Check for updates

Received: 11 December 2020 | Accepted: 28 January 2021
DOI: 10.1111/jfb.14691

BRIEF COMMUNICATION

JOURNAL OF FISH BIOLOGY fsbi

Assessment of hypoxia avoidance behaviours in a eurythermal fish at two temperatures using a modified shuttlebox system

Rasmus Ern[1] | Andrew J. Esbaugh[2]

[1]Department of Chemistry and Bioscience, Aalborg University, Aalborg, Denmark
[2]Marine Science Institute, University of Texas at Austin, Austin, Texas, USA

Correspondence
Rasmus Ern, Department of Chemistry and Bioscience, Aalborg University, Fredrik Bajers Vej 7H, 9220 Aalborg, Denmark.
Email: rasmus@ern.dk.

Funding information
Funding from the Carlsberg Foundation to R.E. (CF15-0321) and from the National Science Foundation to A.J.E. (IOS 2002549)

Abstract
Behavioural avoidance responses of red drum (*Sciaenops ocellatus*) to aquatic hypoxia were investigated at 22 and 30°C using a modified shuttlebox system. Fish movement between a control side maintained at normoxia and a hypoxic side with stepwise decreasing water oxygen tension was analysed for entries into the hypoxic side, residence time per entry into the hypoxic side and total time in the hypoxic side. Acclimation to 30°C increased the oxygen threshold for the onset of hypoxia avoidance behaviours for entries and total time, while residence time per entry was unchanged.

KEYWORDS
avoidance oxygen tension (P_{avoid}), red drum (*Sciaenops ocellatus*), water oxygen tension (PO_2)

Early studies on the behavioural responses of fishes to aquatic hypoxia used changes in the swimming activity in animals exposed to inescapable hypoxia as a quantifiable trait for hypoxia avoidance behaviour (cf. Domenici *et al.*, 2000; Herbert and Steffensen, 2005; Schurmann and Steffensen, 1994). In such studies, increased swimming activity is typically interpreted as an avoidance response to relocate to less hypoxic conditions, while decreased swimming activity is interpreted as a conservation strategy aimed at minimizing or offsetting metabolic stress (Chapman and McKenzie, 2009). The shuttlebox system (Petersen and Steffensen, 2003) enabled behavioural studies on fishes with the opportunity to move into more favourable oxygen conditions (Cook *et al.*, 2011; Cook and Herbert, 2012; Herbert *et al.*, 2011; Poulsen *et al.*, 2011; Skjaeraasen *et al.*, 2008). In addition to swimming activity, the shuttlebox allows researchers to assess behavioural traits such as residence time in hypoxia and re-entries into hypoxia (Poulsen *et al.*, 2011).

Authors have speculated that the behavioural responses of fishes to aquatic hypoxia are triggered directly by changes in PO_2 or indirectly by changes in the physiological state of the fish (Farrell and Richards, 2009; Herbert *et al.*, 2011). The latter of these hypotheses is supported by data on snapper (*Pagrus auratus*), showing an anaemia-induced decline in cardiorespiratory tissue oxygen supply and the PO_2 that triggers the onset of hypoxia avoidance behaviour (Cook *et al.*, 2011). Water temperature affects the state of the cardiovascular system in fishes *via* changes in metabolic oxygen requirement and tissue oxygen supply capacity (Fry, 1971; Norin and Speers-Roesch, 2021). Water temperature may therefore also affect the behavioural responses of fishes to aquatic hypoxia (Ern, 2019; Killen *et al.*, 2013; McKenzie *et al.*, 2015). Here, we used a modified shuttlebox system from Loligo Systems (Viborg, Denmark) (Figure 1) to test the hypothesis that temperature acclimation affects the behavioural characteristics of *S. ocellatus* moving freely between a chamber maintained at normoxia and a chamber with stepwise decreasing PO_2 from normoxia down to 20 mmHg, which is below the critical water oxygen tension (P_{crit}) of *S. ocellatus* (Ackerly and Esbaugh, 2020; Ern *et al.*, 2016; Pan *et al.*, 2016).

Juveniles were reared and raised in water at 22 ± 1°C and 35 ppt salinity at the University of Texas Marine Science Institute. At the beginning of the study, 16 fish were divided into a control tank (fish 1–8) and an acclimation tank (fish 9–16) with biofiltered water. Water temperature in the control tank was maintained at 22 ± 0.1°C. Water temperature in the acclimation tank was raised incrementally to 30°C at a rate of 2°C day^{-1} and the fish were acclimated to 30 ± 0.1°C for 3 weeks. Control and acclimation temperatures were based on *S. ocellatus* ecology. Optimal rearing temperatures are 22–25°C (Holt *et al.*, 1981) and juvenile fish experience daily temperatures up to 30°C (ASMFC, 2013) and tolerate temperatures up to 33.5°C (Able and Fahay, 2010). Animals were fed to satiation on Otohime B1-C2

 © 2021 Fisheries Society of the British Isles

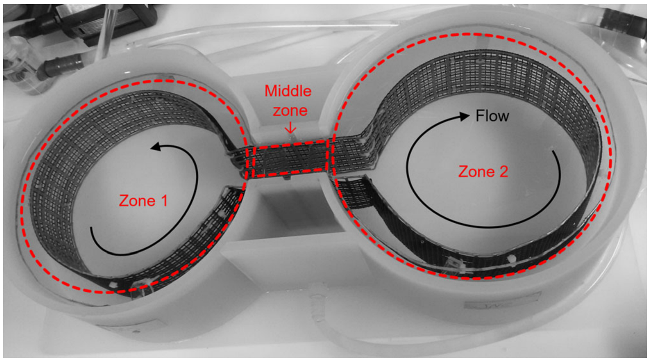

FIGURE 1 Modified shuttlebox with net arena and Plexiglas lid. Also shown are the borders of the three zones (red dashed lines) and the direction of water flow (black arrows) in the left and the right chambers

Fish Diet (reedmariculture.com) daily and food was withheld for 24 h prior to experimentation. At the end of the study, animals were anaesthetised using a buffered MS-222 bath (250 mg l^{-1} MS-222, 500 mg l^{-1} $NaHCO_3$) and euthanized by spinal transection. Experimental procedures were performed in accordance with policies of the Institutional Animal Care and Use Committee of the University of Texas at Austin.

The shuttlebox system consisted of two cylindrical chambers (50 cm in diameter) connected by a channel (10 cm long × 7.5 cm wide). Each chamber had one inlet, one outlet and one overflow. The distance from bottom of the chambers to the overflow was 8.5 cm. Each chamber was supplied with water from a dedicated buffer tank. The bottom of each buffer tank had one outlet, and the top had one inlet and one overflow. Water flowed gravitationally from the buffer tanks into the chambers and it was pumped back into the buffer tanks using two Eheim universal 300 pumps. The inlets and outlets of water generated a continuous circular current in each chamber. The connecting channel minimized mixing of water between the two chambers. Each buffer tank was also supplied with fresh normoxic water at 22 ± 0.1°C and 35 ppt salinity. All four outlets exited into a drain. In each buffer tank, the PO_2 was monitored using fibre optic oxygen sensors and meters from PreSens (Regensburg, Germany), controlled using AutoResp software and DAQ-M instruments from Loligo Systems (Viborg, Denmark), and regulated using solenoid systems that bubbled air and nitrogen (N) gas into the water. In addition to the two buffer tanks, PO_2 was also monitored in the water stream being pumped from the chamber outlets back into the buffer tanks. Water temperature in the buffer tanks was maintained using 300 W titanium tube heaters from Finnex (Chicago, United States) and Wh1436a temperature controllers from Willhi (Shenzhen, China). Fish movement was recorded using an 1640-C uEye camera and uEye Cockpit software from IDS Imaging Development Systems (Obersulm, Germany), and ShuttleSoft motion tracking software (v.2.6.4) from Loligo systems (Viborg, Denmark). A backlighting panel with an infrared light source was used for recording in darkness.

Before each trial, the water PO_2 in the control chamber, the avoidance chamber and the two buffer tanks was stabilized at normoxia. Trials were started by placing the fish in the channel connecting the two chambers, turning off the light in the room, and turning on the camera and motion tracking software. Each trial consisted of an initial 7 h baseline period with both chambers at normoxia followed by seven consecutive 2 h test periods of declining water PO_2: 120, 95, 80, 65, 50, 35 and 20 mmHg in the avoidance chamber. The duration of the baseline and test periods varied slightly because the experimental setup was operated manually. As a result, the duration (mean ± 1SD) was 414 ± 14 min for baseline periods and 105 ± 10 min for test periods. During the baseline period, the water in both chambers was maintained at normoxia. At the end of the baseline period, the PO_2 in the buffer tank connected to the avoidance chamber was reduced to 120 mmHg and allowed to equilibrate with the chamber. The first test period was started once the PO_2 in the water exiting the avoidance chamber through the outlet reached 120 mmHg. At the end of the first test period the PO_2 in the avoidance chamber was reduced from 120 to 95 mmHg following the same procedure. This step was repeated for 80, 65, 50, 35 and 20 mmHg. Fish movement was recorded continuously during the baseline period and the seven test periods. The tracking software was paused at the end of each period and restarted at the beginning of the next. The duration (mean ± 1SD) of these intermediate periods was 15 ± 6 min. The entire trial lasted ~21 h. During trials, the water temperature in the buffer tanks and shuttle box was maintained at 22 ± 0.1°C or 30 ± 0.1°C. Mean body mass ± 1SD was 0.31 ± 0.11 g for fish were measured at 22°C (fish 1–8) and 0.41 ± 0.04 g for fish measured at 30°C (fish 9–16), with no significant difference between the two groups (Student's *t*-test, $P = 0.513$).

Preliminary trials revealed two experimental design issues. These issues prevented accurate assessments of hypoxia avoidance responses, and they were both solved *via* modifications to the commercially available shuttlebox system. The first issue was caused by individuals swimming against the circular current and exhausting themselves at the beginning of trials. Placing a plastic net arena inside the system eliminated the issue by preventing fish from moving into the currents running along the sides of the chambers (Figure 1). The net arena was 8.0 cm tall and consisted of two cylindrical arenas (44 cm in diameter) connected by a channel (16 cm long × 7.0 cm wide). The second issue was caused by oxygen diffusing from the air into the water in the avoidance chamber and by fish engaging in aquatic surface respiration (ASR) (Kramer & Mehegan, 1981). ASR is observed in many fish species during hypoxia (Perry *et al*., 2009; Richards, 2011) and it may influence hypoxia avoidance trials if fish at unfavourable PO_2 levels alleviate respiratory distress by engaging in ASR rather than exiting the avoidance chamber. The issue was solved by adding a customized Plexiglas lid to the experimental setup (Figure 1). The lid, which had negative buoyancy, rested on top of the net arena and was covered by 1–2 mm of water. The lid was 3 mm thick and consisted of two circular disks (48 cm in diameter) connected by a rectangular bridge (11 cm long × 7.0 cm wide).

Preliminary tests of the modified shuttlebox system showed the water PO_2 outside and inside the net arena equilibrated during the intermediate periods. Adding a dye to the water revealed a slight disruption of the circulating water passing through the net arena at the chamber entrances and, as a result, a slight mixing of the waters in the two chambers. Test trials also proved it was challenging to perfectly balance the amount of water circulating through the inlets and outlets of the control chamber and the avoidance chamber over the 21 h trial period. We believe the gradual decline in PO_2 in the control zone during the 21 h trial periods was cause by these two issues (Figure 2a,b). However, any such mixing was to slow to constrain fish movement, as virtually all individuals were observed motionless in the connecting channel during baseline periods. Furthermore, the lowest PO_2 in the control chamber (~100 mmHg) was well above the highest PO_2 at which significant avoidance responses were observed (~50 mmHg). It is therefore reasonable to assume the decline in PO_2 in the control zone during trials did not influence the hypoxia avoidance responses of the fish.

For analysis of behavioural data, the shuttle box was divided into three zones: (a) a control zone covering the area in the control chamber, including the entrance area, (b) an avoidance zone covering the area in the avoidance chamber, including the entrance area, and (c) a middle zone covering the channel between the two chambers, excluding the two entrance areas. For individual fish, three behavioural hypoxia responses (Poulsen *et al*., 2011) were calculated for each of the three zones during the baseline period and the seven consecutive test periods: (a) entries into zone (number per hour), (b) residence time in zone (seconds per entry) and (c) total time in zone (minutes per hour). Percentage changes in the responses during the seven test periods relative to the responses during the baseline period were calculated for each zone (Figure 2). Using absolute values from fish in the avoidance zone, the onset of hypoxia avoidance behaviour was determined for each response by comparing the baseline period to each of the seven test periods (one-way RM ANOVA followed by Holm–Sidak pair-wise multiple comparison *versus* control group procedures). Fish #2 was inactive during test periods and excluded as an outlier from the statistical analysis (Grubbs' test based on residence time, $P < 0.001$). With the exception of individual #2, all fish exhibited regular shuttling between the two chambers by the end of the 7 h baseline period. Data not meeting the assumptions of normality and homoscedasticity were transformed. The significance level for all tests was $P < 0.05$. Calculations were done using Excel and statistical analyses were done using SigmaPlot 12.5 (systatsoftware.com). Results and statistical analysis are presented in Table 1, together with the PO_2 in the water exiting the control and avoidance chambers *via* the outlets.

Fish acclimated to 30°C increased the PO_2 threshold for the onset of hypoxia avoidance behaviours for entries into zones per hour and total time in zones per hour, while residence time in zones per entry was unchanged (Figure 2). At both temperatures, entries into zones per hour, residence time in zones per entry and total time in zones per hour were equally divided between the control zone and the avoidance zone during the normoxic baseline periods. In fish kept at 22°C, entries in the avoidance zone per hour, residence time in the avoidance zone per entry and total time in the avoidance zone per hour were maintained with declining PO_2 from normoxia down to 20, 35 and 35 mmHg, respectively, where the traits became significantly reduced relative to the normoxic baseline period. In fish acclimated to 30°C, entries in the avoidance zone per hour, residence time in the avoidance zone per entry and total time in the avoidance zone per hour were maintained with declining PO_2 from normoxia down to 35, 35 and 50 mmHg, respectively, where the traits became significantly reduced relative to the normoxic baseline period. Prolonged exposure to aquatic hypoxia can affect the expression and oxygen affinity of haemoglobin (Pan *et al*., 2017), which in turn may enhance or blunt the hypoxia sensitivity of behavioural responses (Cook *et al*., 2011). Because the order of hypoxia exposure was from highest to lowest PO_2, the responses observed in *S. ocellatus* may deviate from the responses of fishes exposed to more abrupt changes in PO_2. Future studies randomizing the PO_2 exposure levels may reveal the extent of this potential issue.

The order of these responses is comparable to the order of responses identified in rainbow trout (*Oncorhynchus mykiss*) using the two-current choice flume system (Poulsen *et al*., 2011). In that study, entries into the hypoxic side declined from 47 mmHg, residence time per entry declined from 88 mmHg and total time declined from 125 mmHg. While the order of the responses is comparable between *S. ocellatus* and *O. mykiss*, the oxygen sensitivity of the responses is not. *S. ocellatus* exhibited the first significant response at 50 mmHg *versus* 125 mmHg in *O. mykiss*. The differences in the oxygen sensitivity may be species-specific or they may be linked to the systems and protocols used to study the two species, including the relative ease with which animals can move between normoxia and hypoxia in the two-current choice flume system when compared to the shuttlebox system.

The increases in entries into zones per hour and decreases in residence in zones per entry suggest the overall swimming speed of *S. ocellatus* increased from 22 to 30°C (Table 1). Intraspecies differences in fish swimming speed during trials may affect the behavioural response to hypoxia (Cook and Herbert, 2012). Water temperature affects the swimming speed of fishes (Claireaux *et al*., 2006) and the behavioural parameters identified in *S. ocellatus* were therefore normalized using the values of individual fish in the initial baseline period at normoxia as the starting reference (Herbert and Steffensen, 2005). As a result, the increases in the PO_2 threshold for the onset of hypoxia avoidance behaviours in *S. ocellatus* from 22 to 30°C should not be affected by temperature-induced increases in fish swimming speed.

The shuttlebox system is one of two popular systems used in studies on the behavioural characteristics of fishes (Jutfelt *et al*., 2017). The other system is the two-current choice flume system, which is the predominant system used in studies on the behavioural responses of fishes to hypoxia (Cook *et al*., 2011; Cook and Herbert, 2012; Herbert *et al*., 2011; Poulsen *et al*., 2011). The choice flume system requires that experimental fish maintain swimming speed for several hours during trials, making it ideal for assessing pelagic migratory species (Cook *et al*., 2011; Cook and Herbert, 2012;

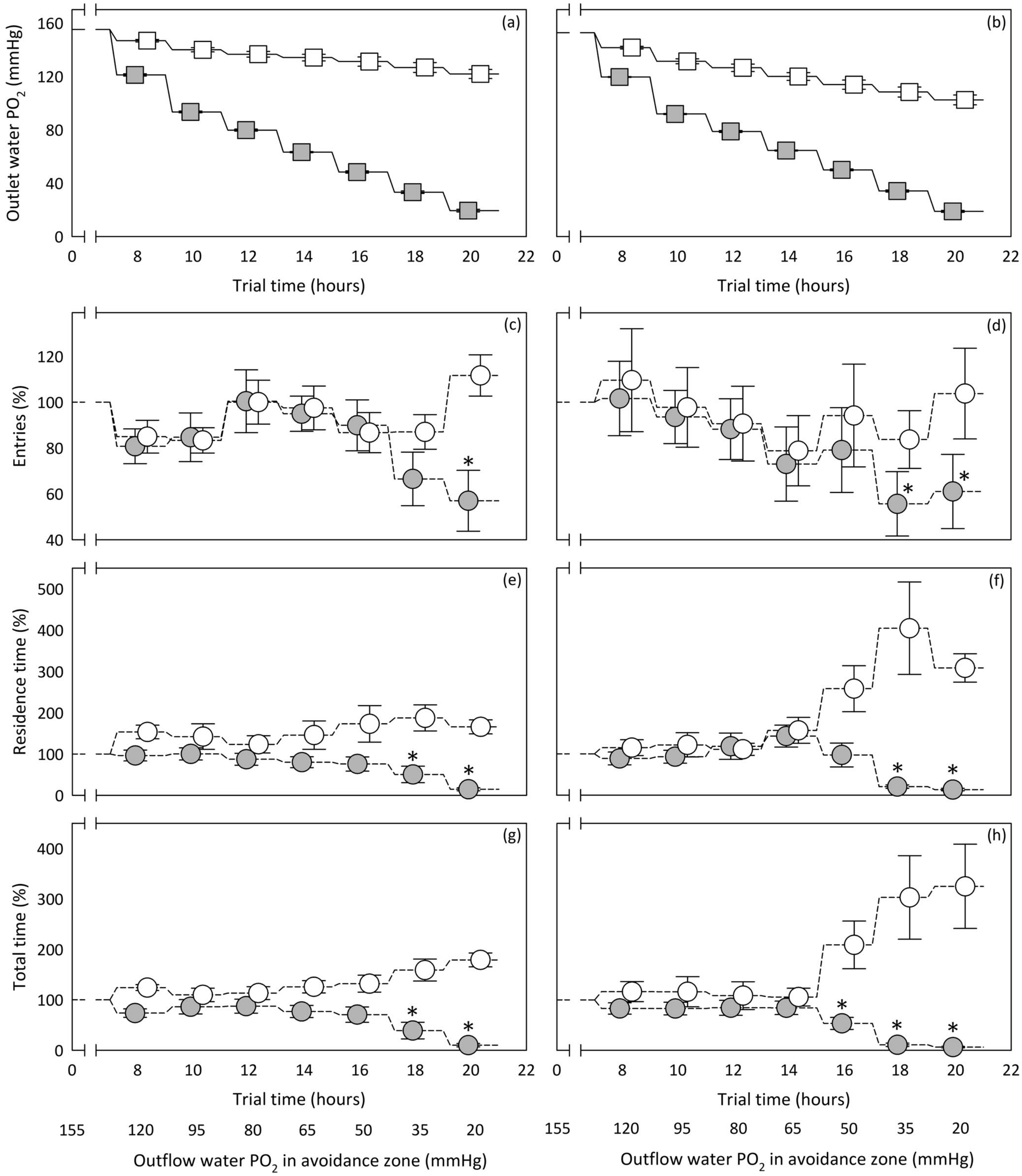

FIGURE 2 Hypoxia avoidance behaviours in *Sciaenops ocellatus* in a modified shuttlebox system: (a, c, e, g) fish kept at 22°C and (b, d, f, h) fish acclimated to 30°C. (a, b) PO_2 (mmHg) in the outlet water from the control zone (white squares) and the avoidance zone (grey squares) during the initial 7 h baseline period at normoxia and the seven subsequent 2 h test periods of declining PO_2. Percentage changes in (c, d) entries (number per hour), (e, f) residence time (seconds per entry) and (g, h) Total time (minutes per hour) in the control zone (white circles) and in the avoidance zone (grey circles) during the seven 2 h test periods relative to the initial 7 h baseline period. For the avoidance zone, asterisks (*) indicate a significant difference from the baseline period (one-way RM ANOVA, $P < 0.05$). Values are mean ± 1SE

TABLE 1 Water temperature and outlet PO_2 in the modified shuttlebox system, and the behavioural characteristics of *S. ocellatus*, including entries, residence time and total time in the control zone, the avoidance zone and the middle zone during the initial 7 h baseline period at normoxia and the seven subsequent 2 h test periods of declining water PO_2

		Baseline	1st	2nd	3rd	4th	5th	6th	7th
22°C									
Outlet PO_2 (mmHg)	Control	Normoxia	147 ± 3	140 ± 5	137 ± 6	135 ± 7	132 ± 10	128 ± 11	123 ± 10
	Avoidance	Normoxia	121 ± 3	94 ± 2	80 ± 2	64 ± 1	48 ± 3	34 ± 3	20 ± 3
Entries (number per hour)	Control	23.5 ± 8.4	19.3 ± 6.0	19.5 ± 7.4	22.3 ± 5.5	22.6 ± 8.1	21.4 ± 11.5	20.8 ± 9.6	26.2 ± 10.5
	Avoidance	23.2 ± 8.6	17.8 ± 5.5	19.9 ± 9.1	21.9 ± 6.0	21.7 ± 7.6	21.9 ± 11.9	16.3 ± 10.5	13.8 ± 9.4*
	Middle	46.0 ± 17.1	37.4 ± 11.1	39.6 ± 16.0	44.7 ± 11.1	44.8 ± 15.6	43.5 ± 23.4	37.3 ± 19.8	40.3 ± 16.7
Residence time (seconds per entry)	Control	79.9 ± 38.6	118.2 ± 53.0	119.0 ± 105.5	92.4 ± 61.7	121.6 ± 113.2	152.0 ± 147.0	152.0 ± 109.5	129.9 ± 66.8
	Avoidance	67.7 ± 21.1	59.5 ± 13.0	63.6 ± 25.9	53.4 ± 20.9	49.4 ± 20.7	48.3 ± 34.3	31.3 ± 38.3*	8.5 ± 4.3*
	Middle	12.4 ± 4.3	14.5 ± 5.5	15.1 ± 5.7	12.8 ± 5.4	10.2 ± 2.2	11.5 ± 3.4	15.1 ± 61	15.5 ± 5.3
Total time (minutes per hour)	Control	27.2 ± 4.6	33.7 ± 6.2	30.1 ± 11.4	30.5 ± 9.2	34.1 ± 10.1	35.5 ± 12.3	42.1 ± 13.8	47.6 ± 6.0
	Avoidance	23.7 ± 3.5	17.7 ± 6.7	21.0 ± 10.7	20.8 ± 9.3	18.3 ± 8.1	16.9 ± 10.2	9.3 ± 10.7*	2.5 ± 2.2*
	Middle	9.1 ± 2.9	8.6 ± 2.9	8.9 ± 2.3	8.7 ± 1.4	7.5 ± 3.1	7.5 ± 3.2	8.5 ± 4.1	9.9 ± 4.8
30°C									
Outlet PO_2 (mmHg)	Control	Normoxia	142 ± 3	131 ± 5	126 ± 8	120 ± 8	114 ± 10	108 ± 10	102 ± 10
	Avoidance	Normoxia	120 ± 1	92 ± 1	79 ± 2	65 ± 1	50 ± 2	34 ± 2	19 ± 2
Entries (number per hour)	Control	34.8 ± 12.2	35.2 ± 16.5	30.2 ± 10.0	28.0 ± 9.5	25.4 ± 12.3	29.6 ± 14.8	27.6 ± 11.1	32.0 ± 10.8
	Avoidance	39.0 ± 15.3	38.6 ± 19.0	33.7 ± 11.0	31.0 ± 9.2	25.1 ± 12.0	27.1 ± 15.0	18.7 ± 11.2*	19.7 ± 12.8*
	Middle	72.9 ± 27.8	68.1 ± 28.2	60.5 ± 18.0	56.0 ± 16.9	48.5 ± 25.6	54.5 ± 25.9	45.5 ± 21.0	49.3 ± 17.5
Residence time (seconds per entry)	Control	41.1 ± 27.8	59.2 ± 73.0	50.4 ± 45.9	46.8 ± 34.6	75.6 ± 92.1	82.0 ± 29.8	130.2 ± 82.2	111.8 ± 54.0
	Avoidance	57.4 ± 39.9	44.5 ± 30.3	50.0 ± 31.0	60.4 ± 46.5	73.9 ± 49.2	48.6 ± 42.9	8.9 ± 2.9*	5.5 ± 0.8*
	Middle	5.1 ± 2.6	6.7 ± 5.7	10.1 ± 11.1	11.3 ± 11.8	9.8 ± 14.1	11.4 ± 10.8	11.9 ± 10.7	8.3 ± 8.7
Total time (minutes per hour)	Control	22.3 ± 12.3	25.2 ± 17.1	22.6 ± 16.0	22.3 ± 16.8	24.2 ± 18.2	35.5 ± 10.5	47.7 ± 9.1	51.7 ± 5.3
	Avoidance	30.9 ± 11.5	26.7 ± 14.6	25.7 ± 12.1	26.8 ± 15.8	28.1 ± 18.5	15.4 ± 9.7*	2.9 ± 2.1*	1.8 ± 1.1*
	Middle	6.8 ± 5.6	8.1 ± 8.4	11.7 ± 14.7	10.9 ± 12.3	7.7 ± 10.9	9.1 ± 9.9	9.4 ± 9.5	6.5 ± 5.8

For the avoidance zone, asterisks (*) indicate a significant difference from the baseline period (one-way RM ANOVA, $P < 0.05$). Values are mean ± 1SD.

Herbert *et al*., 2011; Poulsen *et al*., 2011), but less suitable for studying sedentary benthic species. By contrast, fish assessed in the shuttlebox system can move freely between the two chambers at their own leisure, making the shuttlebox system in this study a suitable alternative to the choice flume system when studying the behavioural responses of sedentary benthic fishes to aquatic hypoxia.

In conclusion, this study showed a temperature-induced increase in the PO_2 avoidance threshold for two of the three behavioural parameters identified in *S. ocellatus*. Aquatic oxygen-deficient (hypoxic) "dead zones" and marine heat waves are changing the distribution of fishes, with more pronounced effects expected in the future (IPCC, 2018; IUNC, 2019). These results suggest the occurrence of marine heat waves in regions already affected by an oxygen-deficient "dead zone" may exacerbate the impacts of aquatic hypoxia on the distribution of *S. ocellatus*. Our results also suggest that the onset of hypoxia avoidance behaviours in *S. ocellatus* occur at PO_2 levels above their P_{crit} (Ackerly & Esbaugh, 2020; Ern *et al*., 2016; Pan *et al*., 2017). A similar pattern is reported for *O. mykiss* (McKenzie *et al*., 2007; Poulsen *et al*., 2011), indicating these responses are triggered by mechanisms other than the transition to anaerobic metabolism and accumulation of anaerobic metabolites occurring in many fishes at PO_2 levels below their P_{crit} (Farrell & Richards, 2009). The study also described an effective method for determining the behavioural responses of fishes to aquatic hypoxia, including several modifications to the commercially available shuttlebox system. Adopting these modifications will enable accurate assessments of hypoxia avoidance behaviours in sedentary benthic fishes, including smaller individuals with body mass <1 g. This information can help to provide a better understanding of the physiological and organismal responses of fishes to aquatic hypoxia. This in turn may contribute to conservation planning by providing the necessary mechanisms to forecast changes in species distributions with anthropogenic changes in water oxygen and temperature (Evans *et al*., 2015; McKenzie *et al*., 2016).

ACKNOWLEDGEMENTS

We gratefully acknowledge financial support from the Carlsberg Foundation to R.E. (CF15-0321) and from the National Science Foundation to A.J.E. (IOS 2002549).

CONTRIBUTIONS

Conceptualization: R.E. and A.J.E. Methodology: R.E. Formal analysis: R.E. Investigation: R.E. Resources: A.J.E. Data curation: R.E. Writing – original draft: R.E. Writing – review and editing: A.J.E. Visualization: R.E. Supervision: A.J.E. Project administration: A.J.E. Funding acquisition: R.E. and A.J.E.

ORCID

Rasmus Ern https://orcid.org/0000-0002-4202-476X

REFERENCES

Able, K. W., & Fahay, M. P. (2010). *Ecology of estuarine fishes: Temperate waters of the Western North Atlantic*. Baltimore, Maryland: John Hopkins University Press.

Ackerly, K. L., & Esbaugh, A. J. (2020). The additive effects of oil exposure and hypoxia on aerobic performance in red drum (*Sciaenops ocellatus*). *Science of the Total Environment*, *737*, 140174.

Atlantic States Marine Fisheries Commission (ASMFC) 2013. Addendum I to Amendment 2 to the Red Drum fishery management plan: habitat needs and concerns. http://www.asmfc.org/species/red-drum.

Chapman, L. J., & McKenzie, D. J. (2009). Behavioural responses and ecological consequences. In J. G. Richards, A. P. Farrell, & C. J. Brauner (Eds.), *Fish physiology: Hypoxia* (pp. 26–79). London, NY: Academic Press.

Claireaux, G., Couturier, C., & Groison, A. L. (2006). Effect of temperature on maximum swimming speed and cost of transport in juvenile European sea bass (*Dicentrarchus labrax*). *Journal of Experimental Biology*, *209*, 3420–3428.

Cook, D. G., & Herbert, N. A. (2012). The physiological and behavioural response of juvenile kingfish (*Seriola lalandi*) differs between escapable and inescapable progressive hypoxia. *Journal of Experimental Marine Biology and Ecology*, *413*, 138–144.

Cook, D. G., Wells, R. M. G., & Herbert, N. A. (2011). Anaemia adjusts the aerobic physiology of snapper (*Pagrus auratus*) and modulates hypoxia avoidance behaviour during oxygen choice presentations. *Journal of Experimental Biology*, *214*, 2927–2934.

Domenici, P., Steffensen, J. F., & Batty, R. S. (2000). The effect of progressive hypoxia on swimming activity and schooling in Atlantic herring. *Journal of Fish Biology*, *57*, 1526–1538.

Ern, R. (2019). A mechanistic oxygen- and temperature-limited metabolic niche framework. *Philosophical Transactions of the Royal Society B*, *374*, 20180540.

Ern, R., Norin, T., Gamperl, A. K., & Esbaugh, A. J. (2016). Oxygen dependence of upper thermal limits in fishes. *Journal of Experimental Biology*, *219*, 3376–3383.

Evans, T. G., Diamond, S. E., & Kelly, M. W. (2015). Mechanistic species distribution modelling as a link between physiology and conservation. *Conservation Physiology*, *3*, cov056.

Farrell, A. P., & Richards, J. G. (2009). Defining hypoxia: An integrative synthesis of the responses of fish to hypoxia. In J. G. Richards, A. P. Farrell, & C. J. Brauner (Eds.), *Fish physiology* (pp. 487–503). New York, NY: Academic Press.

Fry, F. E. J. (1971). The effect of environmental factors on the physiology of fish. In W. S. Hoar & D. J. Randall (Eds.), *Fish physiology* (pp. 1–98). New York, NY: Academic Press.

Herbert, N. A., Skjæraasen, J. E., Nilsen, T., Salvanes, A. G. V., & Steffensen, J. F. (2011). The hypoxia avoidance behaviour of juvenile Atlantic cod (*Gadus morhua* L.) depends on the provision and pressure level of an O_2 refuge. *Marine Biology*, *158*, 737–746.

Herbert, N. A., & Steffensen, J. F. (2005). The response of Atlantic cod, *Gadus morhua*, to progressive hypoxia: fish swimming speed and physiological stress. *Marine Biology*, *147*, 1403–1412.

Holt, J., Godbout, R., & Arnold, C. (1981). Effects of temperature and salinity on egg hatching and larval survival of red drum *Sciaenops ocellatus*. *Fishery Bulletin*, *79*, 569–573.

IPCC (2018). In V. Masson-Delmotte, P. Zhai, H.-O. Pörtner, D. Roberts, J. Skea, P. R. Shukla, et al. (Eds.), *Global Warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty*. Geneva, Switzerland: IPCC.

IUNC (2019). In D. Laffoley & J. M. Baxter (Eds.), *Ocean deoxygenation: Everyone's problem - Causes, impacts, consequences and solutions* (p. 580). Gland, Switzerland: IUCN.

Jutfelt, F., Sundin, J., Raby, G. D., Krång, A. S., & Clark, T. D. (2017). Two-current choice flumes for testing avoidance and preference in aquatic animals. *Methods in Ecology and Evolution*, *8*, 379–390.

Killen, S. S., Marras, S., Metcalfe, N. B., McKenzie, D. J., & Domenici, P. (2013). Environmental stressors alter relationships between physiology and behaviour. *Trends in Ecology & Evolution*, *28*, 651–658.

Kramer, D. L., & Mehegan, J. P. (1981). Aquatic surface respiration, an adaptive response to hypoxia in the guppy, *Poecilia reticulata* (*Pisces, Poeciliidae*). *Environmental Biology of Fishes*, *6*, 299–313.

McKenzie, D. J., Axelsson, M., Chabot, D., Claireaux, G., Cooke, S. J., Corner, R. A., et al. (2016). Conservation physiology of marine fishes: state of the art and prospects for policy. *Conservation Physiology*, *4*, cow046.

McKenzie, D. J., Belao, T. C., Killen, S. S., & Rantin, T. F. (2015). To boldly gulp: standard metabolic rate and boldness have context-dependent influences on risk-taking to breathe air in a catfish. *Journal of Experimental Biology*, *218*, 3762–3770.

McKenzie, D. J., Pedersen, P. B., & Jokumsen, A. (2007). Aspects of respiratory physiology and energetics in rainbow trout families that differ in size-at-age and condition factor. *Aquaculture*, *263*, 280–290.

Norin, T., & Speers-Roesch, B. (2021). Metabolism. In S. Currie & D. H. Evans (Eds.), *The physiology of fishes* (5th ed., pp. 129–141). Boca Raton, FL, USA: CRC Press.

Pan, Y. K., Ern, R., & Esbaugh, A. J. (2016). Hypoxia tolerance decreases with body size in red drum *Sciaenops ocellatus*. *Journal of Fish Biology*, *89*, 1488–1493.

Pan, Y. K., Ern, R., Morrison, P. R., Brauner, C. J., & Esbaugh, A. J. (2017). Acclimation to prolonged hypoxia alters haemoglobin isoform expression and increases hemoglobin oxygen affinity and aerobic performance in a marine fish. *Scientific Reports*, *7*, 7834.

Perry, S. F., Jonz, M. G., & Gilmour, K. M. (2009). Oxygen sensing and the hypoxic ventilatory response. In J. G. Richards, A. P. Farrell, & C. J. Brauner (Eds.), *Fish physiology* (pp. 193–253). San Diego, CA: Academic Press.

Petersen, M. F., & Steffensen, J. F. (2003). Preferred temperature of juvenile Atlantic cod *Gadus morhua* with different haemoglobin genotypes at normoxia and moderate hypoxia. *Journal of Experimental Biology*, *206*, 359–364.

Poulsen, S. B., Jensen, L. F., Nielsen, K. S., Malte, H., Aarestrup, K., & Svendsen, J. C. (2011). Behaviour of rainbow trout *Oncorhynchus mykiss* presented with a choice of normoxia and stepwise progressive hypoxia. *Journal of Fish Biology*, *79*, 969–979.

Richards, J. G. (2011). Physiological, behavioral and biochemical adaptations of intertidal fishes to hypoxia. *Journal of Experimental Biology*, *214*, 191–199.

Schurmann, H., & Steffensen, J. F. (1994). Spontaneous swimming activity of Atlantic cod *Gadus morhua* exposed to graded hypoxia at 3 temperatures. *Journal of Experimental Biology*, *197*, 129–142.

Skjæraasen, J. E., Nilsen, T., Meager, J. J., Herbert, N. A., Moberg, O., Tronci, V., … Salvanes, A. G. V. (2008). Hypoxic avoidance behaviour in cod (*Gadus morhua* L.): the effect of temperature and haemoglobin genotype. *Journal of Experimental Marine Biology and Ecology*, *358*, 70–77.

How to cite this article: Ern R, Esbaugh AJ. Assessment of hypoxia avoidance behaviours in a eurythermal fish at two temperatures using a modified shuttlebox system. *J Fish Biol*. 2021;1–7. https://doi.org/10.1111/jfb.14691