different hosts, and (vi) dispersal is low even among nearby conspecific hosts and is consistently low over several years. Although we have not proved that sympatric speciation has caused the divergence of this complex, the weight of the present evidence supports this view better than an allopatric mechanism.

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8. S. I. Guttman *et al.*, unpublished observation. Of 689 *Enchenopa* from four *Ptelea* marked and followed over an 8-week period, only 13 individuals moved between trees. Eleven of these moved between two *Ptelea* approximately 1 m apart; the other two treehoppers moved approximately 25 m.
9. Females were collected after oviposition started and mating was completed. Females were confined to branches covered with nylon cages. All host plants were grown in a garden plot to minimize the effect of environmental conditions on host phenology.
10. We would like to thank M. Taylor, B. Kintzer, and K. Olmstead for their help. This study was funded by National Science Foundation grant DEB-8023086. Delaware Experiment Station Miscellaneous Paper No. 1000. Contribution No. 518 of the Department of Entomology and Applied Ecology, University of Delaware, Newark 19711.

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Breathing Gas Mixtures Different from Air: An Adaptation for Survival Under the Ice of a Facultative Air-Breathing Fish

Abstract. *Gaseous respiration by central mudminnows (*Umbra limi*), particularly their use of bubbles composed of gas mixtures other than air, may have evolved as an adaptation to the oxygen-depleted, carbon dioxide–rich water of winterkill lakes. Under simulated winterkill conditions, mudminnows frequently engulfed gaseous bubbles. Use of bubbles was not related to varying methane or nitrogen content (0 to 80 percent) when all bubbles contained 20 percent oxygen. When the oxygen content of bubbles varied (0 to 20 percent), fish visited bubbles randomly but remained longer and took fewer "breaths" at bubbles with high oxygen content. High temperature (16° to 34°C) and low* pH *(6.8 to 4.5) did not stimulate increased air-breathing when dissolved oxygen was sufficient.*

Central mudminnows, *Umbra limi* (Kirtland), are continuous but facultative air breathers that use the highly vascularized swim bladder as an accessory respiratory organ (*1*). How this form of respiration evolved in the Umbridae is not known. Most species of air-breathing fishes live in warm, stagnant, hypoxic waters, although some live in well-oxygenated waters that are very acid, rich in CO_2, or subject to seasonal drought (*2*). We hypothesize that gaseous respiration by central mudminnows, particularly the ability to "breathe" from bubbles with gas mixtures markedly different from those of air, is an adaptation to the winterkill lakes that the fish commonly inhabit, in which the waters are depleted of O_2 and are rich in CO_2.

In winterkill lakes, ice prevents the fish from having direct contact with the atmosphere for 4 to 5 months of the year, but mudminnows may use bubbles trapped beneath the ice (*3*). Klinger *et al.* (*3*) found that mudminnows held in field enclosures with air bubbles in a winterkill lake, in which dissolved oxygen (DO) approached 0.0 mg per liter, survived longer than fish in enclosures without air bubbles. The average oxygen content of the naturally occurring bubbles was 3 percent (range, 0 to 11 percent) when DO was 0.5 mg per liter; bubbles also contained 1 to 75 percent methane and 23 to 98 percent nitrogen.

The severe environment (*4, 5*) of winterkill lakes was simulated in the laboratory (*6*). In a series of six experiments (Table 1) we examined how mudminnows behaved toward (i) air bubbles under both low DO–high CO_2 and high DO–low CO_2 conditions and (ii) bubbles composed of gas mixtures (nitrogen, methane, and oxygen) different from air but similar to mixtures that occur under natural conditions. To simulate ice cover, we inserted a panel of white, translucent fiber glass into each of six molded fiber glass aquariums (inside dimensions, 60 by 28 cm, and 35 cm deep). Five 4-mm holes were drilled through the fiber glass surface and countersunk; gas bubbles (2 ml) injected through holes simulated bubbles beneath the ice. We simulated cracks by leaving the holes unplugged; mudminnows could ventilate at the holes and pull oxygenated water (DO, 5 to 10 mg per liter) from above the fiber glass but could not gulp air.

Each experiment consisted of five 30-minute observation periods, and new gas bubbles were injected between these periods (*7*). Twelve fish (one large fish, 88 to 112 mm, and one small fish, 65 to 75 mm, in each of six aquariums) were placed in the aquarium before the start of experiment 1. The location and behavior of fish were recorded at 30-second intervals. Horizontal lines on the glass divided each tank into three equal volumes in the bottom, middle, and top. In addition to these three positions, fish could be at the simulated "ice" (with a part of the body touching it), at a hole, at a bubble, or at a plugged hole. We usually set DO and CO_2 concentrations the night before an experiment and measured both before the observation periods (*8*).

During experiment 1, with low DO and high CO_2 concentrations (Table 1), naïve mudminnows (those not previously exposed in the laboratory to low DO, high CO_2, or bubbles) moved up to the "ice" (62 percent of the time observed) and were often near bubbles (18 percent of the time) (Fig. 1a). After approaching a bubble a fish often raised its head and engulfed part of the bubble; additional breaths were frequently taken during the course of a visit. When a fish visited a hole it often inserted its head into the hole and pumped water from above across the gills. Although fish occasionally visited open holes, they were not recorded there (Fig. 1a), indicating that the mudminnows preferred to use bubbles than to pump oxygenated water down through holes.

The fish in experiment 1 were also tested under high DO–low CO_2 conditions (Table 1, experiment 2). When oxygen was abundant mudminnows spent a substantial percentage of time in the bottom third of the aquariums (47 percent), relatively little time at the "ice" (18 percent), and were rarely near holes or bubbles (3 percent) (Fig. 1b).

When the mudminnows were returned to low DO–high CO_2 conditions (Table 1, experiment 3), they again moved up to the "ice" (65 percent of the time observed) and were often near bubbles (25 percent of time) (Fig. 1c). The behavior of mudminnows was similar in experiments 1 and 3, indicating that the response to low DO–high CO_2 and air bubbles did not change as a result of experience.

That mudminnows frequently engulfed air bubbles when DO was low and CO_2 high but rarely when DO was high and CO_2 low, suggests that the fish used bubbles as an oxygen source under hypoxic-hypercarbic conditions. Gee (*9*) observed that the gas volume in swim bladders of mudminnows decreased more rapidly in hypoxic conditions than in normoxic conditions.

Oxygen depletion generally proceeds from the bottom of the lake to the top. Our results, as well as those of Gee (*9*), indicate that mudminnows respond to low DO–high CO_2 conditions by moving toward the surface, which is similar to the response observed in many other freshwater species (*3, 4, 10, 11*). However, because they apparently can take gaseous oxygen from bubbles into the swim bladder, mudminnows, unlike most other freshwater fishes, would not have to remain near the highly oxygenated water just beneath the ice. Mills (*10*) found that the vertical and horizontal distributions of mudminnows in a winterkill lake were independent of the distribution of DO during winter.

With bubbles composed entirely of air, nitrogen, or methane (Table 1, experiment 4), mudminnows visited bubbles indiscriminately but spent significantly ($P < .05$) more time near those composed of air (25 percent) than near those composed of nitrogen (4 percent) or methane (3 percent) (Fig. 1d) (*12*). In experiment 5, the methane and nitrogen content of bubbles varied from 1 to 80 percent, with oxygen constant at 20 percent. The percentage of time fish spent at bubbles was independent ($P \geq .05$) of methane or nitrogen content (Fig. 1e) (*12*), indicating that mudminnows were able to use bubbles composed of gases substantially different from air as long as the bubbles contained oxygen.

When the oxygen content of bubbles varied (Table 1, experiment 6), mudminnows visited bubbles randomly ($P \geq .05$) (*12*) and did not discriminate among bubbles before engulfing a portion of one. However, the fish remained longer ($P < .05$) at bubbles with higher percentages of oxygen than at bubbles with no oxygen (Fig. 1f) (*12*) and took fewer ($P < .05$) breaths per minute at bubbles with 5, 10, or 20 percent oxygen than at bubbles with 0 or 2.5 percent (*12*).

Data on frequency of visits, percentage of time at bubbles, and rate of breaths suggest that mudminnows are not able to detect the oxygen content of a bubble before engulfing it. The fish sampled bubbles of varying oxygen content with equal frequency but altered their behavior after a physiological response. There were no statistically significant ($P \geq .05$) differences in the percentage of time spent at bubbles with 2.5 to 10 percent oxygen (*13*), suggesting that bubbles with only 2.5 percent oxygen are sufficient to meet a mudminnow's physiological needs at low temperatures (< 4°C). The percentage of time spent at bubbles with 20 percent oxygen was less ($P < .05$) than that at bubbles with 5 and

Table 1. Environmental conditions of six experiments on the behavior of mudminnows (*18*) to air bubbles under simulated ice with cracks (open holes) and with high or low concentrations of dissolved oxygen (DO) and CO_2 and to bubbles varying in gas composition (*19*). All experiments were conducted under winter water temperatures (1.6° to 3.9°C) in the laboratory between 24 January and 20 February 1972. The order in which the experiments were conducted was 1, 6, 4, 5, 2, and 3.

Experiment	Fish alive (*N*)	DO range (mg/liter)	CO_2 range (mg/liter)	Conditions
1. Low DO–high CO_2	12	0.23– 0.29	10–18	Two air bubbles; two open holes; one plugged hole
2. High DO–low CO_2	9	10 –11	2– 5	Same as Exp. 1
3. Low DO–high CO_2	9	0.15– 0.31	27–28	Same as Exp. 1
4. Major gases vary	10	0.15– 0.27	20–27	One bubble each of air, CH_4, and N_2; two plugged holes
5. O_2 constant, CH_4 and N_2 vary	10	0.16– 0.38	12–16	Five bubbles each with 20 percent O_2 and 0 to 80 percent CH_4 and N_2
6. O_2 varies	11	0.12– 0.27	18–27	Five bubbles with O_2 from 0 to 20 percent

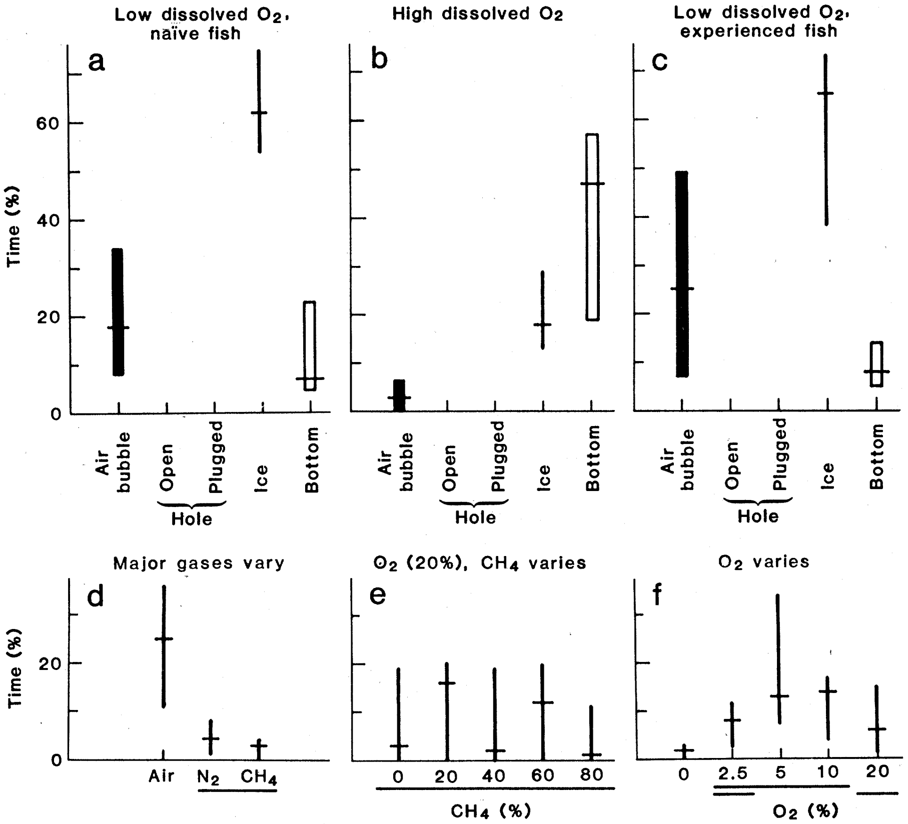

Fig. 1. Time (mean and 95 percent confidence interval) spent by mudminnows at air bubbles, open holes, plugged holes, the "ice," and the bottom under three experimental conditions: (a) low DO–high CO_2 (naïve fish), (b) high DO–low CO_2, and (c) low DO–high CO_2 (experienced fish). Time spent by mudminnows at bubbles with various gas mixtures: (d) air, nitrogen, and methane bubbles; (e) bubbles varying in nitrogen and methane content; and (f) bubbles varying in oxygen content. Values that do not share a common underline are significantly different ($P < .05$) from each other (*13*).

10 percent oxygen (*13*), indicating that mudminnows may have been able to obtain sufficient oxygen more quickly at bubbles with 20 percent oxygen.

Selection for the ability to use gaseous oxygen would be intense during the hypoxic and hypercarbic conditions of winter. Fish using gaseous respiration not only obtain more oxygen but can also avoid high amounts of dissolved hydrogen sulfide, which can reach lethal concentrations in winterkill lakes (*4, 5, 14*). Although selection pressures for gaseous respiration are obviously great in winter, air-breathing by mudminnows might have evolved as an adaptation to low *p*H conditions in summer. Mudminnows in northern Wisconsin often inhabit waters with moderately low *p*H (*15*). In experiments examining effects of *p*H and elevated temperatures on air-breathing in mudminnows, there were no statistically significant differences ($P \geq .05$) observed in rates of air-breathing among fish held in water from a dystrophic (*p*H 4.5), oligotrophic (*p*H 5.6), or mesotrophic (*p*H 6.8) lake (0.7, 1.4, and 0.9 breaths per fish per hour, respectively) (*6*). Also, frequency of air-breathing did not increase significantly ($P \geq .05$) as water temperatures increased from 16°C to a lethal level (34°C) regardless of the type of lake water (*16*).

By using the gaseous oxygen in bubbles, mudminnows are able to survive concentrations of DO an order of magnitude lower than those reported as stressful to most fishes that rely on aquatic respiration (*17*). Moreover, the ability of mudminnows to respond differently to bubbles varying in oxygen content would enhance their ability to survive in winterkill lakes, where bubbles differ greatly in composition. Whether the bubbles come from sediments, from gases extruded in water when it freezes, or from gases exhaled or escaping from the fur of aquatic mammals, they are a critical winter resource for mudminnows, allowing them to survive in environments that are lethal to other fishes.

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7. The observer (in an observation area with low light intensity) viewed the fish through a one-way mirror set at an angle. Observations were usually made between 0915 and 1445. Each aquarium was lit by incandescent bulbs controlled by timers with day length simulated for winter conditions.
8. Temperature and chemical conditions were measured at least once a day in each aquarium from 2 hours before to 1/2 hour after observations. Water samples were siphoned from the aquariums; DO was measured by Winkler titration (azide modification) and CO_2 by titration with sodium carbonate. Replicate chemical measurements made on 3 days agreed closely.
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20. We thank S. Steffel, R. Ozane, R. Stein, B. Richardson, S. Brandt, S. Petty, and S. Klinger for assistance in observations or analyses. Supported by NSF grant GB 18272.

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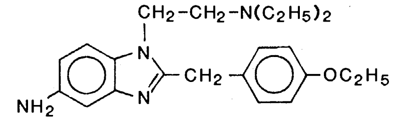
Irreversible Ligands with High Selectivity Toward δ or μ Opiate Receptors

Abstract. *Alkylating agents that display strong selectivity for opiate receptor types δ or μ were prepared by appropriate modification of the structures of the strong analgesics fentanyl, etonitazene, and endoethenotetrahydrooripavine. The availability of these substances should facilitate studies of the structural basis of receptor specificity and of the physiologic roles of these receptors.*

The existence of several endogenous opioid peptides (*1*) and data suggestive of separate types of opiate receptors (*2*) have raised questions about the relations of the peptides to the receptors and about the structure and function of the various types of receptors. Of the opiate receptor types so far proposed, δ and μ are the best documented (*2*) and together account for 70 percent of the opiate receptors of rat brain (*3*).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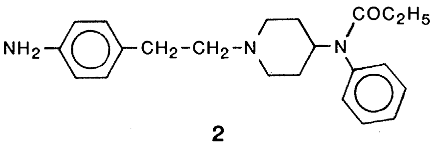
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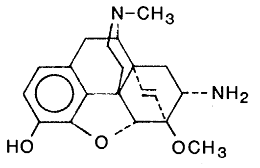
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One approach to the study of these questions requires selective, site-directed alkylating agents for each receptor type. Probes with exclusive specificity for δ receptors have been heretofore unavailable, although several affinity reagents based on enkephalin have been prepared (*4*). In this report, we describe alkylating derivatives of several opiate analgesics and show that some of these are highly selective, and irreversible, ligands for δ receptors, whereas others are equally selective for μ receptors.

Irreversible ligands with apparent specificity toward μ (and perhaps κ) receptors have been prepared by modification of epoxymorphinan structures characteristic of morphine, the prototypical μ ligand (*5*). We used amino derivatives of strong analgesics based on etonitazene (structure **1**), fentanyl (structure **2**), and endoethenotetrahydrooripavine (structure **3**) to prepare alkylating ligands of greater selectivity. These amines were converted to the corresponding isothiocyanato-, bromoacetamido-, and methylfuramido- derivatives and assayed for covalent interaction with receptors. Opiate receptor assays were carried out with rat brain membranes, which have μ and δ receptors and with membranes from the neuroblastoma-glioma hybrid NG108-15 cells, which have only δ receptors (*6*).