Claireaux *et al.*, 2013) species. This HT variance and its implications for tness are also contextually dependent upon the physiology, behaviour and environment of the shes (Richards, 2011). For example, La Pointe *et al.* (2014) show that striped bass *Morone sa atilis* (Walbaum 1792), infected with a common Chesapeake Bay bacterium, have a higher critical oxygen tension and greater loss of aerobic scope than uninfected conspeci cs. The co-familiar European sea bass *Dicentrarchus labra* (L. 1758) showed disorientation and reduced ability to respond to stimuli under moderate hypoxia [50% air saturation (AS)] , which would affect predator and prey interactions (Lefrancois & Domenici, 2006). Furthermore, *D. labra* were also shown to increase their risk-taking behaviour under hypoxic conditions in a manner that was dependent on individual RMR (Killen *et al.,* 2011). Thus, differential survival (mortality selection) and the ability to carry on routine biological functions leading to fecundity (*e.g.* growth) in waters that experience hypoxia may depend on relative HT.

HT has generally been tested under minimal ow conditions, either with large groups of sh together or with an individual sh in a respirometer or sh box (Robb & Abrahams, 2003; Farwell *et al.*, 2007; McKenzie *et al.*, 2008; Claireaux *et al.*, 2013). Such experiments usually result in a wide range of HTs for individuals of similar size and from the same population (Miller *et al.*, 2002; Davies *et al.*, 2011; Killen *et al.*, 2011; Claireaux *et al.*, 2013). Loss of equilibrium (LOE) is one commonly used endpoint in these tests, which, if done carefully, are not lethal, allowing multiple tests on the same individual. While this type of trial may accurately assess HT in relatively inactive, demersal or small species, pelagic schooling species such as *M. sa atilis* are more likely to encounter hypoxia while moving through hypoxic layers or trying to escape advancing hypoxic zones (Rice *et al.*, 2013).

Juvenile *M. sa atilis* are commonly found in hypolimnetic regions of Chesapeake Bay, an important nursery ground for the entire Atlantic stock (Waldman *et al.*, 1997), where hypoxic zones occur annually from April to October (Hagy *et al.*, 2004; Kemp *et al.*, 2005). Thermal strati cation, combined with salinity gradients, creates a hypolimnion that is isolated from atmospheric oxygen by a turbid epilimnion for much of this time. Density differences between the two layers create a stable pycnocline that limits mixing and oxygenation. By combining this with minimal hypolimnetic photosynthetic oxygen production and organic matter fallout from the epilimnion, net oxygen depletion can occur in the hypolimnion. Hypoxia created thus in estuarine environments has dramatically increased in recent years as a result of cultural eutrophication (Diaz & Breitburg, 2009). These hypoxic zones are not static and can be driven suddenly into normoxic waters by winds (seiches) and tidal currents (Breitburg, 1990), overwhelming a rst line of hypoxia defence, behavioural avoidance, leading to the death of sh (Rice *et al.,* 2013).

Due to the commercial and recreational importance of *M. sa atilis*, it is imperative to have a realistic understanding of this species' ability to cope with and respond to hypoxia, as hypoxic regions are predicted to expand with climate change (Keeling *et al.*, 2010). While studies of how hypoxia in uences swimming performance are legion and have been performed on many sh species (Chapman & McKenzie, 2009; Domenici *et al.*, 2012), studies of how HT is in uenced by swimming are almost unknown (McKenzie *et al.*, 2007). Here, hypoxia challenge tests were performed on the same individuals under two ow regimes in Brett-type swim tunnels (Nelson, 1989) to test the hypothesis that individual *M. sa atilis* have identical HT regardless of swimming activity.

A total of 13 juvenile *M. sa atilis*, 123–183 mm total length (L_T), were collected by the Maryland Department of Natural Resources trawl survey from the main channel of the Chesapeake Bay and transported to Towson University in Chesapeake Bay water at 4° C. Fish were brought to the experimental temperature of $20{\cdot}1 \pm 1{\cdot}0$° C

decreased to 10% AS as an exponential function with an average instantaneous slope of $3{\cdot}12 \pm 0{\cdot}38\%$ AS min^{-1}. If an animal did not lose equilibrium after 4 h at 10% AS, the oxygen concentration was lowered further by 2% AS every hour until they did. Two galvanic oxygen-sensing probes were used to determine the level of AS in the swim tunnel (one anterior and one posterior to the swimming section). The probes were calibrated before each trial. One probe was connected through a digital converter box to a solenoid valve attached to an air stone, which maintained dissolved oxygen saturation at the desired level (Oxy-Reg System, Loligo Systems; www.loligosystems.com). HT was recorded as cumulative oxygen de cit (D_{CO}). If oxygen concentration is plotted as a function of time, D_{CO} is the difference between the area under the curves of a hypothetical animal remaining at 100% AS throughout the experiment and the experimental animal's actual oxygen exposure until the time that it lost equilibrium (LOE). D_{CO} is recorded in the units of per cent times minutes and included the initial period of reduction to 10% AS. For example, a theoretical animal that lost equilibrium at exactly 4 h at 10% AS after a 30 min reduction to 10% AS during which the experimental animal had an oxygen de cit of 1350% times minutes (difference between 100% saturation and the area under the exponential reduction curve) would have a D_{CO} of: D_{CO} $((30T \times 100\%X) - 350\%T) + (240T \times 100\%X) - (240T \times 10\%X) = 23\,250\%T$, where T is time in min and X is AS.

Immediately as an animal lost equilibrium, it was removed, measured, weighed and transferred to a recovery tank at 100% AS. Trial order for both tests was randomly determined, but a minimum separation time between trials for an individual of at least 2 weeks was adopted. The mean time between trials for each individual was 8·4 weeks.

All statistical analyses were conducted with an α level of 0·05 in SPSS (www.01.ibm.com/software/analytics/spss) or Statistica 5.0 (www.statsoft.com). The D_{CO} values

resting *M. sa atilis*. If *M. sa atilis* are only oxyregulating while swimming, this coupled with the increased oxygen demand while swimming would account for the much more rapid advancement to equilibrium loss. The results are also predictable from the 'limiting oxygen concentration' modelling of Claireaux & Lagardere (1999) for juvenile *D. labra* . This reduced HT while swimming is not necessarily an intuitive result. LOE from at least one other environmental stressor (heat) is unaffected by swimming activity in *M. sa atilis*. The critical thermal maximum (CT_{max}) of 11 similar-sized *M. sa atilis* was not affected by swimming at 50% of U_{crit}, despite the fact that dissolved oxygen was also decreasing with the rising temperature of the CT_{max} test (J. A. Nelson, unpubl. data).

An important nding of this study was that there was no relationship between an individual's HT in a minimal ow environment and while swimming (insigni cant rank order correlation; Spearman $P > 0{\cdot}05$), which suggests different mechanisms whereby equilibrium is lost under the two conditions. This relates well to the different metabolic scaling coef cients seen between resting and exercising animals (Darveau *et al.*, 2002; Glazier, 2009). Because different metabolic processes such as protein turnover and Na^+/K^+ ATPase activity are the dominant consumers of energy at rest, and processes such as myosin and Ca^{++} ATPases dominate during exercise, there is no *a priori* reason to expect an animal to have equal relative HT at each of the activity levels. As reduction in oxygen consumption can be observed in hypoxic shes both at rest (Speers-Roesch *et al.*, 2010) and during exercise (Fu *et al.*, 2011), there may be further intraspeci c variation in how they selectively arrest metabolic processes and make cardiovascular adjustments, producing further shuf ing of their relative HTs (*i.e*. a hypoxia response−swimming interaction term) at different levels of hypoxia and exercise. Supporting this idea, Urbina & Glover (2013) showed a change in the aerobic metabolic scaling coef cient in a large number and size range of an oxyconforming galaxid under hypoxia as the animals differentially transitioned into anaerobic metabolism to meet their energy needs.

HT in shes is a complex function of an animal's ability to extract oxygen from the environment and supply it to key tissues, control aerobic metabolic rate and to recruit anaerobic metabolism. Thus, in various studies, HT has been found to be related to resting metabolic rate (Claireaux & Lagardere, 1999; McKenzie *et al.*, 2008), ability to depress aerobic metabolism (Corkum & Gamperl, 2009) and recruit anaerobic metabolism (Almeida-Val *et al.*, 2000). Marras *et al.* (2010) showed that a cohort of *D. labra* undergoing repetitive constant acceleration tests had much larger variation in their anaerobic capacity than in their aerobic capacity. Whether that result applies to the larger variability of *M. sa atilis* HT while swimming is material for future study.

Size did not affect HT under either ow condition. These results conform to Chittenden (1971) who found no effect of sh size on oxygen levels at LOE or death in *M. sa atilis*. Although various studies have reported divergent effects of body size on HT, Nilsson & Ostlund-Nilsson (2008) reviewed the subject and concluded that there is no scaling of HT until sh transition to anaerobic metabolism, when larger sh would have an advantage due to their lower mass-speci c metabolic rate and ability to store fermentable substrates.

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References

Almeida-Val, V. M. F., Val, A. L., Duncan, W. P., Souza, F. C. A., Paula-Silva, M. N. & Land, S. (2000). Scaling effects on hypoxia tolerance in the Amazon sh *Astronotus ocellatus*

Pihl, L., Baden, S. P. & Diaz, R. J. (1991). Effects of periodic hypoxia on distribution of demersal sh and crustaceans. *Marine Biolog* **108,** 349–360.