# Behavioral responses of juvenile weakfish (*Cynoscion regalis*) to diel-cycling hypoxia: swimming speed, angular correlation, expected displacement, and effects of hypoxia acclimation

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**Abstract:** Areas of low flushing and high nutrient loading in shallow estuaries are susceptible to diel-cycling hypoxia and also represent important nursery habitat for juvenile estuary-dependent fishes. Swimming speed, angular correlation, and expected displacement were measured in juvenile weakfish (*Cynoscion regalis*) in response to diel-cycling hypoxia (cycling between 7.0 and 0.4 mg  $O_2 \cdot L^{-1}$ ). Saturation-acclimated (7.0 mg  $O_2 \cdot L^{-1}$ ) weakfish exhibited an active response by increasing swimming speed (to a maximum at 2.8 mg  $O_2 \cdot L^{-1}$ ) and angular correlation (to a maximum at 1.4 mg  $O_2 \cdot L^{-1}$ ) as dissolved oxygen (DO) decreased, after which weakfish exhibited a passive response and both swimming speed and angular correlation decreased by ~50% and 70%, respectively, at 0.4 mg  $O_2 \cdot L^{-1}$ . Weakfish acclimated to hypoxia (cycling between 2.0 and 11.0 mg  $O_2 \cdot L^{-1}$  for 10 days) did not vary swimming speed during decreasing DO or DO recovery (increasing DO) and had an overall swimming speed 46% lower than saturation-acclimated weakfish at 7.0 mg  $O_2 \cdot L^{-1}$ . At the end of DO recovery, saturation- and hypoxia-acclimated weakfish had recovered 60% and 80% of their initial swimming speeds, respectively. The relationship between previous hypoxia exposure and behavior may be an important determinant of habitat utilization in estuarine nursery areas impacted by diel-cycling hypoxia.

**Résumé :** Les zones de vidange réduite et d'apports importants de nutriments dans les estuaires peu profonds sont sujettes à une hypoxie à cycle journalier; ils représentent aussi des habitats de nourricerie précieux pour les jeunes chez les poissons associés aux estuaires. Nous avons mesuré la vitesse de nage, la corrélation angulaire et le déplacement attendu chez des jeunes acoupas royaux (*Cynoscion regalis*) en réaction à une hypoxie à cycle journalier (variant de 7,0 à 0,4 O<sub>2</sub>·L<sup>-1</sup>). Des acoupas acclimatés à la saturation (7,0 O<sub>2</sub>·L<sup>-1</sup>) réagissent de manière active en augmentant leur vitesse de nage (jusqu'à un maximum à 2,8 O<sub>2</sub>·L<sup>-1</sup>) et leur corrélation angulaire (jusqu'à un maximum à 1,4 O<sub>2</sub>·L<sup>-1</sup>) à mesure que l'oxygène dissous (OD) décroît, pour ensuite réagir passivement; la vitesse de nage et la corrélation angulaire décroissent alors respectivement de ~50 % et de 70 % à 0,4 O<sub>2</sub>·L<sup>-1</sup>. Les acoupas acclimatés à l'hypoxie (variant de 2,0 et 11,0 mg O<sub>2</sub>·L<sup>-1</sup>) pendant 10 jours) ne changent pas leur vitesse de nage durant la décroissance d'OD et la récupération d'OD (augmentation d'OD) et ont une vitesse globale de nage de 46 % inférieure à celle des acoupas acclimatés à la saturation à 7,0 mg O<sub>2</sub>·L<sup>-1</sup>. À la fin du rétablissement d'OD, les acoupas acclimatés à la saturation et à l'hypoxie avaient récupéré respectivement 60 % et 80 % de leur vitesse initiale de nage. La relation entre l'exposition antérieure à l'hypoxie et le comportement peut être un facteur déterminant important de l'utilisation des zones estuariennes de nourricerie affectées par des cycles journaliers d'hypoxie.

[Traduit par la Rédaction]

## Introduction

Estuaries provide nursery habitat for many economically and ecologically important fishes (Weinstein 1979; Able 1999; Minello et al. 2003). Many studies have emphasized the importance of tidal headwaters as nursery areas for estu-

Received 10 April 2008. Accepted 5 December 2008. Published on the NRC Research Press Web site at cjfas.nrc.ca on 21 February 2009. J20505

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<sup>1</sup>Corresponding author (e-mail: damian@udel.edu). <sup>2</sup>Present address: Department of Civil and Environmental Engineering, University of Delaware, 301 DuPont Hall, Newark, DE 19716, USA. ary-dependent fishes (Weinstein 1979; Ross 2003; Meng et al. 2004). These environments provide high productivity (Deegan 2002) and a possible refuge from predators (Ross 2003; Manderson et al. 2004), but they are also increasingly afflicted with diel-cycling hypoxia because they have longer hydraulic residence times and higher surface area to volume ratios (Mallin and Lewitus 2004) and are closer to freshwater-derived nutrient sources than open estuary areas (D'Avanzo and Kremer 1994; Tyler et al. 2009). Diel-cycling hypoxia is driven primarily by the 24 h cycle of net daytime dissolved oxygen (DO) production by algae and nighttime respiration by all biota.

Hypoxia can induce a variety of behaviors in fishes, depending on physiological adaptations. Kramer (1987) categorized these behavioral responses as (*i*) changes in activity level, (*ii*) changes in vertical and horizontal position, (*iii*) increased use of air breathing in bimodal breathers, and (*iv*) increased aquatic surface respiration. Changes in activity level can be further categorized as either active or passive strategies for coping with declining oxygen (Van Raaij et al. 1996). A passive strategy is characterized by a decrease in activity level to save energy, although the chance of encountering more oxygenated waters decreases. An active strategy involves increased activity, thereby increasing the chance of encountering more oxygenated water, although this strategy is more energetically expensive (Benhamou and Bovet 1989).

The relationship between these strategies is species (Herbert and Steffensen 2005, 2006), context (Sloman et al. 2006), age/size (Bejda et al. 1987), and environment specific. Some studies have reported active responses (Metcalfe and Butler 1984 (dogfish (Scyliorhinus canicula)); Schurmann and Steffensen 1994 (Atlantic cod (Gadus morhua)); Dalla Via et al. 1998 (sole (Solea solea))), whereas others have observed passive responses (Dizon 1977 (skipjack tuna (Katsuwonus pelamis) and yellowfin tuna (Thunnus albacares)); Bejda et al. 1987 (red hake (Urophycis chuss)); Shoji et al. 2005 (red sea bream (Pagrus major))). Most studies demonstrate that fish use multiple behavioral strategies over the course of low-DO exposure and that the decision to employ one strategy over another is based on energetic constraints (Herbert and Steffensen 2006). For example, Dalla Via et al. (1998) described the temporal response in activity of sole over a large range of oxygen saturation. As DO levels declined from 80% to 20% saturation, sole reduced activity. Severe hypoxia (20%-6% saturation) resulted in a switch to anaerobic metabolism and the most severe hypoxia (5% saturation) resulted in burst and panic-like swimming. In contrast, Domenici et al. (2000) observed an increase in swimming speed of Atlantic herring (Clupea harengus) during severe hypoxia (34%-15% saturation) followed by a decrease in swimming speed and eventual school disruption. Domenici et al. (2000) suggested that different swimming responses might be due to differences in hypoxia tolerance and overall activity level among species. Specifically, hypoxia-tolerant and sluggish species might primarily employ the passive strategy, whereas hypoxia-intolerant and active species might primarily use the active strategy.

Fishes that increase their swimming speed in response to decreasing DO can decrease their residence time in that environment (Turchin 1998; Benhamou 2004*a*). Fishes that respond passively to decreasing DO might save energy but do not reduce residence time. Most studies of fish movement in response to hypoxia employ path analysis to calculate residence time, and they concentrate exclusively on path length (swimming speed) and rarely determine path curvature (turning angles) (Schurmann and Steffensen 1994; Lefrancois et al. 2005). Maintaining a straight swimming path is less energetically expensive than increasing speed and the interaction of changes in swimming speed and turning angle distribution may ultimately determine the ability of fish to escape low-DO events.

Depending on energetic state (e.g., enough excess energy to avoid low-DO conditions or to risk foraging losses by emigrating from preferred habitat), inherent physiological tolerance, and previous DO exposure history, among other factors, an individual of a given species may employ different behavioral strategies. Interindividual variability in fish behavior was long thought of as statistical noise until its importance in regulating population flexibility was recognized (Magurran 1993; Gregory and Wood 1998; Kolok 1999). Maintaining high levels of interindividual variability is particularly important in spatially and temporally heterogeneous environments because a range of behavioral strategies may be needed to optimize fitness (Komers 1997). Studies that examine whether there is consistent individual variability over time and how hypoxia affects that variability will create a more detailed model of fish behavior than exclusively examining the mean response of a number of individuals.

Can. J. Fish. Aquat. Sci. Vol. 66, 2009

Diel-cycling hypoxia presents unique challenges to juvenile estuary-dependent fishes because it varies at multiple temporal and spatial scales (Tyler et al. 2009). At the within-day temporal scale, fish are faced with the choice of staying within an area as DO decreases (generally from 2 h before sunset to 2 h after sunrise) or attempting to escape. Within a season, the behavioral strategies of fish result in acclimation to diel-cycling hypoxia and (or) making daily temporary emigrations from otherwise preferred habitat. Variability in the behavior of fishes and in the spatial extent of hypoxia in these systems is the primary determinant of whether fish are exposed to lethal or growth-limiting DO levels. Regardless of estuarine dependency, juvenile fishes are under strong selective pressure to grow during the nursery seasons (Houde 1987, 1989; Sogard 1997) and this energetic constraint intensifies the consequences of their behavioral decisions. No previous studies have examined swimming behavior in response to decreasing DO in juvenile estuary-dependent fishes. Additionally, no study has examined fish responses to the diel DO cycle, from initial DO decline through DO recovery, as well as the effect of acclimation to a diel DO cycle.

The overall objective of this study was to quantitatively characterize changes in swimming speed, angular correlation, and expected displacement of juvenile weakfish (*Cynoscion regalis*), an estuary-dependent fish, in response to a complete diel cycle similar to the DO dynamics observed during mid- to late summer in the field. Additionally, some juvenile weakfish were acclimated (10 days) to a less severe diel DO cycle common in late spring to early summer to examine the impact of acclimation on swimming behavior and survival during exposure to the more extreme diel-cycling DO that occurs later in the summer. Finally, interindividual variability in swimming response to diel-cycling DO was examined in acutely exposed (saturation-acclimated) and chronically exposed (hypoxia-acclimated) juvenile weakfish.

## Materials and methods

Weakfish were collected from nursery areas in the Delaware Coastal Bays using an otter trawl. Fish were maintained in recirculating aquaria at 25 °C, 20 ppt salinity, and a 14 h light: 10 h dark photoperiod for at least 10 days before observation. Fish were fed frozen mysid shrimp (*Mysis relicta*) ad libitum. Weakfish were separated into three experimental groups prior to observation based on DO exposure during the 10 day holding period: control, saturation acclimated, and hypoxia acclimated. Control and saturationacclimated weakfish were exposed to saturated DO (7.3 mg  $O_2 \cdot L^{-1}$ ) conditions during the holding period. Hypoxia-acclimated weakfish were exposed to diel-cycling DO (2.0– 11.0 mg  $O_2 \cdot L^{-1}$ ) in a computer-controlled DO system (Grecay and Stierhoff 2002). Minimum (2.0 mg  $O_2 \cdot L^{-1}$ ) and maximum (11.0 mg  $O_2 \cdot L^{-1}$ ) DO concentrations coincided with the beginning of the light (0700) and dark (2100) periods, respectively. DO treatments were chosen to closely approximate natural DO conditions in shallow Mid-Atlantic Bight estuaries (D'Avanzo and Kremer 1994; Tyler et al. 2009). Mean (±SE) lengths of weakfish for control, saturation-acclimated, and hypoxia-acclimated experiments were 10.4 (±0.22), 10.7 (±0.25), and 9.79 (±0.44) cm, respectively.

A video camera (Hitachi KP-MN) attached to a frame grabber (PIXCI) and associated image analysis software (XCAP) was used to record the position of individual weakfish in a mesocosm tank (2.4 m diameter  $\times$  0.2 m depth) every second. Before each trial, fish were not fed for 12 h to avoid any postprandial effects on behavior. Fish were introduced into the tank and acclimated for 1 h prior to a trial. Control weakfish (n = 10) were observed in saturated conditions to determine if behavior changed without stimuli associated with DO change. Saturation-acclimated (n = 18) and hypoxia-acclimated (n = 10) weakfish were exposed to stepwise decreases in DO. Each trial began at 7.0 mg  $O_2 \cdot L^{-1}$ (~100% saturation) and DO was decreased by 1.4 mg  $O_2 \cdot L^{-1}$ (~20% saturation) increments to 1.4 mg O2·L<sup>-1</sup> (~20% saturation), at which point the DO was reduced to 0.8 mg  $O_2 \cdot L^{-1}$  (~15% saturation) and finally 0.4 mg  $O_2 \cdot L^{-1}$  (~10% saturation). Fish were exposed to each DO level for 30 min and transitions between each period lasted 3-5 min (Fig. 1). Subsequently, DO was raised in the reverse fashion to examine swimming activity following hypoxia exposure. DO was continuously measured with a YSI 5100 DO meter and continuously adjusted remotely by adding compressed nitrogen (decreasing DO) or oxygen (increasing DO) to an outer ring in the mesocosm tank. The DO probe was located at the edge of the observation area. By measuring DO with four DO probes simultaneously before observation, it was determined that multiple pumps in the outer ring mixed water into the inner ring (2 m diameter observation area) ensuring homogeneous DO exposure.

Mean swimming speed and the distribution of changes in direction (turning angles) were averaged for each half hour of constant DO exposure using the 1 s interval positioning data recorded by the video camera. From the distribution of turning angles, the angular mean and concentration around the mean were extracted. Weakfish were equally likely to move left or right (balance tendency); therefore, the angular mean was zero. The concentration of turning angles around the angular mean was calculated by taking the cosine of the turning angles  $(r = \cos(\theta))$ , which represents the angular correlation between successive turns. High angular correlation indicates a straight-line path (r = 1) and low angular correlation indicates random movement (r = 0). Since weakfish often swim along the edges of a circular tank, it should be noted that swimming in a circle could also result in high angular correlation. For example, a fish swimming clockwise in a circle making 10° turns to the right would have an angular correlation of 1.0. Although observed angular correlation in a mesocosm may not exactly reflect search patterns

**Fig. 1.** Mean  $\pm$  SE (*a*) swimming speed, (*b*) angular correlation, and (*c*) expected displacement of saturation-acclimated surviving ( $\bigcirc$ ) and hypoxia-acclimated ( $\bigcirc$ ) weakfish during the DO treatment and recovery period. Points show the mean response over the previous 30 min period. The broken line indicates DO exposure.



in situ, relative changes in angular correlation are suggestive of behavioral strategy changes that fish may utilize in the field. Swimming speed (step length) and angular correlation were used to compute expected displacement  $(E(D)_{app})$  for each half hour exposure period based on the approximation proposed by Benhamou (2004*b*):

$$E(D)_{\rm app} = 0.886\lambda \left[ \frac{n(1+r)}{1-r} \right]^{0.5}$$

where  $\lambda$  represents step length, *n* represents the number of steps (in this case the number of seconds), and *r* represents angular correlation. This model was used to quantify the influence of simultaneous changes in swimming speed and path straightness on the relative changes in each individual's expected displacement.

Control, saturation-acclimated, and hypoxia-acclimated trials were tested for the omnibus null hypothesis that no changes in swimming speed, angular correlation, and expected displacement occurred over time using a one-way repeated-measures ANOVA with Greenhouse–Geisser adjustment. Each half hour period represented a factor (Maxwell and Delaney 1990). If any trial deviated from the omnibus null hypothesis, polynomial contrasts were fit to the difference scores to characterize the overall response.

To examine interindividual variability in swimming

speed, Kendall's coefficient of concordance (W) was used to test whether fish maintained their rank order through time ( $\alpha < 0.05$ ). In conjunction with Kendall's coefficient of concordance, the coefficient of variation (CV = standard deviation/mean) in swimming speed was calculated to determine whether changes in concordance were linked to changes in relative swimming speed variability.

# Results

#### Saturation versus hypoxia acclimation

Swimming speed, angular correlation, and expected displacement of control weakfish (observed in saturated DO conditions) did not change significantly over time (Table 1). In contrast, hypoxia significantly altered several aspects of saturation-acclimated weakfish behavior during DO treatment and recovery phases (Table 1). Swimming speed of saturation-acclimated weakfish during DO treatment followed a quadratic relationship (Table 1; Fig. 1a). Speed increased to a relative maximum at 2.8 mg O<sub>2</sub>·L<sup>-1</sup>, a 42% increase from the swimming speed observed at 7.0 mg  $O_2 \cdot L^{-1}$  (DO saturation). Below 2.8 mg  $O_2 \cdot L^{-1}$ , swimming speed decreased as DO decreased and reached a minimum at 0.4 mg O<sub>2</sub>·L<sup>-1</sup>, a 54% drop from swimming speed at 7.0 mg O<sub>2</sub>·L<sup>-1</sup>. Significant changes in swimming speed occurred immediately after DO began decreasing and swimming speed remained faster than at DO saturation even at DO as low as 1.4 mg  $O_2 \cdot L^{-1}$ .

Angular correlation also followed a quadratic pattern with decreasing DO (Table 1; Fig. 1*b*). Angular correlation increased slightly until 0.8 mg  $O_2 \cdot L^{-1}$  before dropping precipitously at 0.4 mg  $O_2 \cdot L^{-1}$ . Simultaneous changes in swimming speed and angular correlation resulted in a significantly quadratic relationship between decreasing DO and expected displacement (Table 1; Fig. 1*c*). Expected displacement reached a maximum of 28.6 m·30 min<sup>-1</sup> at 2.8 mg  $O_2 \cdot L^{-1}$ , a 52% increase from expected displacement at 7.0 mg  $O_2 \cdot L^{-1}$  (18.8 m·30 min<sup>-1</sup>). Concomitant decreases in swimming speed and path straightness resulted in a 69% reduction in expected displacement at 0.4 mg  $O_2 \cdot L^{-1}$  compared with the expected displacement at 7.0 mg  $O_2 \cdot L^{-1}$ .

Hypoxia-acclimated weakfish experienced no mortality during DO treatment and had lower swimming speed than their saturation-acclimated counterparts (Fig. 1a). Interestingly, hypoxia-acclimated weakfish displayed no significant changes in mean swimming speed during treatment (Table 1; Fig. 1a). However, overall mean swimming speed of hypoxia-acclimated weakfish was also 46% lower than for control weakfish (observed in saturated DO conditions). Angular correlation decreased until DO reached 2.8 mg O<sub>2</sub>·L<sup>-1</sup>; however, when DO decreased to below the minimum DO concentration at which hypoxia-acclimated weakfish had been held during the 10 day pretreatment holding period (2.0 mg  $O_2 \cdot L^{-1}$ ), angular correlation increased throughout the rest of the treatment period (Fig. 1b). Increasing swimming speed of saturation-acclimated weakfish with declining DO down to 2.8 mg  $O_2 \cdot L^{-1}$  (Fig. 1*a*), coupled with decreasing angular correlation (path straightness) of hypoxia-acclimated fish, resulted in hypoxia-acclimated weakfish exhibiting only ~45% of the expected displacement of saturation-acclimated fish at 2.8 mg  $O_2 \cdot L^{-1}$  (Fig. 1*c*).

Saturation-acclimated and hypoxia-acclimated weakfish both showed a latent effect of hypoxia exposure on behavior as DO increased during the DO recovery phase. Swimming speed of saturation-acclimated weakfish during the recovery phase followed a significantly cubic relationship (Table 1; Fig. 1a). That is, swimming speed continued to decrease even as DO increased from 0.4 to 1.4 mg  $O_2 \cdot L^{-1}$  and then increased as DO increased from 1.4 to 4.2 mg O<sub>2</sub>·L<sup>-1</sup> until plateauing at ~0.9 body length (BL)·s<sup>-1</sup>. Angular correlation of saturation-acclimated weakfish also followed a cubic relationship (Table 1; Fig. 1b). However, whereas swimming speed only recovered to 52% of the initial speed at 7.0 mg  $O_2 \cdot L^{-1}$  (Fig. 2), angular correlation at the end of the DO recovery phase matched that at the beginning of the DO treatment phase (Fig. 1b). Although angular correlation decreased to a minimum of 0.49 at 1.4 mg  $O_2 \cdot L^{-1}$  during the DO recovery phase, the coefficient of variation among individuals was seven times greater than at 0.4 mg  $O_2 \cdot L^{-1}$ , which is also reflected in the standard errors in Fig. 1b. Individuals recovered at different rates but the decreased variation in angular correlation of saturation-acclimated weakfish at the end of the DO recovery period indicates that all fish eventually straightened their swimming paths by the end of the recovery phase.

Saturation-acclimated weakfish recovered faster than hypoxia-acclimated weakfish as evidenced by their greater expected displacement by 4.2 mg  $O_2 \cdot L^{-1}$  in the DO recovery phase (Fig. 1c). The reason for the recovery disparity between saturation- and hypoxia-acclimated research was twofold: the individual saturation-acclimated weakfish (n = 10)observed during recovery represented a hardier subset of the original subjects (n = 18) and a direct comparison of the two acclimation types gives a biased assessment of recovery. In contrast with basing recovery on mean swimming speed, by dividing recovery swimming speed by initial swimming speed observed at 7.0 mg  $O_2 \cdot L^{-1}$  (for each acclimation type), hypoxia-acclimated weakfish showed a less severe decline in swimming speed than did saturation-acclimated weakfish and ended the DO recovery phase at 79% of their baseline speed (Fig. 2).

#### Survivors versus nonsurvivors

Saturation-acclimated weakfish were more susceptible to hypoxia-induced mortality than were hypoxia-acclimated weakfish. Forty-four percent of saturation-acclimated weakfish died during DO treatment, whereas no hypoxia-acclimated or control weakfish died in any experiments. All mortalities occurred at the end of exposure to 0.4 mg  $O_2 \cdot L^{-1}$ , so those individuals did not contribute to analyses of the DO recovery phase. Survivors and nonsurvivors also differed in key aspects of their behavior. In general, swimming speed and expected displacement of both survivors and nonsurvivors paralleled one another in a quadratic relationship (Figs. 3a and 3c). Nonsurvivors were on average 31% less active than survivors during DO treatment. In addition, the degree of response differed between survivors and nonsurvivors. Nonsurvivors increased swimming speed by 78% as DO decreased from 7.0 to 2.8 mg  $O_2 \cdot L^{-1}$  compared with survivors' increase of only 25% over the same DO range (Fig. 3a). Nonsurvivors decreased angular correlation by 31% when DO decreased from 0.8 to 0.4 mg

Acclimation	Period	Attribute	F	р	Contrast	F	р
Control	Treatment	SS	0.84	0.42			
		r	2.11	0.15			
		E(D)	0.69	0.55			
	Recovery	SS	0.50	0.62			
		r	1.00	0.41			
		E(D)	0.25	0.76			
Saturation acclimated	Treatment	SS	21.5	< 0.001	Quadratic	52.1	< 0.001
		r	6.64	0.003	Quadratic	11.5	0.003
		E(D)	15.0	< 0.001	Quadratic	39.1	< 0.001
	Recovery	SS	4.84	0.03	Cubic	26.2	0.001
		r	3.81	0.041	Cubic	5.78	0.04
		E(D)	6.71	0.015	Cubic	12.3	0.007
Hypoxia acclimated	Treatment	SS	0.65	0.52			
		r	1.53	0.24	Order 4	6.86	0.028
		E(D)	0.69	0.55			
	Recovery	SS	0.93	0.48			
		r	0.59	0.51			
		E(D)	0.88	0.44			

 Table 1. Results of repeated-measures ANOVA to test for the effects of hypoxia on weakfish behavioral attributes.

Note: Degrees of freedom were adjusted by the Greenhouse–Geisser coefficient owing to violations of the sphericity assumption. The attributes tested were swimming speed (SS), cosine of the turning angle (r), and expected displacement (E(D)). The polynomial contrast shown was the contrast that explained the greatest amount of variation in attribute change through time.

**Fig. 2.** Percentage of initial swimming speed (at 7.0 mg  $O_2 \cdot L^{-1}$ ) that individual saturation-acclimated ( $\bigcirc$ ) and hypoxia-acclimated ( $\bigcirc$ ) weakfish attained during the DO recovery period. Points show the mean response over the previous 30 min period. The broken line indicates DO exposure.



 $O_2 \cdot L^{-1}$  compared with a survivor decrease of only 14% over the same DO range (Fig. 3*b*). Finally, survivors continued to increase their expected displacement from 23.6 to 31.0 m·30 min<sup>-1</sup> during DO decline down to 1.4 mg  $O_2 \cdot L^{-1}$  compared with only 2.8 mg  $O_2 \cdot L^{-1}$  for nonsurvivors (Fig. 3*c*). Survivors were generally more active and exhibited a more correlated swimming pattern than nonsurvivors (Figs. 3*a* and 3*b*).

#### Interindividual variability

The range of swimming speeds exhibited by individuals was reduced by exposure to hypoxia, which consequently led to a reduction in interindividual variability as measured by maintenance of rank order in swimming speed. Kendall's coefficient of concordance was highly significant for the control weakfish (observed in saturated DO conditions) (W = 0.86, p < 0.001), indicating that relatively slow and fast individuals remained so throughout the treatment period (Fig. 4a). Saturation-acclimated weakfish also maintained significant rank order in swimming speed during the whole treatment period (W = 0.66, p < 0.001) (Fig. 4b). However, acute hypoxia exposure decreased the maintenance of rank order as weakfish decreased swimming speed. When swimming speed was increasing during the DO decline from 7.0 to 2.8 mg  $O_2 \cdot L^{-1}$  (Fig. 1*a*), Kendall's coefficient of concordance was similar to that for control weakfish (Figs. 4). However, when swimming speed was decreasing during the DO decline from 2.8 to 1.4 mg  $O_2 \cdot L^{-1}$  (Fig. 1*a*), Kendall's coefficient of concordance decreased by 38% (W = 0.53, p < 0.530.001) (Fig. 4b). While Kendall's coefficient of concordance was still significant, there was much more crossing over between individuals as mean swimming speed across individuals decreased than when mean swimming speed was increasing. Finally, recovery of preexposure interindividual variability in saturation-acclimated and hypoxia-acclimated weakfish proceeded similarly for the two groups. Kendall's coefficient of concordance in swimming speed decreased to 0.38 (p = 0.10) in saturation-acclimated fish and to 0.30 (p =0.28) in hypoxia-acclimated weakfish during the first half of recovery when DO was still below 2.8 mg  $O_2 \cdot L^{-1}$ . As DO increased above 2.8 mg  $O_2 \cdot L^{-1}$  to a value of 7.0 mg  $O_2 \cdot L^{-1}$ , at the end of the recovery phase, the coefficient increased to a value of 0.76, similar to that for control weakfish (exposed to saturated DO).

Since no hypoxia-acclimated weakfish died as a result of treatment, maintenance of rank order in swimming speed was assessed over three DO categories: decreasing DO above 2.8 mg  $O_2$ ·L<sup>-1</sup>, DO below 2.8 mg  $O_2$ ·L<sup>-1</sup>, and increas-



ing DO above 2.8 mg O<sub>2</sub>·L<sup>-1</sup>. Maintenance of rank order was very similar to that of control weakfish as DO initially declined from 7.0 to 2.8 mg O<sub>2</sub>·L<sup>-1</sup> (W = 0.83, p < 0.001) (Fig. 5). When DO was 2.8 mg O<sub>2</sub>·L<sup>-1</sup> and falling and then recovering back to 2.8 mg O<sub>2</sub>·L<sup>-1</sup>, Kendall's coefficient of concordance revealed rank order that was highly discordant (W = 0.18, p = 0.27) (Fig. 5). Finally, during the recovery period when DO was above 2.8 mg O<sub>2</sub>·L<sup>-1</sup>, maintenance of rank order rebounded to W = 0.79 (p < 0.001). Although hypoxia-acclimated weakfish exhibited no significant change in swimming speed across DO levels, individual weakfish altered their behavior relative to their initial swimming speeds.

## Discussion

Saturation-acclimated weakfish increased their swimming speed as soon as DO began to decrease, long before DO reached levels known to be lethal or even growth limiting for this species (Stierhoff 2005). This active response is potentially a behavior to facilitate escape to a more oxygenated environment. In contrast, hypoxia-acclimated weakfish showed no change in mean swimming speed as DO decreased. It has been shown in rainbow trout (*Oncorhynchus mykiss*) that chromaffin cells secrete catecholamines (stress **Fig. 4.** Swimming speed of (*a*) individual control weakfish (observed in saturated DO conditions) and (*b*) saturation-acclimated weakfish during the DO treatment period. Kendall's coefficient of concordance (*W*) is included for the entire period for control fish and for each half of the saturation-acclimated weakfish treatment period (separated by the vertical line). The top of the shaded areas indicates DO exposure level.



**Fig. 5.** Swimming speed of individual juvenile hypoxia-acclimated weakfish during the DO treatment and recovery periods. The top of the shaded areas indicate DO exposure level.



hormones) in response to hypoxia exposure (30 min at 25% DO saturation) and that plasma catecholamine secretion decreases after the fourth day of exposure (McNeill and Perry 2006). It is possible that 10 days of exposure of hypoxia-acclimated weakfish to diel-cycling hypoxia was sufficient to eliminate the stress hormone mediated escape response of these fish in the present study. The present results suggest that juvenile weakfish residing in tidal tributaries affected by diel-cycling hypoxia have a suite of behavioral options (i.e., active strategy (increase swimming speed), passive strategy (decrease swimming speed), change in turning frequency, and acclimation). The response difference between

saturation-acclimated and hypoxia-acclimated weakfish highlights the importance of context for predicting fish distributions from observation of hypoxia-induced behavioral changes.

Recent studies on two species, Atlantic herring (Herbert and Steffensen 2006) and Atlantic cod (Herbert and Steffensen 2005), using the same DO exposure protocol on fish acclimated to saturated DO conditions for 2-3 weeks also emphasize the importance of context. Herbert and Steffensen (2006) found that Atlantic herring increased swimming speed as DO decreased in a stepwise fashion, but only when DO was decreasing (unsteady) and only below 8.5 kPa PO<sub>2</sub> (~40% saturation). After DO stabilized following the decline step, Atlantic herring resumed swimming at the initial swimming speed exhibited at DO saturation. In contrast, Atlantic cod decreased their swimming speed as DO decreased in the same exposure protocol, although they briefly increased swimming speed during the initial PO2 decrease from 19.9 to 13.2 kPa PO<sub>2</sub> (~94%-62% saturation) (Herbert and Steffensen 2005). Both species increased swimming speed only when DO was in a declining, unsteady state. Herbert and Steffensen (2006) hypothesized that during steady-state low-DO conditions, the optimal behavioral strategy includes not changing swimming speed. They speculated that static DO conditions indicate a spatially extensive hypoxic zone wherein increased swimming speed will not facilitate escape. They attributed the speciesspecific differences in behavioral responses of Atlantic herring and Atlantic cod to differences in schooling behavior, general levels of activity, and hypoxia tolerance. Atlantic herring, an active pelagic schooling fish, displayed an active response at DO below ~40% saturation, presumably because of the combination of being relatively hypoxia tolerant (i.e., higher haemoglobin oxygen affinity) and the necessity of maintaining oxygen delivery to tissue because of their more active lifestyle.

It is difficult to directly compare the response of weakfish with the responses of either Atlantic herring or Atlantic cod because the DO reduction rate of the present study was twice as fast and the lowest DO of exposure was ~5% saturation compared with ~30% saturation in the Herbert and Steffensen (2005, 2006) studies. However, saturation-acclimated weakfish significantly changed swimming speeds at each step in the DO exposure protocol (irrespective of DO being steady or unsteady). The overall faster DO decrease and minimum DO used in the present study and observed in the field (Tyler et al. 2009) may help to explain why swimming speed changed in saturation-acclimated weakfish regardless of whether DO conditions were steady state. For instance, Moss and McFarland (1970) observed that northern anchovies (Engraulis mordax) increased their swimming speed when DO was decreased rapidly but observed no change in swimming speed if DO was decreased slowly. Furthermore, juvenile weakfish displayed both active (DO >2.8 mg  $O_2 \cdot L^{-1}$ ) and passive (DO < 2.8 mg  $O_2 \cdot L^{-1}$ ) responses. The rapid DO decline induced an active response and the continued DO decline (<2.8 mg  $O_2 \cdot L^{-1}$ ) in conjunction with the initial active response resulted in the observed passive response. Atlantic herring might also have displayed a passive response at low DO if DO had been decreased faster and ended below 6 kPa PO<sub>2</sub> (~30% saturation).

The role of escape probability advocated by Herbert and Steffensen (2005, 2006) to explain behavioral changes in Atlantic herring and Atlantic cod taking place only when DO was in an unsteady state may also help explain differences between hypoxia-acclimated and saturation-acclimated weakfish. Escape probability is regulated by the spatial extent of hypoxia, which in estuarine nursery areas experiencing diel-cycling hypoxia depends on the geomorphology of the system (Tyler et al. 2009). For instance, tide pools (Congleton 1980) and estuarine ponds (Beck and Bruland 2000) may offer no chance of escape as DO decreases, whereas tributaries of coastal lagoons are usually adjoined to less dynamic and higher (depending on the time of day) DO conditions in open bay sites. Weakfish acclimated to saturation and diel-cycling hypoxia had different preconditioning experience. As a result of 10 days of exposure to diel-cycling hypoxia, hypoxia-acclimated weakfish displayed no significant changes in swimming speed throughout the initial exposure. The preconditioning period either trained hypoxia-acclimated weakfish to expect no chance of escape or reduced the stress associated with low DO. Either way, determining the role that expected escape probability plays in dictating the behavioral response of weakfish to decreasing DO should be an important component of future study. McNeill and Perry (2006) demonstrated that hypoxia acclimation made rainbow trout less responsive to further exposure after 4 days of acclimation. Hypoxia-acclimated weakfish were less active and less responsive in terms of changing swimming speed during DO decline and recovery, but they were also less susceptible to hypoxia-induced mortality than were saturation-acclimated weakfish. Routley et al. (2002) preconditioned epaulette sharks (Hemiscyllium ocellatum) to hypoxia before measuring their ventilatory and metabolic responses in a closed respirometer and found that preconditioned fish reduced their critical oxygen concentration from 2.2 to 1.7 mg  $O_2 \cdot L^{-1}$ . Owing to low variance in the measured oxygen consumption rate (VO<sub>2</sub>) of preconditioned sharks, Routley et al. (2002) hypothesized that reduced routine metabolic rate rather than enhanced oxygen delivery was the primary mechanism of acclimation. The reduced variance in VO<sub>2</sub> indicated that the shark's routine metabolism had become closer to their basal metabolism as a result of hypoxia preconditioning. Results of the current study are consistent with this hypothesis for juvenile weakfish. Hypoxia-acclimated weakfish were less active, even at DO saturation, than control weakfish (observed in saturated DO conditions). Indeed, their limited range of activity was most apparent at 0.4 mg  $O_2 \cdot L^{-1}$ , where variability was threefold lower than at 7.0 mg  $O_2 \cdot L^{-1}$ . The implications for reducing routine metabolism as a mechanism of acclimation for weakfish in the field depend mainly on the relevant concurrent biological conditions (i.e., food resources and predation).

In addition to changes in swimming speed, hypoxia-acclimated weakfish also adjusted angular correlation, an energetically cheaper form of behavioral enviroregulation. Angular correlation of hypoxia-acclimated weakfish decreased as DO decreased down to 2.8 mg  $O_2 \cdot L^{-1}$ , at which point swimming paths became straighter as DO continued to decrease below minimum DO experienced during the 10 day acclimation period. The initial decrease in angular correlation may have been part of a passive response seen in species more sluggish than weakfish (e.g., crucian carp (*Carassius carassius*); Nilsson et al. 1993). Wingfield (2003) pointed out that a stressor is only as stressful as it is unpredictable. In the current study, although DO routinely dropped below the US Environmental Protection Agency's acute DO criterion (2.3 mg  $O_2 \cdot L^{-1}$ ; US Environmental Protection Agency 2000) during the 10 day pretreatment holding period, physiological adjustments may have primed hypoxia-acclimated weakfish for this stressor. However, when DO fell below 2.0 mg  $O_2 \cdot L^{-1}$ , hypoxia-acclimated fish may have been limited to adjusting (increasing) path straightness rather than increasing swimming speed to facilitate escape.

Saturation-acclimated weakfish increased swimming speed quickly once DO increased above 1.4 mg  $O_2 L^{-1}$  during the recovery phase; however, speed did not recover to 100% of the pretreatment level. This suggests that hypoxia may have a latent effect on swimming behavior. In nursery habitats impacted by diel-cycling hypoxia, a series of days with low early morning DO levels may not be independent events and reductions in weakfish swimming speed may carry over. Lewis et al. (2007) found that after exposure to low DO, the Amazon cichlid Astronotus occelatus required ~2 h to repay the oxygen debt incurred during low-DO exposure. In diel-cycling hypoxia systems, there is a 6-10 h window when DO is normoxic to hyperoxic (Tyler et al. 2009). The rate at which weakfish recover swimming speed may play an important role in determining foraging activity. Swimming paths of saturation-acclimated weakfish became significantly less straight only when DO decreased to 0.4 mg O<sub>2</sub>·L<sup>-1</sup>. As the DO recovery period started, mean angular correlation continued to decrease until DO increased to 1.4 mg  $O_2 \cdot L^{-1}$ , at which point angular correlation was 40% lower than in control fish (observed in saturated DO conditions). The coefficient of variation for angular correlation was sevenfold greater at 1.4 mg  $O_2$ ·L<sup>-1</sup> than at 7.0 mg O2·L-1. Half of the recovering saturation-acclimated weakfish never decreased angular correlation below r = 0.8, whereas the other half recovered at differing rates, and 28% of the saturation-acclimated weakfish never became disoriented (r > 0.8). Such relatively hardy individuals may be able to take advantage of otherwise suboptimal habitat for foraging opportunities and also maintain greater predator avoidance capabilities.

Maintaining behavioral plasticity gives individuals more options when faced with capricious environments (Lomnicki 1978, 1999). Saturation-acclimated weakfish exhibited one behavioral strategy, reduced activity, when DO was lower than 2.8 mg  $O_2 \cdot L^{-1}$ . As each individual's swimming speed became more similar to every other individual's, Kendall's coefficient of concordance decreased. Specifically, as mean swimming speed approaches zero, the range of swimming speeds decreases. As the range of swimming speeds decreases, the individual swimming speeds "cross" more over time, thereby reducing Kendall's coefficient of concordance. Hypoxia-induced reduction in maintenance of rank order in swimming speed was even more pronounced for hypoxia-acclimated weakfish (~80% less than for control weakfish exposed to saturated DO conditions when DO was below 2.8 mg  $O_2 \cdot L^{-1}$ ). The more pronounced reduction in Kendall's coefficient of concordance for hypoxia-acclimated weakfish is consistent with the constraints that they were under at the beginning of DO treatment. Although there was no significant change in the mean swimming speed of hypoxia-acclimated weakfish, individuals reacted differently depending on their initial swimming speed. In addition, it is noteworthy that the turning point between a significantly high Kendall coefficient of concordance to a breakdown of maintained rank order in swimming speed came when DO decreased below the minimum DO exposure experienced during the 10 day preconditioning period (2.0 mg  $O_2 \cdot L^{-1}$ ). As DO decreased below this level, those individuals that began treatment swimming faster than the overall mean swimming speed decreased their speed and those individuals that began the trial swimming slower than the mean swimming speed increased their speed.

Interindividual variability was also apparent in survivorship. Juvenile weakfish that survived hypoxia treatment (down to 0.4 mg  $O_2 \cdot L^{-1}$  (~10% saturation)) were those individuals that were generally more active and less responsive in terms of changing swimming speed during hypoxia treatment than were nonsurvivors. Similarly, Van Raaij et al. (1996) observed that rainbow trout that survived hypoxia treatment employed a passive strategy, whereas nonsurvivors employed an active strategy. The degree of response to hypoxia appears to increase both species' susceptibility to hypoxia-induced mortality under conditions where escape is not possible. Another potential reason for the mortality disparity between active and less active weakfish is related to resource allocation. That is, some individuals may have invested more energy in growth rate and less in swimming performance. Billerbeck et al. (2001) found that Atlantic silversides (Menidia menidia) partition energy between growth and swimming performance and provided evidence that growth rate was not the only trait under heavy selective pressure. In another study on juvenile weakfish, Stierhoff (2005) found no significant difference in growth rate between weakfish fed ad libitum under diel-cycling hypoxia  $(2-11 \text{ mg } O_2 \cdot L^{-1})$  and DO saturation (7 mg  $O_2 \cdot L^{-1})$  for 7 days in the laboratory. Perhaps under ad libitum feeding conditions, and no predation, reduced activity and the resulting reduced interindividual variability is the preferred strategy to maintain high growth rates.

Recent studies of juvenile weakfish in the Delaware Coastal Bays shed light on potential behavioral strategies employed in response to diel-cycling hypoxia in the field. For example, Tyler and Targett (2007) found very low densities of juvenile weakfish in trawl catches during early morning when DO was below 2 mg  $O_2 \cdot L^{-1}$ ; however, later in the day when DO had recovered, weakfish densities had returned to relatively high levels. These findings suggest that weakfish employ an active escape strategy as DO decreases. In a laboratory study of juvenile weakfish growth, Stierhoff et al. (2009) observed no decrease in growth rate of weakfish exposed to diel-cycling hypoxia (2-11 mg  $O_2 \cdot L^{-1}$ ) compared with fish at DO saturation (7 mg  $O_2 \cdot L^{-1}$ ). In contrast, Stierhoff (2005) found that weakfish experiencing diel-cycling hypoxia in a tributary of the Delaware Coastal Bays had low growth rates (measured in situ using the RNA:DNA method) that were related to low-DO conditions. Given these results, it appears that juvenile weakfish

may gain a significant energetic savings by decreasing swimming speed (by 46% in the current study) during longterm (several days) exposure to diel-cycling hypoxia in the laboratory, which may be responsible for the observed constant growth rate down to 2.0 mg  $O_2 \cdot L^{-1}$ . In the field, however, weakfish must forage and avoid predators and may not have the behavioral flexibility of substantial reductions in swimming speed. Additionally, the minimum DO exposure in the laboratory acclimation protocol was 2.0 mg  $O_2 \cdot L^{-1}$ ; however, in the field, DO is routinely anoxic, which substantially increases the risk of hypoxia-induced mortality regardless of acclimation status. As a consequence, juvenile weakfish in the field appear to behave more like saturationacclimated weakfish from the current study and employ an active response (Tyler and Targett 2007; Brady 2008). The attendant consequences of this avoidance may include less foraging time, more overlap with predators, the energetic cost associated with the increased frequency of long distance movements, and the increased time exposed to size-dependent predation owing to low growth rates.

## Acknowledgements

The authors greatly appreciate K. Stierhoff and R. Tyler for their perspective and comments during the course of this study. This research was supported by funding from the Delaware Sea Grant Program, NOAA, US Department of Commerce, under grant No. NA03OAR4170011 (project R/F-23) to T.E. Targett.

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