementary material). The mean duration within shelters for the alanine treatment was 32.6±10.3 s with a maximum of 224 s during which time the snappers did not appear to display any foraging behaviors such as nipping the substrate or attacking the toadfish model. Given that preyfish (toadfish) are most likely to naturally excrete a suite of amino acids and that the only statistically significant differences among treatments were for duration, we proceeded with the 2.5 mol N l^{-1} PAG mixture in amino acid trials 4 and 5.

Table 1. *Response of Lutjanus griseus to ammonia-N and/or urea-N*

	Behavior							
	0–25 cm*		25–50 cm*		Visual scan		Enter shelter	
	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
Trial 1 (*N*=10)								
Baseline	0.3±0.2[a]	0±0	0.6±0.2[a]	0.9±0.3	0±0[a]	0±0	0±0[a]	0±0
Ammonia	7.2±1.0[b]	0.9±0.5[†]	6.2±1.8[b]	0.1±0.4[†]	6.5±0.1[c]	0.4±0.2[†]	0.4±0.3[b]	0±0
Urea	4.3±0.9[b,c]	0.8±0.5[†]	2.3±0.9[b]	0.4±0.3[†]	2.8±0.9[b]	0.6±0.3[†]	0.3±0.2[b]	0±0
Ammonia/urea	3.9±0.8[c]	0.5±0.4[†]	3.6±1.0[b]	1.4±0.7[†]	2.9±0.7[b]	0.5±0.3[†]	0.0±0.0[b]	0±0
Positive control	16.0±2.4[d]	2.9±1.4[†]	3.0±1.1[b]	2.1±1.3	15.7±2.2[d]	2.0±1.1[†]	1.8±0.7[c]	0±0[†]
Trial 2 (*N*=4)								
Baseline	0±0[a]	0±0	0±0[a]	0±0	0±0.0[a]	0±0	0±0[a]	0±0
Ammonia-N (12 μmol l^{-1})	4.5±4.0[b]	0.3±0.5[†]	1.3±0.7[b]	0.8±0.7	4.0±2.4[b]	0±0[†]	0±0[a]	0±0
Ammonia-N (24 μmol l^{-1})	2.9±3.0[b]	0.3±0.3[†]	2.5±2.2[b]	0.8±0.7	3.0±2.4[b]	0±0[†]	0±0[a]	0±0

Behavioral categories include distance (*) gray snapper passed from experimental shelters delivering odorants; visual scan refers to when fish ceased locomotion for >1 cycle of pectoral fin sweeps and visually scanned the internal shelter chamber. The duration of each odorant challenge was 60 min.

Baseline is a control for pump noise prior to odorant delivery, the control columns represent simultaneous seawater delivery during odorant challenges and the positive control is clarified toadfish homogenate.

Values are presented as means ± s.e.m.; [†]significant differences between treatments and controls; significant differences among treatment groups are indicated by different lower case letters.

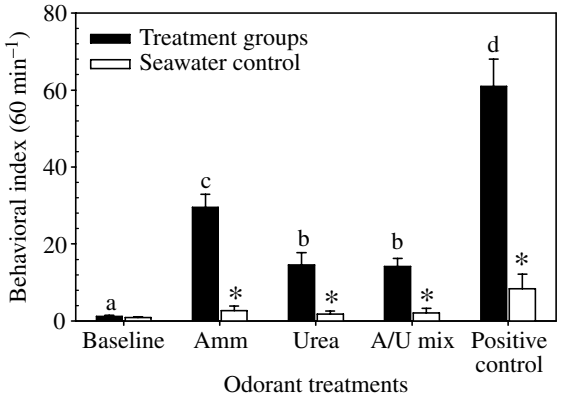

Fig. 2. Behavioral responses to 25 μmol N l^{-1} ammonia-N (Amm), urea-N, a 1:1 ammonia-N/urea-N mixture (A/U Mix) and controls during 60 min observation periods. Clarified toadfish homogenate, a positive control, was delivered last. The behavioral index is the sum of snapper responses characterized by entry into the shelter, passing 0–25 cm from shelter opening, passing 25–50 cm from shelter, and hovering while visually scanning shelter interior, which were awarded 5, 2, 1 and 1 points, respectively. The baseline reflects activity prior to odorant delivery. Data were log(x+1) transformed for equal variance. The paired t-test was used to examine each treatment and its alternate seawater control, and significant differences are denoted by an asterisk. Differences among treatment group where determined with a repeated measures ANOVA (F=14.509, P<0.001, N=10) and significantly different groups are indicated by different letters.

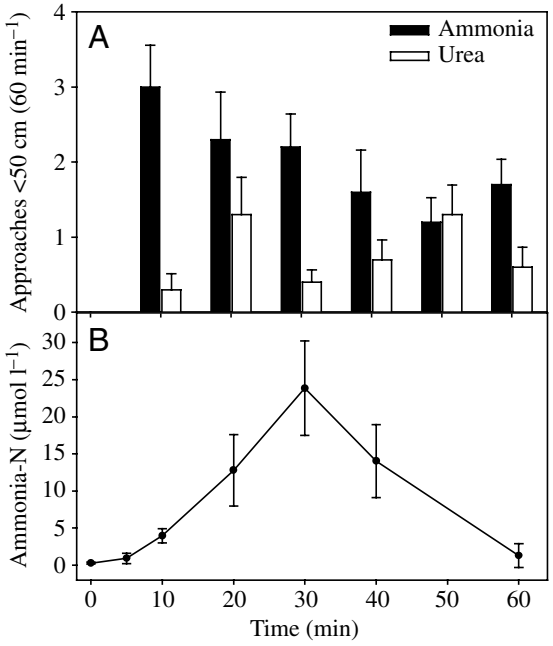

Fig. 3. (A) Number of approaches by snappers to <50 cm from the experimental shelter during the ammonia and urea treatments, separated into 10-min intervals. There were no significant differences among time intervals for either odorant with repeated measures ANOVA. (B) Ammonia concentration (mean ± s.e.m., N=10) from the calibration of the odorant delivery system. 33 mmol N l^{-1} NH_4Cl was delivered for 30 min (1.67 ml min^{-1}) followed by ambient seawater. The shelter's internal ammonia concentration (after mixing) was sampled *via* the access port.

In odorant trial 4, gray snappers exhibited a significantly greater response to PAG + ammonia than to either PAG without waste-N, or PAG + ammonia/urea mix with behavioral index scores of 16.8±1.8, 6.5±1.8 and 7.1±0.8, respectively (Fig. 4A). The same overall trend was seen among components of the index score (Table 3). The fifth odorant trial examined differences in snapper behavioral responses between the PAG + urea and PAG without waste-N with no statistical differences between treatments (Fig. 4B, Table 3).

Discussion

Our study placed special emphasis on simulating native gray snapper habitat to maximize the ecological relevance. Additionally, methodologies for scoring snapper behavior reflect previous field observations (Starck and Schroeder, 1971), hence the behavioral index gives the highest score to individuals entering shelters and striking the toadfish model. Although the time spent at the odorant source is considered a valid quantification of behavior (Hay et al., 1998), our results indicate that duration was not necessarily the most appropriate characteristic (Table 2). In trial 3, gray snapper entered shelters and rested passively on the substrate for up to 3.5 min when presented with 2.5 μmol N l^{-1} alanine. However, when responding to positive controls (toadfish homogenate), snappers entered shelters for 8.7±1.5 s and generally struck rapidly at the toadfish model or substrate.

Our results indicate that gray snappers respond to ammonia and that urea appears to function as a cloaking or masking agent as seen in the blunting of gray snapper behavioral responses by mixtures of either ammonia/urea (Fig. 2) or ammonia/urea + amino acid (Fig. 4A). However, no discernable urea blunting effect was noticed in response to the urea + amino acid mixture (Fig. 4B) suggesting that the cloaking effect of urea is specific to the ammonia odor. We believe that these results are important in at least two regards: first waste ammonia elicits a prey attack response; and second that a co-excreted waste molecule (i.e. urea) masks this response.

Ammonia was shown to elicit shoaling in silversides (*Hepsitia stipes*) nearly five decades ago (Steven, 1958), but it has not received research focus comparable to amino acids or bile salts, presumably owing to its higher threshold sensitivity. We demonstrate that behavioral sensitivity to ammonia is in fact close to that of these other compounds within the biologically relevant range of excreted/exuded values in fish (see Kajimura et al., 2004). The threshold for the response of gray snapper to ammonia occurs at low concentration (<5 μmol N l^{-1}, Fig. 3B) and gray snapper approached from distances beyond 50 cm where the calculated concentration was 55 nmol N l^{-1}, based on our dilution experiment. The behavioral responses displayed by snapper also represent the

Table 2. *Response of Lutjanus griseus to amino acids*

	0–25 cm*		Visual scan		Enter shelter		Duration in shelter (s)	
	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
Trial 3								
Baseline	0.5±5.0[a]	0.2±0.1	0±0[a]	0.2±0.2	0±0[a]	0±0	0±0[a]	0±0
Alanine (A)	8.8±3.4[b]	1.3±0.6[†]	12.7±5.8[b]	1.0±0.6[†]	4.8±2.4[a]	0±0	158±79[b]	0±0[†]
Glycine (G)	10.5±3.1[b]	0.5±0.2[†]	10.8±3.7[b]	0.2±0.2[†]	0.5±0.5[a]	0±0	3±3[a]	0±0
Proline (P)	4.5±1.8[b]	0.3±0.3[†]	3.8±1.8[b]	0±0[†]	0.2±0.2[a]	0±0	1±1[a]	0±0
AG	5.5±3.3[b]	2.5±1.0	4.0±3.0[b]	1.3±0.5[†]	0.3±0.3[a]	0±0	6±6[a]	0±0
PAG	7.9±1.8[b]	1.0±0.7[†]	7.4±1.9[b]	0.6±0.4[†]	1.4±1.3[a]	0±0	6±5[a,b]	4±4

Amino acid treatments included 2.5 μmol N l^{-1} L-alanine (A), L-glycine (G) and L-proline (P), with a 1:1 AG mix and a 1:1:1 PAG mixture. Behavioral categories include distance (*) gray snapper passed from experimental shelters delivering odorants; visual scan refers to when fish ceased locomotion for >1 cycle of pectoral fin sweeps and visually scan the internal shelter chamber. The duration of each odorant challenge was 60 min.

The baseline is a control for pump noise prior to odorant delivery; the control columns represent simultaneous seawater delivery during odorant challenges.

Values are presented as means ± s.e.m.; [†]significant differences between treatments and controls; significant differences among treatments groups are indicated by different lower case letters.

stereotypical phases of both arousal and search normally associated with prey localization (Jones, 1992) as opposed to odorant detection during electrophysiological recordings of olfactory or other neurons.

The generalized concept of odorants in the aquatic medium is that simple basic molecules are preferred since solubility is critical rather than volatility as in air (Sorensen, 1992). This description fits ammonia better than amino acids or bile salts, which are generally the subjects in prey detection studies (Hara, 1992; Caprio, 1984; Døving et al., 1980). However, given that the threshold sensitivities for amino acids, as measured by electro-olfactograms, are quite low (ranging from 10^{-9} to 10^{-5} mol l^{-1}) (see Hara, 1992; Caprio, 1984), a role for amino acids cannot be discounted.

We suggest that prey detection in fish may result from an initial attraction by readily excreted ammonia and subsequent 'assessment' of the prey's susceptibility to predation by amino acid detection, since amino acids leak rates are generally much lower but their relative proportions can change in response to stress (Kajimura et al., 2004). It is tempting to hypothesize that if predatory fishes are able to discriminate the ratio of ammonia to specific amino acids, they could assess the stress level of prey, which would be consistent with optimal foraging strategy; however, experimental work is needed.

This present study of snapper chemoreception and our prior documentation of co-excretion of urea and ammonia in wild toadfish (Barimo et al., 2004) represent the first case of a 'waste' chemical agent that can be excreted by an individual to cloak or mask its own chemical signal from potential predators.

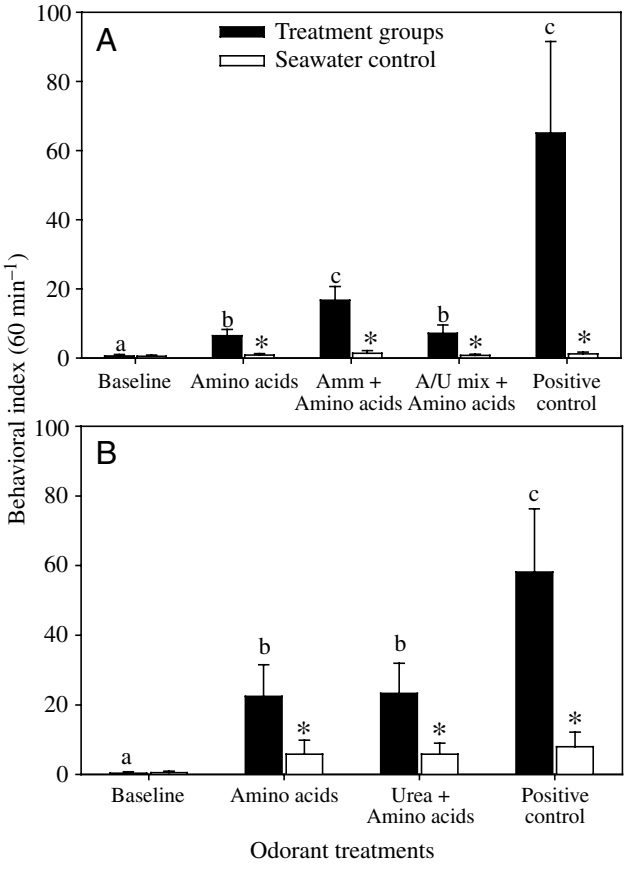

Fig. 4. (A) Response to a 2.5 μmol N l^{-1} mixture (1:1:1) of L-proline, L-alanine and L-glycine (Amino Acids; PAG); 2.5 μmol N L^{-1} PAG with 25 μmol N L^{-1} ammonia-N (Amm + Amino acids); 2.5 μmol N l^{-1} PAG with 25 μmol N l^{-1} ammonia-N + urea-N (1:1; A/U mix + Amino acids); and toadfish homogenate (positive control). Significant differences were found between treatment groups (F=7.393, P<0.001, N=8) as indicated by the different letters. (B) Behavioral assay to measure response to a 2.5 μmol N l^{-1} mixture (1:1:1) of PAG; 2.5 μmol N l^{-1} PAG with 25 μmol N l^{-1} urea-N; and seawater controls. Significant differences were found (F=9.293, P=0.005, N=6). Controls, the tabulation of the behavioral index, data transformations and subsequent statistics were identical to Fig. 2.

Table 3. *Response of Lutjanus griseus to PAG + ammonia-N, urea-N and ammonia-N/urea-N (1:1)*

	0–25 cm*		25–50 cm*		Visual scan		Enter shelter		Duration in shelter (s)	
	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
Trial 4 (*N*=8)										
Baseline	0.2±0.2^{a}	0±0	0.1±0.1^{a}	0.2±0.2	0±0^{a}	0±0	0±0^{a}	0±0	0±0^{a}	0±0
PAG	1.5±1.6^{b}	0.3±0.2†	1.6±0.6^{b}	0.3±0.2†	1.3±0.4^{a,b}	0.1±0.1†	0.1±0.1^{a}	0±0	0.4±0.4^{a}	0±0
PAG+Ammonia	4.4±1.6^{c}	0.4±0.2†	2.9±0.9^{b}	0.1±0.1†	3.1±1.2^{b}	0.3±0.2†	0.1±0.1^{a}	0±0	0.3±0.2^{a}	0±0
PAG+Ammonia/urea	1.6±0.7^{b}	0.3±0.2†	1.4±0.3^{b}	0.1±0.1†	1.0±0.5^{a,b}	0.1±0.1†	0±0^{a}	0±0	0±0^{a}	0±0
Positive control	11.9±4.3^{c}	0.4±0.2†	2.6±0.8^{b}	0.1±0.1†	15.3±6.5^{c}	0.3±0.2†	4.8±2.5^{b}	0±0†	52±30^{b}	0±0†
Trial 5 (*N*=6)										
Baseline	0.2±0.2^{a}	0.2±0.1	0.1±0.1^{a}	0.2±0.2	0.1±0.1^{a}	0.2±0.2	0±0^{a}	0±0	0±0^{a}	0±0
PAG	7.2±3.1^{b}	2.3±1.8†	5.8±1.6^{b}	0.8±0.3†	4.5±1.9^{b}	0.5±0.5†	0.3±0.3^{a}	0±0	0±0^{a}	0±0
PAG+urea	5.7±2.2^{b}	2.2±1.5†	7.0±2.3^{b}	0.8±0.4†	5.0±2.6^{b}	0.3±0.2†	0±0^{a}	0±0	0±0^{a}	0±0
Positive control	16.2±5.5^{c}	3.0±1.7†	9.7±3.4^{b}	1.8±0.9†	13.0±5.6^{b}	0.2±0.2†	0.7±1.3^{a}	0±0	5±4^{a}	0±0

PAG=2.5 μmol N l^{-1} mixture (1:1:1) of L-alanine, L-glycine and L-proline; PAG+Ammonia=2.5 μmol N l^{-1} PAG + 25 μmol N l^{-1} ammonia-N; PAG+Urea=2.5 μmol N l^{-1} PAG + 25 μmol N l^{-1} urea-N; PAG+Ammonia/urea=2.5 μmol N l^{-1} PAG with 25 μmol N l^{-1} amm-N/urea-N (1:1).

Categories include the distance (*) gray snapper passed from odorant source; visual scan refers to when fish ceased locomotion for >1 cycle of pectoral fin sweeps and visually scan the internal shelter chamber. The duration of each odorant challenge was 60 min.

Baseline controls for pump noise prior to odorant delivery, the control columns represent simultaneous seawater delivery during odorant challenges and the positive control was clarified toadfish homogenate.

Values are presented as means ± s.e.m.; †significant differences between treatments and controls; significant differences among treatments groups are indicated by different lower case letters.

Thus far, in the aquatic environment, only disruptions of chemoreception by environmental contaminants have been documented, where, for example metals can disrupt homing behaviors necessary for migration among salmonids (Hara, 1992; Sutterlin and Gray, 1973). Additionally, male toadfish guarding offspring within nests excrete ~50% of waste-N as urea-N (Barimo et al., 2004), which should further increase the individual's fitness by cloaking progeny since embryos excrete 89% of their measured waste-N as ammonia-N (Barimo and Walsh, 2005). Urea has been exploited for a variety of functions besides 'waste excretion' where the selective values are presumed to offset the considerable bioenergetic cost of ureogenesis (Withers, 1998). This study presents yet another novel role for urea in animal evolution to the aquatic environment.

The mechanism for masking of ammonia by urea could be competitive binding directly with ammonia at receptor sites. Alternatively, a separate pathway such as the trigeminal system, which is associated with the detection of noxious smells and has dedicated receptors separate from olfactory epithelium may be affected (Silver, 1987). Since urea does not appear to mask amino acid scents (PAG), but suppresses the ammonia response, the direct competitive interaction with ammonia is more likely. It is also possible that ammonia and urea are detected *via* separate receptor sites with sensory information processed in the olfactory bulb as a unique odor. Furthermore, binary odorants administered to a mammalian model were found to stimulate neurons in olfactory cortex that were not stimulated when either odorants was presented independently (Zou and Buck, 2006). It also seems unlikely that gray snapper perceived the urea odor as noxious since they did approach the odorant source, albeit significantly less than for ammonia. Additional study is needed to determine whether urea may also cloak known prey fish pheromones such as sex steroids or prostaglandins (Hara, 1992; Sorensen, 1992).

Except for a brief window during early development, urea synthesis and excretion have not been kept 'turned on' in most teleosts. Therefore, the metabolic cost of this strategy must offset an unusually high attractiveness of this group to predators in the absence of urea cloaking or other countermeasures to predation. Male toadfish and midshipman (family Batrachoididae) produce loud advertisement calls to attract mates (Tavolga, 1971; Barimo and Fine, 1998). Toadfish predators are believed to intercept these acoustic signals (Myrberg, Jr, 1981). We speculate that whereas other subfamilies within this group may have evolved either venomous spines (Thalassophryninae) or bioluminescence (Porichthyinae) as countermeasures to predation (Collette, 1966; Harper and Case, 1999), the subfamily that includes *O. beta* (Batrachoidinae) does neither, suggesting urea cloaking is one of several, advanced predator defense strategies within the arsenal of this family.

We dedicate this study to the late Authur Myrberg Jr for his inspiration and enthusiasm. We thank Chris Wood and Tom Mommsen for their advice, Angel Li for technical help, and Peter Frezza for field assistance. We thank also Suliban Cabal for first bringing to our attention the concept of cloaking. This

work was funded by National Science Foundation (IOB-0455904) and National Institute of Environmental Health (ESO5705) grants to P.J.W.

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