Air-breathing behaviour of the African anabantoid fish

_Ctenopoma muriei_

A. M. Randle* and L. J. Chapman†

Department of Zoology, University of Florida, Gainesville, FL 32611, U.S.A.

(Received 19 February 2004, Accepted 8 February 2005)

Frequency of aerial respiration in _Ctenopoma muriei_ increased as dissolved oxygen (DO) decreased; breathing behaviour was spatially synchronous, and there was no difference in air-breathing frequency between populations from a high- and low-DO site. Although _C. muriei_ survived without access to the surface, its loss of buoyancy and equilibrium under these conditions suggested that it is a functionally obligatory air breather.

© 2005 The Fisheries Society of the British Isles

Key words: Anabantidae; bimodal breather; synchronous air breathing; tropical freshwater fish.

All air-breathing fishes are bimodal, and thus use a combination of aerial and aquatic respiration. This is partially because air-breathing fishes continue to rely on their gills for gas exchange, nitrogen excretion and ionic regulation (Claiborne, 1998; Gilmour, 1998; Karnaky, 1998). There is a large range, however, in the use of aquatic respiration among air-breathing fishes. Fishes that regularly use aerial respiration over a range of dissolved oxygen (DO) levels are considered continuous air breathers (Graham, 1997). Continuous air breathers can be either obligatory or non-obligatory. Obligatory continuous air breathers have reduced gills and are unable to meet their oxygen demands by aquatic respiration alone, even when DO is high. Non-obligatory continuous air breathers also use aerial respiration over a range of DO, but do not require it for survival under normoxic conditions (Graham, 1997). In contrast, facultative air breathers only use aerial respiration when DO is low or when their oxygen demands are high (Graham _et al._, 1977; Bevan & Kramer, 1987; Graham, 1997). Air-breathing fishes also seem to vary in ventilation mode. Some bimodal species are known to exhibit triphasic ventilation where stale air is forced out the air chamber with inhaled air, while others use quadruphasic ventilation where stale air is forced out of the air chamber by flushing with water (Liem, 1987). In

*Author to whom correspondence should be addressed at present address: Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260, U.S.A. Tel.: +1 412 648 7687; fax: +1 412 624 4759; email: apr8@pitt.edu

†Present address: Department of Biology, McGill University, 1205 Avenue Docteur Penfield, Montreal, PQ, H3A 1B1, Canada

© 2005 The Fisheries Society of the British Isles
some species of clariid catfishes they use both modes depending on ecological circumstance (Liem, 1987). Thus, there is great diversity in air-breathing behaviours and modes of respiration among bimodal species. Variation in air-breathing behaviour among populations, however, has received far less attention, but may occur when widespread species inhabit a range of environmental conditions. In this study the breathing behaviour of the widespread African anabantoid *Ctenopoma muriei* (Boulenger) was investigated and variation in air-breathing behaviour between two populations experiencing different DO regimes explored.

The anabantoid fishes are bimodal breathers with paired suprabranchial chambers containing a bony element covered by a highly vascularized layer of tissue called the labyrinth apparatus (Graham, 1997). They are continuous air breathers, and most appear to be obligatory; however, the group exhibits variation in development of the suprabranchial organ, and some anabantoids, including species of *Ctenopoma*, seem to be non-obligatory (Pinter, 1986; Graham, 1997). Some anabantoids including *Ctenopoma* and *Helostoma* are capable of ventilating either triphasically or quadruphasically, while *Anabas* uses the triphasic mode exclusively (Peters, 1978; Liem, 1980, 1987). *Ctenopoma muriei* is an anabantid native to the Lake Victoria basin and is common in Nilotic waters, ranging from the White Nile, south to Lake Victoria and Lake Edward (Norris, 1988). The breathing behaviour of this species has not been well described; however, it is a good candidate for studies of interdemic variation, because it is a widely dispersed species that is common in hypoxic wetlands but can also be found in the well-oxygenated littoral zone of some lakes. In addition, given potentially high aerial predation pressure in some populations (Randle & Chapman, 2004), *C. muriei* may exhibit breathing behaviours that minimize predation risk such as spatial or temporal synchrony in air breathing. The specific objectives of this study were to: (1) examine variation in respiratory behaviour among populations of *C. muriei* from sites that differ in oxygen availability, (2) describe its mode of ventilation, (3) determine if it exhibits synchronous air-breathing behaviour and (4) explore if it is an obligatory or non-obligatory air breather.

Specimens of *C. muriei* were collected in Uganda, East Africa, in May and August of 1998 from two sites known to differ in DO concentration: Lake Kabaleka (high oxygen site) and the Lwamunda Swamp (low-oxygen site). Lake Kabaleka (0°16′N; 30°15′E) is located in the southern portion of Kibale National Park, Uganda. Melnychuk & Chapman (2002) reported a strong vertical gradient of DO in Lake Kabaleka, with a well-oxygenated upper stratum [7-9 mg l⁻¹ (c. 142 mm Hg), 28-7° C] and a moderately oxygenated lower stratum [2-8 mg l⁻¹ (c. 50 mm Hg), 23-7° C]. Specimens of *C. muriei* were captured in the upper stratum of the main lake or in the lake margin where DO was relatively high. The Lwamunda Swamp surrounds Lake Nabugabo, Uganda (31°50′E to 31°56′E; 0°20′S to 0°25′S) and separates it from Lake Victoria. Within the wetland there are several small lagoons (generally <2 m of depth; temperature range 21-5-22-6° C). Randle & Chapman (2004) reported very low levels of DO in swamp lagoons [mean morning DO = 0-49 mg l⁻¹ (c. 10 mm Hg); midday = 1·15 mg l⁻¹, (c. 24 mm Hg), upper 20 cm of the water column], where specimens of *C. muriei* were captured for this study. Fish from both sites were transferred to the Makerere University Biological Field

Station in Kibale National Park and kept in separate holding tanks for the duration of the experiments outlined below. Storage tanks were maintained at ambient temperatures at 17-0 ± 0.2°C and 5-0 ± 0.2 mg l⁻¹ (mean ± s.e.) of dissolved oxygen. Water temperature and DO concentration did not differ significantly among holding tanks ($t = 0.92$, d.f. = 49, $P = 0.370$ and $t = 0.33$, d.f. = 36, $P = 0.148$, respectively). Fish were fed a diet of Tetra Flake fish food daily. Fish were held in these conditions for a minimum of 2 weeks prior to experimentation to facilitate acclimation to the cooler water temperatures at Kibale relative to the lower-altitude collection sites.

Twelve individuals from each population were uniquely marked with tattoo ink and placed into one of three randomly selected groups (three groups of four fish from each population). A group of four fish from one population was placed in an experimental tank (55 × 22 × 30 cm) c. 12 h prior to the beginning of each experiment. Two hours prior to the experiment tanks were aerated for 1 h. The mean ± s.e. total length ($L_T$) and mass of experimental fish were 5.9 ± 0.21 cm and 3.8 ± 0.41 g, respectively, and did not differ among the six groups of fish (ANOVA, length: $F_{5,18}$, $P = 0.81$; mass: $F_{5,18}$, $P = 0.99$). Variation in respiratory behaviour among populations of *C. muriei* was examined in response to progressively lowered oxygen partial pressure ($P_{O_2}$). Dissolved oxygen was lowered progressively to 0 mm Hg using a small amount of sodium sulphite (Lewis, 1970; Olowo & Chapman 1996; Melnychuk & Chapman 2002). Water temperature and DO concentration (YSI Model 51B or Model 95), and frequency of air-breathing bouts were recorded over a 15 min period followed by a 10 min break before the next set of observations. Behavioural trials averaged 4.3 h in length and each trial was repeated three times for each of the three groups from both high (Kabaleka) and low (Lwamunda) oxygen sites. The linear relationship between air-breathing frequency and $P_{O_2}$ was examined for the mean of three trials for each group. A repeated measures ANOVA (with Greenhouse-Geisser correction) was used to detect effects of population, $P_{O_2}$ level and their interaction on air-breathing frequency. The timing of exhalent air was recorded during aerial respiration events to explore the ventilation mode of *C. muriei* (triphasic or quadruphasic) in the context of this study. Air released prior to *C. muriei* reaching the surface is evidence of quadruphasic ventilation, whereas, air released after the fish reaches the surface is evidence of triphasic ventilation. The social breathing tendency (synchronous respiration) of *C. muriei* was examined in a group context by observing each group of four fish and quantifying the mean number of air breaths (at $P_{O_2} = 0$ mm Hg) taken within the immediate vicinity (<1 fish $L_T$) of a conspecific and comparing that to the mean number of air breaths taken alone (>1 fish $L_T$ from conspecifics). An independent sample *t*-test was used to compare means.

To determine whether *C. muriei* is an obligatory or facultative air breather, observations were made of the respiratory behaviour of individuals denied access to the surface. Four fish were placed in an experimental tank with well aerated water and allowed to acclimate as above. Normal air-breathing behaviour was recorded during the first 15 min interval, after which, a perforated lid was lowered over the surface to prevent aerial respiration. Two trials, one for each population, were conducted in which fish were denied surface access for 24 h.
The air-breathing attempts and behaviour were recorded every hour for the first 7 h and last 3 h of each 24 h period.

In this study there was no difference in breathing rates between the two populations of *C. muriei* over the range of PO$_2$, nor was there a significant interaction between population and PO$_2$ ($F_{1,4}, P = 0.438$). The effect of PO$_2$ on air-breathing frequency, however, was significant ($F_{1,4}, P = 0.006$). The rate of air-breathing increased from an average of 10.5 breaths per 15 min at 100 mm Hg to 14.3 breaths per 15 min at 0 mm Hg, and was directly correlated with PO$_2$ ($r = -1.0, n = 5, P < 0.001$). During the progressive hypoxia trials, *C. muriei* released exhalent air prior to reaching the surface, which is evidence of quadruphasic ventilation. *Ctenopoma muriei* also exhibited spatial synchrony in air-breathing behaviour. Individuals were more likely to respire aerially in the immediate presence of conspecifics than alone ($t$-test, d.f. = 10, $P < 0.002$; Fig. 1). Individuals would slowly approach the surface as a tight group, often alternating the lead position. One or more individuals would break the surface to breathe; then the whole group would descend rapidly to the bottom. Although groups of individuals would approach the surface in this manner, often only a single individual would breathe.

In the two trials where fish were denied access to the surface, they were able to survive in well-oxygenated water for 24 h without using aerial respiration. Thus, according to conventional categorizations of air breathers, *C. muriei* would be considered a non-obligatory continuous air-breather. Under these conditions, however, fish immediately lost equilibrium and became negatively buoyant for the duration of the experiment. Fish continued to make aerial respiration attempts over a 24 h period, but their behaviour was abnormal (*i.e.* lying on their side at the bottom of the tank), activity was minimal and upward swimming movements were strained. After 24 h, fish were allowed to break the surface, and instantly regained buoyancy and resumed normal behaviour.

**Fig. 1.** The mean ± s.e. number of air breaths for *Ctenopoma muriei* when solitary ([■]) and when in groups ([□]) (*i.e.* with one or more conspecifics within one body length) over a 30 min period at PO$_2$ = 0 mm Hg (**, $P < 0.005$).
Ctenopoma muriei used aerial respiration over a wide range of DO; however, the frequency of air-breathing bouts was negatively related to $P_{O_2}$ suggesting greater use of aquatic respiration at higher $P_{O_2}$. Changes in respiratory partitioning in response to dissolved oxygen have been demonstrated in several other bimodal breathing species, e.g., Trichogaster trichopterus (Pallas), (Burggren, 1979) Clarias macrocephalus Günther (Bevan & Kramer, 1987) and Rhinelepis strigosa (Valenciennes) (Takasusuki et al., 1998), and may reduce costs of air breathing (e.g., travel and predation risk). The release of exhaled air prior to reaching the surface is evidence of a quadruphasic mode of ventilation (Peters, 1978; Liem, 1980). Liem (1980) described the quadruphasic pattern of air ventilation in the helostomatid anabantoid Helostoma temmincki Cuvier and found that air was expelled from the mouth, mostly when the fish had its mouth above the water. The observation of bubble release in C. muriei prior to breaking the surface in the present study, however, is consistent with observations by Peters (1978) on Ctenopoma damasi (Poll & Damas), C. muriei, Ctenopoma nigropammosum Reichenow and Ctenopoma kingsleyae Günther. Ctenopoma muriei may exhibit triphasic ventilation when water levels are inadequate for the quadruphasic mode; however this was not explored in the current study.

Several air-breathing species are known to exhibit temporally and spatially synchronous breathing behaviour, e.g., Lepisosteus spp. (Hill, 1972), Umbra limi (Kirtland) (Gee, 1980) and Clarias liocephalus Boulenger (Chapman & Chapman, 1994), which is thought to be an adaptation to aerial predation pressure (Kramer & Graham, 1976). Synchronous air-breathing behaviour is similar in principle to schooling behaviour in that it reduces the probability of predation on an individual compared to air breathing alone (Kramer & Graham, 1976; Chapman & Chapman, 1994). In C. muriei, not all individuals took a breath on approach to the surface, sometimes only a single fish would breathe; however, the spatial synchrony may still afford the benefits of grouping on approach to the surface.

The air-breathing behaviour of C. muriei in response to progressive hypoxia is consistent with the definition for continuous non-obligatory air-breathers (i.e., fish used aerial respiration continuously over a range of DO and could survive without access to the surface in normoxia). When denied access to the surface, however, C. muriei was unable to maintain buoyancy and normal swimming behaviour, which would limit activities such as foraging or predator evasion. Although C. muriei could survive without access to the surface, it should probably be considered a functionally obligatory air breather, because it requires access to the surface to maintain buoyancy and normal swimming behaviour.

There was no evidence for differences in air-breathing behaviour in response to hypoxia between C. muriei captured in the well-oxygenated ecotone of Lake Kabaleka and those captured in the extremely hypoxic lagoons of the Lwamunda Swamp. It is possible that C. muriei move freely between the hypoxic dense swamp interior and ecotonal waters of Lake Kabaleka and therefore experience extreme hypoxia on a regular basis. In addition, Randle (2001) found no difference in the total gill filament length of air-breathing organ mass between C. muriei from Lake Kabaleka and the Lwamunda Swamp, which may limit flexibility in behavioural responses to hypoxia. In C. muriei, the air-breathing organ (and potentially the swimbladder) appears to be
particularly important in maintaining buoyancy, and the air-breathing organ in anabantoids may also be important in auditory sensitivity and vocalization (Yan, 1998; Ladich & Yan, 1998). It is possible that the multiple functions of the air-breathing organ and surface respiration in anabantoids constrain the degree of morphological response to divergent oxygen environments and consequently limit the degree of variation in respiratory behaviour among populations.

Funding for this research was provided from the Wildlife Conservation Society, the National Science Foundation (DEB-9622218), Sigma Xi, Raney Award and the International Women’s Fishing Association. Permission to conduct research in Uganda was acquired from the National Council for Science and Technology, the Office of the President, and Makerere University (Uganda). We thank C. Chapman, J. Paul and F. Nordlie, the field assistants at Lake Nabugabo, and our colleagues at the Fisheries Resources Research Institute of Uganda for assistance with various aspects of this project.

References


