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The behavioural and physiological response of Atlantic cod *Gadus morhua* L. to short-term acute hypoxia

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The average rate of swimming speed and the physiological status or stress of individual Atlantic cod *Gadus morhua* was monitored in response to short-term acute (STA) hypoxia (*i.e.* partial pressure of oxygen, P_{O_2}, reduced from 20·9 to 4·3 kPa within 1 h at 10° C). The STA hypoxic response of Atlantic cod was associated with a large primary increase (+29%) and a large secondary decrease (−54%) in swimming speed as well as major physiological stress (*e.g.* plasma cortisol = 214·7 ng ml^{-1} and blood lactate = 2·41 mmol l^{-1}).

Key words: cod; hypoxia; oxygen; stress; swimming.

The Atlantic cod *Gadus morhua* L. is moderately tolerant of low O_2 (*i.e.* hypoxia) (Plante *et al.*, 1998; Herbert & Steffensen, 2005) and its distribution is known to overlap with some of the hypoxic regions of the Baltic Sea (Neuenfeldt, 2002) and the Canadian Gulf of St Lawrence (D'Amours, 1993; Plante *et al.*, 1998). Progressive hypoxia influences the energetic physiology of Atlantic cod by compressing the aerobic metabolic scope (*i.e.* energy available for non-basal processes) (Claireaux *et al.*, 2000) and disturbing the basal rate of metabolism at a critical partial pressure of oxygen (P_{O_2}) (*i.e.* S_{crit}) (Schurmann & Steffensen, 1997; Claireaux *et al.*, 2000). Hypoxia imposes an energetic challenge on fishes but major physiological stress could be avoided at pressures in excess of S_{crit} if a satisfactory balance is maintained between O_2 supply and demand. For this to be achieved, excessive energetic expenditure (such as high routine rates of swimming) be addre be down-regulated appropriately and in accordance with the inherent level of hypoxia. Contrary to this expectation, Herbert & Steffensen (2005) show that when Atlantic cod are exposed to an initially rapid decline in aquatic P_{O_2} (19·9–13·2 kPa), they exhibit

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an avoidance-like response with an 18% increase in the average rate of speed. By virtue of a secondary (41%) reduction in speed, however, Atlantic cod do seem to retain some form of homeostasis during progressively deep hypoxia because major physiological stress (*i.e.* elevated cortisol and lactate) is not apparent at pressures in excess of S_{crit} (Herbert & Steffensen, 2005).

Herbert & Steffensen (2005) exposed Atlantic cod progressively to a severe level of P_{O_2} (4·3 kPa) during a 7 h long-term acute (LTA) hypoxia experiment but the behavioural and physiological response of Atlantic cod has never been examined in response to short-term acute (STA) hypoxia. The current study therefore aimed to examine the behaviour and physiology of Atlantic cod in response to a highly acute decline in P_{O_2} to 4·3 kPa over a 1 h period and a suitable comparison was made with the LTA hypoxic study of Herbert & Steffensen (2005). Assuming Atlantic cod mount an appropriate behavioural response to STA hypoxia, it might be expected that a more rapid drop in swimming speed and no additional stress at 4·3 kPa (Herbert & Steffensen, 2005) would be observed. Alternatively, Atlantic cod may experience heightened physiological stress as a result of inappropriate swimming speeds and the greater rate in P_{O_2} reduction.

Eight Atlantic cod, mean ± S.D. 447 ± 134 g and 38 ± 5 cm fork length (L_F), were collected by trawl and acclimated in several 450 l tanks at the Marine Biological Laboratory, Helsingør, Denmark, over a 3 week period. Fish were maintained under a 12D:12L regime in near-fully air-saturated sea water (salinity = 30; temperature = 10·0° C, range ± 0·2° C) with regular feeding. The behavioural and physiological response of individual Atlantic cod to an acute hypoxic exposure was assessed using the experimental apparatus of Herbert & Steffensen (2005). In brief, a 1·5 × 2·0 cm black rubber marker was sutured on to the dorsal nasal region of a fish anaesthetized with benzocaine (40 mg l^{-1} bicarbonate buffered sea water). The single fish was subsequently placed in a round experimental tank (129 cm diameter, 60 cm deep), supplied with a continuous flow of near-fully air-saturated sea water (10·0° C, range ± 0·2° C), and left to acclimate without any disturbance for 2 days. During experimental periods, the geometric centre of the black marker was determined using a customized software programme and its x and y co-ordinates transmitted to a data acquisition package at a rate of 10 Hz. The average rate of swimming speed [*i.e.* the cumulative distance swum in body lengths (L_F) per s] was calculated from the positional (x and y) data.

Hypoxia was generated using the re circulatory flow technique of Herbert & Steffensen (2005). In brief, water P_{O_2} was manipulated in a separate 930 l (oxygenating and deoxygenating) mixing tower and transferred back to the experimental tank under gravity without any disturbance to either the fish or the behavioural tracking. Water P_{O_2} was adjusted by bubbling air (for oxygenation) or nitrogen (for deoxygenation) and was monitored with a WTW microprocessor oximeter (OXI 196) equipped with an oxygen probe at one side of the main experimental tank. For continuous deoxygenation, an O_2-regulating unit received the tank P_{O_2} signal and, *via* a solenoid valve, controlled the flow of gas from a compressed bottle of nitrogen. Rapid mixing ensured that oxygen levels were uniform throughout the experimental tank. Re-circulating water (5° C) was periodically passed over cooling coils in the separate mixing chamber

to ensure constant water temperatures (*i.e.* 10·0° C, range ± 0·3° C) were maintained throughout the experimental period.

Behavioural monitoring always commenced at 0900 hours after at least 48 h of acclimation. The experiment ran for a total of 2·5 h and was divided into three main periods. Period 1: the routine speed of Atlantic cod was observed in near-fully air-saturated sea water ($P_{O_2} = 20{\cdot}9$ kPa) over a 1 h period. Period 2: the speed of Atlantic cod in response to a highly acute decline in P_{O_2} (20·9–4·3 kPa) was monitored over the second 1 h period. To assess the speed of Atlantic cod at different levels of hypoxia, period 2 was divided into six 10 min intervals (*i.e.* periods 2A to 2F). These 10 min periods corresponded to the following mean P_{O_2} values: 2A = 19·1 kPa; 2B = 15·0 kPa; 2C = 11·5 kPa; 2D = 8·9 kPa; 2E = 6·9 kPa; 2F = 5·3 kPa. Period 3: Atlantic cod were maintained under deep hypoxia ($P_{O_2} = 4{\cdot}3$ kPa) for a final 30 min period. Fish were held at 4·3 kPa for 30 min because it was compatible with the methodology of Herbert & Steffensen (2005) and effectively enabled the physiological stress of Atlantic cod following STA and LTA to be compared (Fig. 1 provides a graphical description of the three experimental periods). To analyse the effect of acute hypoxia on Atlantic cod swimming speed, the mean (expected) routine speed of fish in period 1 was compared against all other intervals in periods 2 and 3 using one-way ANOVA (Statistica 5·5) and an *a priori* planned comparison. Herbert & Steffensen (2005) have shown that the expected routine activity level of Atlantic cod is constant over the 0900–1130 hours period; it was not necessary therefore to monitor the routine speed of Atlantic cod in near-fully oxygenated sea water over the entire 2·5 h period.

At the end of period 3, individual fish were netted and killed immediately (<20 s) with a sharp blow to the head and a sample of blood (*c.* 1·0 ml) extracted rapidly by caudal venepuncture using a heparinized syringe. Fifty microlitres of whole blood was deproteinized in 100 μl 0·6 mol l^{-1} perchloric acid, spun at 12 000 *g* and 4° C for 5 min and the supernatant stored at −20° C for later analysis of blood lactate. The remaining sample of whole blood was spun for 5 min at 4° C and the supernatant stored at −20° C for <48 h before being analysed for plasma cortisol and glucose, using standard photometric techniques (Herbert & Steffensen, 2005). Osmolality was measured immediately using a Wescor osmometer (Vapro™ 5520). The physiological values from the current experiment are classified hereafter as the STA (4·3 kPa) hypoxic group. To assess the effect of highly acute hypoxia, the current physiological values were statistically compared against the LTA (4·3 kPa) and the near-fully air-saturated (19·9 kPa) values of Herbert & Steffensen (2005) using one-way and Kruskal–Wallis ANOVA (followed by appropriate *post hoc* tests for pairwise comparisons). The current study only examined the response of Atlantic cod to STA hypoxia but all fish in the two studies were 1) caught in the same area of the Danish Øresund, 2) acclimated under the same conditions as Herbert & Steffensen (2005) and 3) monitored using the same apparatus as Herbert & Steffensen (2005). A statistical comparison between STA and LTA hypoxia is therefore valid. Significance was accepted at $P < 0{\cdot}05$.

The STA hypoxia had a highly significant effect on the swimming speed of Atlantic cod (one-way ANOVA, $P < 0{\cdot}01$) (Fig. 1). Fish responded with a significant (29%) increase in speed during period 2A when P_{O_2} was first

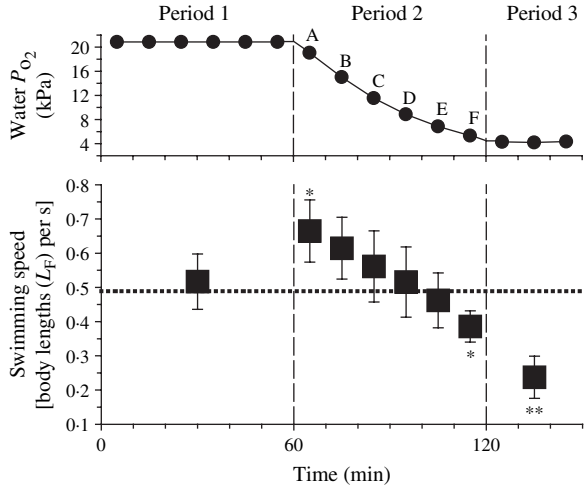

FIG. 1. The mean ± 95% CI rate of swimming speed for Atlantic cod in response to short-term acute hypoxia. Period 1 shows the mean routine swimming speed of fish in near-fully air-saturated sea water (P_{O_2} = 20·9 kPa) over the first 1 h period. Period 2 shows fish swimming speed in response to a rapid 1 h decline in P_{O_2} (20·9–4·3 kPa). Period 2 is divided further into six 10 min intervals (*i.e.* periods 2A to 2F). Period 3 shows the mean speed of fish in deep hypoxia (P_{O_2} = 4·3 kPa) over the final 30 min period. The horizontal dashed line, the mean routine swimming speed of Atlantic cod in the study of Herbert & Steffensen (2005). *$P < 0·05$; **$P < 0·01$.

reduced (Fig. 1) ($P < 0·05$). There was no apparent change in speed of Atlantic cod during periods 2B to 2E but speed was reduced by 25% ($P < 0·05$) and 54% during periods 2F and 3, respectively ($P < 0·01$). Routine swimming speed in the current study did not differ from the routine speed of Atlantic cod in the study of Herbert & Steffensen (2005) (Fig. 1). Based on the observed level of cortisol, lactate and osmolality (and an appropriate comparison with LTA hypoxia), STA hypoxia resulted in a highly severe level of physiological stress (Table I). Compared with fish in LTA hypoxia, STA hypoxia resulted in a pronounced and greater increase in blood lactate and plasma osmolality concentrations ($P < 0·05$) but neither STA nor LTA hypoxia affected blood glucose levels ($P > 0·05$). Although no significant difference was observed ($P > 0·05$), mean cortisol in the STA, 4·3 kPa, group (214·7 ng ml^{-1}) was considerably higher than that in the LTA, 4·3 kPa, group (108·8 ng ml^{-1}) and indicated a severe physiological stress response.

Atlantic cod clearly reduce their swimming speed in response to STA and LTA hypoxia and, since the relationship between speed and O_2 consumption is well known for Atlantic cod and other fish species, this be addre represent a drop in energetic expenditure under progressively low P_{O_2} (Fig. 1; Schurmann & Steffensen, 1994, 1997; Chabot & Dutil, 1999; Petersen & Steffensen, 2003; Herbert & Steffensen, 2005). In response to the initial decline in P_{O_2}, however, Atlantic cod also react with an increase in speed which not only occurs during STA and LTA hypoxia (Herbert & Steffensen, 2005) but also shows a surprising degree of behavioural-P_{O_2} sensitivity. Since this initial increase in speed is almost immediate and there is no evidence of major physiological changes in Atlantic

TABLE I. The physiological stress response of Atlantic cod to short-term acute (STA) hypoxia (4·3 kPa). Data regarding the physiological response of fish in near-fully air-saturated sea water (19·9 kPa) and following long-term acute (LTA) hypoxia (4·3 kPa) are also tabulated for comparative purposes and originate from the study of Herbert & Steffensen (2005). All data are mean ± S.D. (*n*)

Group	Cortisol (ng ml^{-1})	Lactate (mmol l^{-1})	Osmolality (mmol kg^{-1})	Glucose (mmol l^{-1})	Reference
STA, 4·3 kPa	214·7 ± 140 (8)	2·41 ± 1·14 (8)	337·6 ± 7·1 (8)	2·87 ± 1·24 (8)	Present study
LTA, 4·3 kPa	108·8 ± 49·5 (5)	1·05 ± 0·52 (5)	322·0 ± 14·3 (5)	3·53 ± 1·07 (5)	Herbert & Steffensen (2005)
19·9 kPa	22·5 ± 21·6 (7)	0·32 ± 0·18 (8)	317·3 ± 11·2 (8)	3·64 ± 0·90 (7)	Herbert & Steffensen (2005)

cod at pressures >13·2 kPa (Fig. 1; Fritsche & Nilsson, 1989; Perry *et al.*, 1991; Claireaux & Dutil, 1992), it is probably mediated by peripheral O_2 chemoreceptors and has previously been interpreted as an early avoidance response (Herbert & Steffensen, 2005). The behavioural response of Atlantic cod to STA and LTA hypoxia may appear qualitatively similar (Fig. 1; Herbert & Steffensen, 2005) but there are three important quantitative differences. Atlantic cod in STA hypoxia show 1) a greater increase in speed (+29 *v.* +18%) during the first drop in P_{O_2}, 2) a delayed drop in speed (since the first observable reduction occurs at 5·3 kPa rather than at 8·4 kPa) and 3) a greater reduction in speed (−54 *v.* −41%) at the lowest level of P_{O_2} (*i.e.* 4·3 kPa).

Despite major behavioural adjustments, a greater level of physiological stress appears to be manifest by Atlantic cod in STA *v.* LTA hypoxia (Table I). With the exception of glucose, the chosen physiological indicators are all extremely high and approached near-maximal values in the STA, 4·3 kPa, group (Herbert & Steffensen, 2005) (Table I). A short period of time at 4·3 kPa, however, should only yield a slight-moderate level of stress (*v.* near-maximal disturbances in homeostasis) because this P_{O_2} is only slightly lower than the S_{crit} and maybe even the $LC50_{96h}$ (the lethal concentration required to kill 50% of a population in 96 h) of Atlantic cod (Schurmann & Steffensen, 1997; Plante *et al.*, 1998; Claireaux *et al.*, 2000; Herbert & Steffensen, 2005). Rapid behavioural modifications are probably required during STA hypoxia but, as a result of the delayed drop in swimming speed (Fig. 1), Atlantic cod may have involuntarily recruited anaerobic metabolism after exhausting their available aerobic scope (Claireaux *et al.*, 2000). This is certainly feasible since the aerobic metabolic scope of *G. morhua* is compressed by 52% at 8·4 kPa and 10° C (Claireaux *et al.*, 2000) but no noticeable drop in speed, hence O_2 consumption, was observed at that level of P_{O_2} (Fig. 1). It is equally plausible, however, that heightened physiological stress was manifest simply as a result of the greater rate in P_{O_2} reduction. Metabolic scope is relatively unchanged at high P_{O_2} (Claireaux *et al.*, 2000), suggesting that the primary rise in swimming speed at 19·1 kPa was fuelled entirely by aerobic pathways and is unlikely to have caused the elevated

level of stress during STA hypoxia. The different time-dependent response of each physiological variable (*i.e.* cortisol, lactate, glucose and osmolality) is also not likely to account for the generally heightened level of stress.

Atlantic cod exhibit a greater increase and decrease in swimming speed during STA (*v.* LTA) hypoxia and these responses are associated with heightened physiological stress. Depressed rates of activity may confer an energetic advantage in low O_2 but Atlantic cod are only moderately tolerant of low O_2 (Plante *et al.*, 1998; Herbert & Steffensen, 2005) and, with the elevated level of stress in STA hypoxia, are not likely to survive long in an environment that experiences a rapid and long-term reduction in P_{O_2}. Atlantic cod were exposed to an inescapable decline in P_{O_2} but this is a relatively uncommon situation for Atlantic cod in the wild; Atlantic cod typically inhabit a physically heterogeneous environment (Neuenfeldt, 2002) and may theoretically enhance their survival by preferentially selecting regions with lower temperatures (*i.e.* behavioural thermoregulation) or higher P_{O_2} (Claireaux *et al.*, 1995; Petersen & Steffensen, 2003). Since Atlantic cod were not able to avoid hypoxia in the current study, the large primary rise and the secondary delayed drop in swimming speed was probably an inappropriate response to inescapable STA hypoxia. The understanding of Atlantic cod O_2 tolerance has been furthered in the current study but future experimentation should focus on the hypoxia avoidance behaviour of Atlantic cod by offering a choice of environmentally relevant P_{O_2} gradients. Due to conflicting reports in this area (Claireaux *et al.*, 1995; Neuenfeldt, 2002), hypoxia avoidance still remains poorly understood and definitely warrants further investigation.

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