Review

Persisting in papyrus: Size, oxidative stress, and fitness in freshwater organisms adapted to sustained hypoxia☆

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ABSTRACT

Aquatic hypoxia is generally viewed as stressful for aerobic organisms. However, hypoxia may also benefit organisms by decreasing cellular stress, particularly that related to free radicals. Thus, an ideal habitat may have the minimum O2 necessary to both sustain aerobic metabolism and reduce the need to scavenge free radicals and repair free radical damage. The ability of aquatic organisms to sustain aerobic metabolism relates in part to the ability to maximize gas diffusion, which can be facilitated by small body size when O2 uptake occurs across the body surface, by a large gill surface area, or by the ability to use atmospheric air. We use water-breathing organisms in chronically hypoxic papyrus (Cyperus papyrus) swamps of East Africa to test the hypothesis that cellular-level benefits of hypoxia may translate into increased fitness, especially for small organisms. A review of recent studies of fingernail clams (Sphaerium sp.) shows that clams living in sustained hypoxia have minimized oxidative stress and that these cellular-level benefits may lead to increased fitness. We suggest that organisms in the extreme conditions in the papyrus swamps provide a unique opportunity to challenge the conventional classification of hypoxic habitats as ‘stressful’ and normoxic habitats as ‘optimal.’

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This paper was presented at the ‘First International Conference on Oxidative Stress in Aquatic Ecosystems’ held in San Jose del Cabo, Baja California Sur, Mexico On November 20–24, 2012.

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http://dx.doi.org/10.1016/j.cbpa.2013.03.032

Please cite this article as: Joyner-Matos, J., Chapman, L.J., Persisting in papyrus: Size, oxidative stress, and fitness in freshwater organisms adapted to sustained hypoxia, Comp. Biochem. Physiol. A (2013), http://dx.doi.org/10.1016/j.cbpa.2013.03.032
hypoxia in inland and coastal areas through cultural eutrophication and pollution (Diaz and Rosenberg, 1995; Gray et al., 2002; Levin et al., 2009; Keeling et al., 2010). Climate change is likely to exacerbate hypoxic conditions (Keeling et al., 2010; Pörtner, 2010) because O₂ solubility decreases with rising water temperature while ectothermic metabolism increases. This growing awareness that climate change may affect water-breathing ectotherms through O₂ limitation of thermal tolerance (Pörtner, 2010) highlights the importance of hypoxia (solubility, PO₂) as a factor to be considered in global change scenarios. It is thus increasingly important that we understand the consequences of hypoxia for the morphology, physiology, and behavior of aquatic organisms, in order to predict cascading effects on population and community interactions.

Hypoxic habitats typically are defined as those in which dissolved O₂ (DO) concentrations are less than 2 mg L⁻¹ (1.42 mL L⁻¹), or approximately 30% of air saturation (Levin et al., 2009), a designation that aligns well with the median lethal DO concentration (LC₅₀) of more than 200 species of aquatic organisms (Vaquer-Sunyer and Duarte, 2008). The LC₅₀ for DO is highly variable among species and depends on multiple environmental variables (Diaz and Rosenberg, 1995; Gray et al., 2002; Wu, 2002; Vaquer-Sunyer and Duarte, 2008). The consequences of exposure to hypoxia and the strategies employed by aquatic organisms to avoid or tolerate hypoxia have been extensively reviewed (Grieshaber et al., 1994; Diaz and Rosenberg, 1995; Burnett, 1997; Gray et al., 2002; Wu, 2002; Abele et al., 2007; Bickler and Buck, 2007; Storey and Storey, 2007; Larade and Storey, 2009; Levin et al., 2009). Briefly, aquatic organisms use behavioral strategies to avoid hypoxia and use ventilatory and circulatory strategies to attempt to maintain O₂ delivery to their tissues. When O₂ delivery is impared, organisms may rely on anaerobic metabolism, decrease activity, and/or enter metabolic depression.

1.1. Hypoxia may be less stressful to small organisms

Prolonged hypoxia can change the composition of aquatic communities by reducing species richness and diversity, altering dominant functional groups, and (potentially) simplifying the food chain. In general, hypoxia may be accompanied by loss of large predators, demersal fish species, and benthic species that rely on suspension feeding (Wu, 2002; Levin et al., 2009). Hypoxic waters tend to contain organisms with small body size, rapid growth, and annual life cycles (Gray et al., 2002; Wu, 2002; Levin, 2003; Levin et al., 2009). Severely hypoxic waters are frequently characterized by a low richness of highly hypoxia-tolerant fish species (Junk et al., 1983; Chapman et al., 1996a; 1996b; 2002; Anjos et al., 2008) and a high occurrence of fish species with advanced parental care (Roberts, 1973; Chapman and McKenzie, 2009).

Small body size is a widespread characteristic of the invertebrate fauna of hypoxic habitats, likely because the large surface area to volume ratio enhances O₂ diffusion for organisms taking up O₂ through the body surface (Levin, 2003; Levin et al., 2009). This trend is illustrated by the fauna of the open-ocean oxygen minimum zones (OMZs), in which DO is approximately 0.7 mg L⁻¹ (7.5% saturation; Childress and Seibel, 1998; Levin, 2003; Stramma et al., 2008; Keeling et al., 2010). The permanent fauna of OMZs include foraminifera, meiofauna, and a few metazoans, many of which have smaller body size, thinner walls (forams), and larger gill surface area than do related species that live near the ocean surface (Childress and Seibel, 1998; Levin, 2003). The benefits of small body size also are apparent in the repeated documentation of the high survival of meiofauna following hypoxic events and the abundance of meiofauna in anoxic sediments (for review, Gray, 2002; Levin et al., 2009). It has been argued that fish eggs (e.g., Einum et al., 2002; Rombough, 2007), embryos, and/or juveniles (Reardon and Thibert-Plante, 2010) should be smaller under hypoxia, particularly at life-history stages during which O₂ uptake occurs across the egg or body surface. This of course depends on whether or not the whole egg is metabolically active (e.g., Einum et al., 2002; Rombough, 2007). A recent implicit model exploring optimal juvenile size in fishes supported the prediction for small juvenile size under hypoxia (Reardon and Thibert-Plante, 2010). Smaller adult body size under hypoxia is common across fish species, which may explain the observation that hypoxic wetlands serve as refuges for small fish from large piscine predators (reviewed in Chapman and McKenzie, 2009). Two mechanisms have been proposed to explain this pattern, both of which relate to O₂ delivery to the tissues. Small fish may have more efficient gas exchange because of the negative allometric relationship for mass-specific gill surface area (Pauly, 1981; Hughes, 1984), and larger fish may be more limited by the fixed size of red blood cells for gas exchange (West et al., 1997). Although many fish communities persisting under extreme hypoxia are dominated by small species, Nilsson and Ostdlund-Nilsson (2008) argue against scaling of physiological function as a general explanation. They concluded that gill surface area matches metabolic rate over a wide body size range, and therefore body size may not be a powerful determinant of hypoxia tolerance in fishes. Clearly, there is a need not only for additional experimental studies and field observations on body size and hypoxia in adult fishes, but also a broader and more integrative perspective on the balance between sufficient and excess O₂ transport.

1.2. Hypoxia and oxidative stress

As several authors have noted (e.g., Massabuau, 2001; Abele et al., 2007), some small aquatic organisms regulate O₂ delivery to the tissues in a manner that prevents excess O₂ transport, as opposed to maximizing O₂ transport. While meiofauna achieve this regulation by moving into hypoxic sediments, macrofauna decrease ventilation rates (for review, Abele et al., 2007). The apparent preference for environmental and/or tissue hypoxia has been interpreted, in part, as a strategy to minimize free radical production and the oxidative stress that results from an imbalance of free radical production and detoxification/repair (Gray et al., 2004; Abele et al., 2007). This interpretation is strengthened by a series of experiments in Drosophila melanogaster (Zhou et al., 2007, 2008; Ali et al., 2012), in which fruit flies selected for hypoxia tolerance (to 4% O₂) for 200 generations evolved reduced body size/mass, decreased cell number, and reduced mitochondrial superoxide leakage (but no change in lipid or protein oxidative damage).

Although the fitness-related benefits of minimizing oxidative stress are obvious, attempts to link life history patterns with oxidative stress have had mixed success (von Schantz et al., 1999; Sagarin and Somero, 2006; Dowling and Simmons, 2009; Constantini et al., 2010; Isaksson et al., 2011; Guerra et al., 2012). Most of these studies used organisms from terrestrial or normoxic aquatic environments, where the balance between sufficient and excess O₂ delivery to the tissues may be fundamentally different than in organisms adapted to hypoxic environments. If organisms in permanently hypoxic habitats, particularly small organisms that have maximal diffusion abilities, experience DO levels that are close to the optimal level for mitochondrial respiration, then they may gain fitness benefits from not having to regulate O₂ delivery and not experiencing oxidative stress. Testing this prediction is challenging because most permanently hypoxic habitats are not easily accessible, and the most abundant organisms in the habitats tend to be very small (e.g., meiofauna).

Here, we describe a (relatively) accessible, chronically hypoxic habitat that contains a diversity of aquatic organisms, from meiofauna to fishes, which may serve as a useful model system for addressing the above prediction. Although the limnological features and faunal assemblages of this habitat have been characterized (and are reviewed herein), the physiological traits of the fauna are as not well studied. We present evidence from studies with a small bivalve, a fingernail clam, that supports the prediction that organisms may gain a fitness benefit from minimized oxidative stress when they live in sustained hypoxia.

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Fig. 1. Map illustrating the locations of the permanent papyrus swamps in East Africa. Lacustrine swamps are present around the large lakes (Lake Victoria, Lake (Lk.) Kyoga, etc.); valley swamps are present in the Upper Kagera and Kigezi highlands. Inset provides details of Lake Nabugabo, a satellite lake of Lake Victoria (redrawn after Thompson, 1976).
2. Permanently hypoxic swamps in East Africa

There are approximately 85,000 km² of permanent swamps in East Africa, mostly associated with the two East African Rifts and the Lake Victoria basin (Beadle, 1974). These swamps are structured by the emergent sedge papyrus *Cyperus papyrus* and occur in two main forms. Lacustrine swamps are large, floating papyrus mats at the edges of lakes; large lacustrine swamps ring Lake Victoria (Fig. 1) and its satellite lakes (in Uganda, Kenya, and Tanzania), Lake Albert (in Uganda and Congo), Lake George (Uganda), and Lake Kyoga (Uganda). Extensive papyrus swamps also form in river valleys (valley swamps) in the Rwandan highlands and Kigezi highlands and in many river valleys of Uganda where there are good hydraulic gradients. Most valley swamps are fed by intermittent streams and extend into associated floodplains before draining into larger rivers. We focus on two valley swamps in the Mpanga River drainage of western Uganda in this review: the Rwembaita Swamp, a small valley swamp (6 km long with a maximum width of \( \approx 300 \) m, Fig. 2) inside Kibale National Park (KNP), and the much larger Kiaragura Swamp (17 km long with a maximum width of \( \approx 1 \) km, Fig. 2) outside the park.

2.1. Physicochemical characteristics of papyrus swamps

The physicochemical characteristics of permanent swamps are strongly influenced by seasonal rainfall patterns and the growth characteristics of papyrus (Beadle, 1974; Chapman et al., 2001). Equatorial regions of East Africa have two relatively distinct rainy seasons and two dry seasons. Near Lake Victoria, rainfall ranges from 1100 to 1600 mm per year (Kizza et al., 2009); near the valley swamps in and around Kibale National Park (KNP), and the much larger Kiaragura Swamp (17 km long with a maximum width of \( \approx 1 \) km, Fig. 2) outside the park.

Papyrus, a rhizomatous plant, grows to 5 m tall, forming a dense canopy that intercepts up to 90% of the incident light. Water flow rates in papyrus swamps are very low because the canopy blocks wind and the rhizomatous structures reduce water flow. Decaying plant material forms a layer of peat that ranges from 2–3 m deep in lacustrine swamps to 20–30 m deep in valley swamps (Beadle, 1974). High rates of organic matter decomposition produce methane (60%), carbon dioxide (30%) and hydrogen (H₂, 10%) gasses, with carbon monoxide, ethylene, and hydrogen sulfide produced in very small quantities (Chapman et al., 2001 and references therein). Swamp waters tend to have a pH of 6–6.5 while nearby lake or river water has pH closer to 8 (Milburn and Beadle, 1960; Chapman et al., 2001).

The most striking characteristic of the papyrus swamps is the very low DO, which results from high decomposition, low water flow rates, and negligible photosynthesis (Beadle, 1974). In lacustrine swamps around Lake Victoria, DO levels ranged from 0.1 mg L⁻¹ near the shoreline to an average of 2.5 mg L⁻¹ near the open water borders (Carter, 1955; Beadle, 1974; Chapman et al., 2001). In the Rwembaita Swamp in Kibale National Park, DO levels have been recorded since the early 1990s. The Rwembaita Swamp is fed by four major first order streams and drains into the Njuguta River, a tributary of the Mpanga River system, which in turn feeds into Lake George. The tributary streams form relatively stable ecotonal gradients of DO and other physicochemical conditions that vary in location both seasonally and annually (Chapman et al., 2001). DO is very low in the swamp, with an annual average of 1.5 mg L⁻¹ over a 3-year period (1993–1996; Chapman et al., 2000).

In a more recent survey, DO in the swamp averaged 1.35 mg L⁻¹ (range = 0.29 to 4.5; Fig. 3A), very similar to the average levels reported in the mid 1990s and much lower than that of the inflowing Mikana Stream. The swamp outflow tributary flows for 100 m as a forest stream before it joins the Njuguta River, picking up DO as it flows over a rocky substrate (average DO = 4.3 mg L⁻¹). DO in the open waters of the

Fig. 2. Map illustrating the locations of Rwembaita Swamp and Kiaragura Swamp and their respective rivers. Rwembaita Swamp is located in Kibale National Park, Uganda; Kiaragura Swamp is located in the Mpanga River system, outside the park.

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Njuguta River decline during the rainy period when the swamp outflow mixes with the river water (e.g., Julian Day 600–700, Fig. 3A).

DO in the interior of larger valley swamps can be even lower. In the newly characterized Kiaragura Swamp, an enormous valley swamp, annual DO averaged 0.29 mg L\(^{-1}\) at the Kantembwe (swamp) sampling site (Fig. 3B), with low seasonal variation and cool water temperatures (average 19.6 °C). The chronic hypoxia that characterizes this site likely reflects the extent of the swamp, with sites in the dense interior largely isolated from tributary influence. As in the Rwembaita system, swamp water flushes into the main river (Kahunge site) during the wet season, dropping DO levels in the river to well below saturation.

Conductivity and pH data are illustrated in Supplemental Fig. 1 (Rwembaita Swamp system) and Supplemental Fig. 2 (Kiaragura system). Conductivity varies by season and is lower in the swamps than in the outflowing rivers. pH in the two systems agrees well with the natural and anthropogenic gradients in DO level and other limnological variables in these systems. Most of these water-breathing organisms are small, many rely on diffusion for respiration, and they are from taxa in which hypoxia tolerance has been reported. For example, the ciliate Blepharisma undulans survived three days in anoxic conditions (Beadle and Nilsson, 1959) and a nematode (Dorylaimus sp.) survived anoxia for 86 days (Banage, 1966). Members of three invertebrate taxa (Chironomidae, Hirudinea, Oligochaeta) that are present in low abundance in Rwembaita Swamp rely upon gas diffusion across their body surface (Chapman et al., 2004) and reported for the lacustrine swamps in the Victoria basin (Beadle, 1974).

There appears to be a lower diversity of water-breathing invertebrates and protists in papyrus swamps, consistent with other hypoxic habitats. Most of these water-breathing organisms are small, many rely on diffusion for respiration, and they are from taxa in which hypoxia tolerance has been reported.

Air-breathing invertebrates are present in high diversity and abundance in papyrus swamps (Beadle, 1974). Snails that fill their mantle cavities with air (e.g., Pila ovata and Biomphalaria sp.) are abundant in lacustrine (Beadle, 1974) and valley (<38% of fauna; Chapman et al., 2004) swamps in which conductivity is relatively high. Some of these snails estimate in the mud during the dry seasons (Beadle, 1974). The swamp worm (Alma emini), found in lacustrine swamps, can survive in anoxic water for several weeks (Maina and Maloiy, 1998 and references therein) and uses a highly vascularized, flattened fold of tissue near its anus as a tube to trap air bubbles (Beadle, 1957, 1974). Some air-breathing insects use specialized structures that allow them to access atmospheric air; these include the water scorpions (Nepidae) that are very abundant in the Rwembaita Swamp (Chapman et al., 2004) and several types of beetles (Coleoptera) and true bugs (Hemiptera) present in low abundance in Rwembaita Swamp (Chapman et al., 2004) and reported for the lacustrine swamps in the Victoria basin (Beadle, 1974).

2.2. Faunal assemblages and strategies for hypoxia tolerance: Invertebrates

The faunal assemblages of lacustrine and valley swamps are not as diverse as those of the better-oxygenated ecotonal habitats (Chapman et al., 1996a; 1996b; Reid, 2012), reflecting, in part, the strong effect of hypoxia on diversity (Chapman et al., 2001; Levin et al., 2009).

In the Rwembaita Swamp system, DO level was a significant predictor of the relative abundance of several categories of water and air breathing macroinvertebrates, but not of those that rely on diffusion across their body surfaces (Osborne et al., 2001; Chapman et al., 2004). Here we focus on the macroscopic fauna that utilize aerobic metabolism and that are in substantial contact with swamp water, since these are the species with which one could test the predictions of small size, increased fitness, and reduced oxidative stress in individuals living in chronic hypoxia. Table 1 lists the invertebrates and fish inhabiting papyrus swamps in East Africa. Where possible, we identify the organism as a water breather or air breather or ‘mixed development’, which indicates that respiratory strategies differ across life stages.

2.3. Faunal assemblages and strategies for hypoxia tolerance: Fishes

Natural and anthropogenic gradients in DO level and other limnological variables influence fish assemblages in papyrus swamp systems. This is most apparent in the lacustrine swamps around Lake Victoria and its satellite lakes (Fig. 1), in which wetlands adjacent to population centers have rapidly degrading environmental conditions (decreased DO and pH) and lower fish species diversity and richness than do relatively unimpacted wetlands (Naigaga et al., 2011). On a slightly longer time-scale (from the 1950s), the DO gradients from open lake to swamp have formed a barrier to further invasion by the introduced Nile perch (Lates niloticus), enabling some endemic species to find refuge from predation in the lacustrine swamps (Chapman et al., 1996b).
A series of wetland lagoons created behind the fringing papyrus swamp of Lake Victoria (near Jinja, Uganda) during the high Lake Nabugabo, we refer to organisms found in the papyrus choked Juma River, the main tributary of the lake. Note that Chapman et al. (2001) also cites Welcomme (1970) for a

Table 1

<table>
<thead>
<tr>
<th>Order (invertebrates), Family (vertebrates)</th>
<th>Species (if known), or family</th>
<th>Location</th>
<th>Respiratory strategy</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterotrichia (Ciliophora)</td>
<td>Blepharisma undulans</td>
<td>Victoria</td>
<td>Water breather</td>
<td>Beadle and Nilsson (1959)</td>
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<td>Dorylainida (Nematoda)</td>
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<td>Haplotaxida (Oligochaeta)</td>
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<td>Hemiptera</td>
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<td>Coleoptera</td>
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<td>Ephemeroptera</td>
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<td>Architaenioglossa (Gastropoda)</td>
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<td>Veneroida (Bivalvia)</td>
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<td>Decapoda</td>
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<td>Diplodocera (Cladocera)</td>
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<td>Cyclopoida (Copepoda)</td>
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<td>Ostracoda</td>
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<td>Characidae</td>
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<td>Mochokidae</td>
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Schofield and Chapman, 1999; Chapman et al., 2002). Our description of fish assemblages is restricted to lacustrine and valley swamps in which papyrus is the dominant plant and swamps in which papyrus is mixed with *Phragmites mauritianus* (Yala Swamp; Aloo, 2003). For review of fish communities assemblages is restricted to lacustrine and valley swamps in which papyrus is mixed with *Phragmites mauritianus* (Yala Swamp; Aloo, 2003). For review of fish assemblages in nearby, well-oxygenated rivers, see Beadle (1974), Kaufman et al. (1997) and Chapman et al. (2001).

Air-breathing fishes are widespread and present in high biomass in East African papyrus swamps (Table 1). The catfish *Clarias liceophalus* is abundant in Rwembaita Swamp, where its habitat usage is influenced by water depth (presumably to minimize risk of aerial predation) and not by DO level (Chapman, 1995). Some species, like the lungfish *Protopterus aethiopicus* (Chapman and Hulen, 2001). For example, *P. multicolor* from hypoxic swamps are characterized by larger gills and larger heads to accommodate larger gills (Chapman et al., 2008; Crispo and Chapman, 2010, 2011; Friesen et al., 2012). These fish also exhibit behavioral traits similar to the water-breathing invertebrates (Apodaca and Chapman, 2004b), including an extensive use of aquatic surface respiration (ASR). The habitat use patterns of *P. neumayeri* (the only other fish in Rwembaita Swamp besides the catfish) are strongly influenced by DO level, with distribution in the dry season limited to pools and channels with extensive water surface area (Chapman and Liem, 1995).

While there is substantial evidence for traits that maximize gas diffusion in water-breathing fish in papyrus swamps, the degree to which the prediction of smaller body size applies to water-breathing or air-breathing fish in the papyrus swamps needs further study. However, some patterns are emerging. The largest fish found in papyrus swamps are air breathers like the lungfish and clariid catfishes. In general, water-breathing fishes in the dense interior of papyrus swamps tend to be the smaller species of cyprinids, killifishes, and cichlids. Larger cichlids and cyprinids (e.g., *Labeo* species) are more restricted to high DO environments as adults. In the field, offspring from the maternal mouth brooder *P. multicolor* are smaller (eggs, embryos, and juveniles) than in well-oxygenated waters and for adults, the size at maturity of females is smaller under hypoxia (e.g., Reardon and Chapman, 2009, 2012).

### Table 1 (continued)

<table>
<thead>
<tr>
<th>Order (invertebrates), Family (vertebrates)</th>
<th>Species (if known), or family</th>
<th>Location</th>
<th>Respiratory strategy</th>
<th>Reference</th>
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<td><strong>Fish</strong></td>
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<tr>
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<td>Kajagura</td>
<td>Water breather</td>
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* Mixed devel. indicates that respiratory strategy (water versus air-breathing) differs across life stages.
* The reference list is not intended to be exhaustive.

**3. Fingernail clams in a hypoxic habitat have low stress and high fitness**

Fingernail clams (*Sphaerium sp.*) were the dominant water-breathing macroinvertebrate in Rwembaita Swamp but were virtually
absent from stream and river sites in which the DO was 6 mg L\(^{-1}\) or higher and pH was above 7 \(\text{Osborne et al., 2001; Chapman et al., 2004}\). The high clam abundance in the swamp was not surprising since sphaeriids are hypoxia tolerant \(\text{Waite and Neufeld, 1977; Dussart, 1979; Mackie, 1983; Holopainen and Penttinen, 1993; Mackie, 2007; Hornbach et al., 1983; McKee and Mackie, 1981; Hornbach, 1985}\); however, the lack of clams in most stream and river sites was surprising because fingernail clams live in most aquatic habitats, from ephemeral ponds to fast-flowing rivers \(\text{Guralnick, 2005; Mackie, 2007; Funk and Reckendorfer, 2008}\) and the limnological characteristics of the stream and river sites \(\text{higher DO, pH and conductivity, etc.}\) are generally considered beneficial for fingernail clams \(\text{for review, Mackie, 2007}\). However, there was no apparent competitor or predator of clams in stream sites \(\text{Chapman et al., 2004}\) and it is unlikely that resource availability restricts the clams to the swamp \(\text{LJ Chapman, unpublished data}\) or that they are unable to disperse to upstream or downstream sites \(\text{for review of dispersal, Mackie, 2007}\). Since the broad-scale surveys suggested that clams were most abundant in the chronically hypoxic swamp, we conducted two studies, one focusing on fitness and one on stress physiology, to test whether these small clams \((\leq 11\text{ mm shell length})\) gain a fitness benefit from living in sustained hypoxia.

### 3.1. Abundance and reproductive success of clams in hypoxic sites

We conducted a fine-scale survey of clam abundance and limnology along Inlet Stream West (overview in Fig. 2, stations in Fig. 4A), a tributary of the Rwembaita Swamp \(\text{Joyner-Matos et al., 2007}\). DO, water transparency and pH increased along the transect from the swamp sites to the stream; water flow rate, temperature and conductivity did not vary across the sites. Consistent with the broad-scale surveys, clam abundance (catch per unit effort, CPUE) was significantly and negatively associated with DO \(\text{Fig. 4B}\). We confirmed that this relationship between DO and clam CPUE was consistent year-round by measuring clam CPUE and limnological variables twice monthly for 18 months at sites 1 (margin), 6 (ecotone), and 10 (stream) and at one site in the “interior” of the swamp \(\text{Fig. 4C and in Joyner-Matos et al., 2011}\).

Fingernail clams are ovoviviparous, internally brooding larvae in specialized gill pouches \(\text{Meier-Brook, 1977; Mackie, 1978}\) and releasing shelled juveniles that tend to settle close to the parent \(\text{Fig. 5}\). We used two measures of clam fitness \(\text{e.g., Kilgour and Mackie, 1990, 1991}\), population abundance and brood size, to test whether clam fitness varied along the transect \(\text{Joyner-Matos et al., 2011}\). The sizes of the shelled juveniles \((1–4\text{ mm})\) overlapped with the smallest size classes we observed in the dry season survey \(\text{Joyner-Matos et al., 2007}\), confirming that juveniles are abundant at swamp and ecotone sites and that clams live in hypoxia for their entire life cycle. Clams living in hypoxia \((<2\text{ mg L}\^{-1}\) \(\text{exhibited the highest reproductive output over a 12-}\) month period, as indicated by the proportion of clams actively brooding and the number of juveniles per brood \(\text{Fig. 6}\). We collected very few adult clams from the edge of the population’s distribution, the stream site: when this site was removed from the analysis, both brooding metrics were significantly higher in clams from the dense interior of the swamp than in clams from the ecotone.

The sizes of the adult clams \(\text{Joyner-Matos et al., 2007, 2011}\) and brooded larvae \(\text{Joyner-Matos et al., 2011}\) were consistent with the sphaeriid species in North America, in which average shell lengths range from 6 to 12.5 mm \(\text{adults}\) and 1.5–6 mm \(\text{larvae}\) across species \(\text{for review, Mackie, 2007}\). Adult size can vary considerably, even within a species, because sphaeriids tend to have wide spatial distributions, encompassing different latitudes and/or altitudes and because growth is dependent upon temperature, DO, food availability and reproductive status \(\text{Mackie, 2007}\). However, within the swamp–stream system, adult clams from the chronically hypoxic swamp interior were significantly larger than those from the swamp margin or the ecotone sites \(\text{Joyner-Matos et al., 2011}\). This pattern is opposite to that observed in the fishes in the papyrus swamps and may reflect the relatively low metabolical demands of fingernail clams \(\text{for review of scaling of fingernail clam metabolic rate with body size, Hornbach, 1985; for review of metabolic scaling with DO, Mackie, 2007}\). The smaller size of the fingernail clams in the higher DO sites may reflect the stress associated with living at the edge of the optimal habitat \(\text{see below}\). Although the intraspecific size pattern observed in the Rwembaita Swamp system is not consistent with the potential benefits of small size under hypoxia, interspecific trends within Bivalvia do provide some support, in that the fingernail clams \(\text{Sphaerium and Musculium}\) and related “pea clams” \(\text{Pisidium}\) are distinct from other freshwater bivalves on the basis of their small size and prevalence in hypoxic and/or ephemeral habitats \(\text{for review, Mackie, 2007; Cummings and Graf, 2010}\).

### 3.2. Lower oxidative stress in clams in hypoxic sites

To test whether clams along the transect exhibited different levels of steady-state oxidative stress, we measured stress protein expression...
over the 18 months; DO levels in the stream site were 6.2 ± 0.2 mg L

ences with all sites present in the analysis.

ences with all sites present in the analysis.

rative damage in clams living in the swamp interior (Fig. 7B).

duced (B). Data are presented as median with 25th

4. Conclusions

The aquatic organisms in the interior portions of papyrus swamps throughout East Africa live in sustained hypoxia and hypercapnia. Since these organisms are protected from cycles of reoxygenation (even during the rainy season, DO is less than the lethal levels reported in 90% of the experiments reviewed in Vaquer-Sunyer and Duarte, 2008), they likely do not experience stress like that documented in organisms that migrate daily through the OMZ (for review, Tremblay et al., 2012) or that live in intertidal habitats (for review, Freire et al., 2012). These are, therefore, ideal habitats in which to test whether organisms in chronic hypoxia have smaller body size, maximized diffusion abilities, minimal metabolic costs of regulating O2 delivery, and minimized oxidative stress. Unfortunately, all components of this prediction have not been tested in a single species or even in a single taxon. The water-breathing fishes in these habitats show far-reaching adaptations to hypoxia (Chapman et al., 2002) that are consistent with the prediction. These include enlarged gills and shorter diffusion differences, higher hematocrit and (in some) smaller body size. To our knowledge, oxidative stress has not been explored in fish from valley or lacustrine swamp systems.

Although the clam oxidative stress and fitness data sets were collected independently, when considered together (with caution) they illustrate a potential interaction between the DO gradient, cellular-level stress, and fitness. Clams in the swamp interior site (DO < 2 mg L⁻¹) were larger and had lower stress protein expression levels and oxidative damage and the highest reproductive success. These results indicate that stably hypoxic conditions are beneficial for this clam population. Clams near the swamp margin (DO 2–3 mg L⁻¹) had higher stress protein expression and oxidative damage and slightly lower reproductive success. In the ecotone (DO 3–5 mg L⁻¹), stress protein

Fig. 6. Reproductive success of fingernail clams (Sphaerium sp.) from four sites along a swamp–stream transect in Rwembaита Swamp in Kibale National Park, Uganda. Data are the proportion of clams within each collection that were brooding (A) and the brood sizes (B). Dissolved O2 (DO) levels in the swamp interior site averaged 0.904 ± 0.13 mg L⁻¹ (mean ± SEM) over the 18 months; DO levels in the stream site were 6.2 ± 0.2 mg L⁻¹. Sample sizes are the number of sampling events at which adult clams were found (A) and the number of clams dissected (B). Data are presented as median with 25th–75th percentile (box), 10th–90th percentile (whiskers) and outliers (dots); different letters indicate statistically significant differences with all sites present in the analysis. Modified from Joyner-Matos et al. (2011).

Please cite this article as: Joyner-Matos, J., Chapman, L.J., Persisting in papyrus: Size, oxidative stress, and fitness in freshwater organisms adapted to sustained hypoxia, Comp. Biochem. Physiol. A (2013), http://dx.doi.org/10.1016/j.cbpa.2013.03.032
Fig. 7. Factor 1 scores for a principal components analysis of stress protein expression and levels of oxidatively damaged deoxyguanosine (dGuo) and guanosine (Guo) bases in fingernail clams (Sphaerium sp.) collected from four sites in the Rwembaita Swamp–stream system in Kibale National Park. The four collection sites range in dissolved O2 (DO) level. The factor 1 score contained four stress proteins with factor loading scores greater than 0.7: glutathione peroxidase, heat shock protein 60, heat shock protein 70, and manganese superoxide dismutase. Data are presented as median with 25th–75th percentile (box), 10th–90th percentile (whiskers) and outliers (dots). Different letters indicate statistically significant differences. Data in (B) are the proportions of oxidized bases per 10^6 undamaged bases; different letters indicate statistically significant differences. Both graphs are modified from Joyner-Matos et al. (2007).

expression and oxidative damage were elevated (relative to the swamp interior), and reproductive success was lower and more variable. We interpret these patterns as indicating that clams that experience elevated DO levels maintain high stress protein expression levels (but experience higher oxidative damage) at the cost of reduced fitness (and abundance and body size). Finally, the clams living at the extreme distribution edge had low stress protein expression, high oxidative damage, intermittent reproductive success, and very low abundance. If this clam population is adapted to sustained hypoxia, then the clams at the distribution edge may be so stressed that they cannot maintain stress protein expression (e.g., Werner and Hinton, 1999) and perhaps are investing metabolic resources in periodic bursts of reproduction rather than in sustained cellular protection (for review of trade-offs with reproduction, see Speakman, 2008).

The apparent cellular-level benefits of sustained hypoxia demonstrated in this clam population are similar to those proposed for other small organisms (e.g., Gray et al., 2004). However, it is difficult to compare patterns of stress protein expression in the clams with those of other hypoxia-tolerant organisms like the carp, goldfish, garter snake and leopard frog (for review, Hermes-Lima and Zenteno-Savín, 2002; Bickler and Buck, 2007), because these other species eventually experience reoxygenation. These organisms tend to either constitutively express high levels of stress proteins (despite an overall metabolic depression) or upregulate the stress proteins immediately before or after reoxygenation occurs. The inverse relationship between DO and fingernail clam fitness suggests that living in sustained hypoxia in the papyrus swamps allows the clams to allocate greater metabolic resources to brooding, perhaps because they are not maintaining large pools of stress proteins and/or they are not using energy to limit O2 supply to their tissues (e.g., Massabau, 2001; Abele et al., 2007).

The macroinvertebrates and fishes in the valley and lacustrine swamps of East Africa merit further study, including tests of whether they utilize aerobic or anaerobic metabolism (or a mixture) and whether they exhibit the morphological and life history traits predicted to increase success in hypoxic habitats. Since permanently hypoxic (and hypercapnic) habitats are rare and relatively difficult to access, the papyrus swamps in East Africa provide a unique opportunity to challenge the conventional classification of hypoxic/hypercapnic habitats as ‘stressful’ and normoxic habitats as ‘optimal.’ Unfortunately, these habitats, particularly the lacustrine swamps, are being rapidly degraded by conversion to agricultural use and by wastewater discharged from extensive animal, industrial and municipal sources (Chapman et al., 2001; Aloo, 2003; NEMA, 2009; UNEP, 2009; Naigaga et al., 2011). Nonetheless, there is increasing recognition of the value of the swamps as cradles of specialized biodiversity, as important nursery areas and refugia for fishes important to food security, and in providing ecosystem services for human populations that densely populate the waterways of East Africa. The role of swamps as model systems for understanding the pros and cons of hypoxia is only now gaining recognition, but it is clear that these “extreme” environments harbor important opportunities for future investigations.

Acknowledgments

We thank David Julian for suggesting that we re-think our classification of hypoxic habitats as stressful and we thank our two anonymous reviewers for their very helpful suggestions. This paper was presented as a portion of the Climate Regions and Special Habitats session of the First International Congress on Oxidative Stress in Aquatic Ecosystems, which was held in Cabo San Lucas, Mexico in November, 2012. This conference was supported by the generosity of the Centro de Investigaciones Biologicas del Noroeste (CIBNOR), The Company of Biologists, The Academy of Sciences for the Developing World (TWAS), and CaboDolphins. We thank Heidi Richardson and Tammy Sambeli for photography and Carole Verdone-Smith and Mery L. Martinez for assistance with the illustrations. JJM was supported by funds from the Department of Biology at Eastern Washington University. LJC acknowledges support from the Canada Research Chairs program and NSERC (National Sciences and Engineering Research Council of Canada) during the writing phase of this project. We thank the field assistants of the Kibale Fish Project for the dedication to the long-term limnological data collections and contributions to the fish and clam studies in the Rwembaita System over the years.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.cbpa.2013.03.032.

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