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Reduced growth of Atlantic cod in non-lethal hypoxic conditions

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Growth in length and mass, improvements in condition, as well as final condition of *c.* 700 g Atlantic cod *Gadus morhua* were significantly less at 45% and 56% O_2 saturation than at 65%, 75%, 84% and 93% O_2 saturation. Hypoxia decreased food consumption. In turn, food consumption explained 97% of the variation in growth. Conversion efficiency varied slightly, but significantly, with level of dissolved O_2, except that the group reared at 93% O_2 had a lower than expected conversion efficiency. Slow growth in low O_2 was not due to increased activity, because activity decreased in hypoxia. In the Gulf of St Lawrence, waters deeper than 200 m usually are <65% saturated in O_2, and thus should impact negatively on cod growth.

Key words: dissolved oxygen; growth rate; *Gadus morhua*; food ingestion.

INTRODUCTION

Atlantic cod *Gadus morhua* L. from the Gulf of St Lawrence exhibit much slower growth rates than other stocks in the North Atlantic, a fact attributed mostly to the cold waters they inhabit (Brander, 1995; Campana *et al*., 1995). But cod from this stock frequently encounter another unfavourable environmental condition, hypoxia or low levels of dissolved oxygen (O_2). The deep waters of the Gulf of St Lawrence are of oceanic origin, with varying proportions of Labrador and western north Atlantic water entering at the mouth of the Laurentian Channel, and flowing upstream to the estuary (Bugden, 1991). These waters are already hypoxic at the mouth of the Laurentian channel, and O_2 levels decrease further as the waters flow slowly upstream, due to decomposition of organic matter which sinks to the bottom (Coote & Yeats, 1979). Thus waters $\geq$200 m are <65% saturated in O_2 in the Gulf, and <35% saturated in the estuary (D'Amours, 1993; Gilbert *et al*., 1997).

Low levels of dissolved O_2 can be lethal to marine organisms. In the laboratory, mortalities in cod from the Gulf of St Lawrence are observed first at 28% saturation, whereas the lethal threshold (50% mortality) is at 21% saturation between 2 and 6° C (Plante *et al*., 1998). But even above these limits, hypoxia can have an impact on cod. Oxygen is a known limiting factor for fish metabolism, and as such determines growth and activity levels (Fry, 1971; Brett, 1979). Examples of reduced growth in freshwater fish exposed to hypoxia

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abound (Stewart *et al.*, 1967; Andrews *et al.*, 1973; Weber & Kramer, 1983; Cech *et al.*, 1984; Pedersen, 1987), but the extent of growth reduction in hypoxia is unknown for marine species, probably because hypoxia is perceived to be a problem less often in marine environments.

In the present study, to determine the critical level of dissolved O_2 for growth, cod were reared at different levels of dissolved O_2, and changes in length, mass and condition were determined. The impact of dissolved O_2 levels was measured on some physiological variables (haemoglobin, haematocrit, mean cellular haemoglobin content, lactate and glucose concentrations in liver, muscle and plasma, as well as osmotic pressure and Na^+, K^+ and Cl^- concentrations in blood), spontaneous swimming activity, food consumption and food conversion.

MATERIALS AND METHODS

CAPTURE AND HOLDING OF FISH

Cod were caught by trawl near Matane, Québec (58°53′N; 68°30′W) in June 1995 and were maintained under natural photoperiod and artificial lights in 13-m^3 tanks at the Maurice-Lamontagne Institute, Mont-Joli, Québec, Canada until needed for the experiments. Water temperature in the holding tanks varied from 6° C in June to 10° C in late August with an average salinity of 28 (measured by conductivity). Fish were fed capelin *Mallotus villosus* (Müller) *ad libitum* once weekly.

EXPERIMENTAL SET-UP

The set-up consisted of six independent flow-through 800-l circular tanks. Another circular tank (1300 l) was used to hold a separate group of cod in normoxia. Oxygen saturation was adjusted by bubbling a mixture of air and nitrogen into the gas exchange column which flowed into each tank. A computer connected to a polarographic O_2 electrode (ATI Orion, model 870 with DO Probe 087010) and to a series of solenoid valves, monitored O_2 saturation in each tank every 30 min. The flow rate of air and nitrogen was adjusted manually at least once a day to maintain the desired levels of dissolved O_2. The electrode was calibrated daily in water-saturated air. On four occasions, dissolved O_2 levels were also measured by titration using a modification of the Winkler method (Jones *et al.*, 1992).

Temperature was maintained at 10° C by adjusting the temperature of the water entering the experimental tanks at 5 l min^{-1}, and the control tank at 7 l min^{-1}. For each experimental tank, a pump recirculated water through the degassing column at *c.* 25 l min^{-1} to facilitate control of dissolved O_2. In the daytime, dim light was provided by a 60-W incandescent light aimed at the ceiling of the room. In addition, continuous lighting was provided by fluorescent lights covered with red filters.

A custom-built stand supported a video camera *c.* 1·5 m above the water surface. An additional red light (40-W red incandescent bulb) positioned close to the surface, above the central drain of the tank, ensured that all fish were visible on screen. The camera was hooked to a time lapse video-recorder. The light and camera were used periodically to monitor spontaneous swimming activity.

GROWTH EXPERIMENT

On 11 October 1995 a baseline group of 20 cod was transferred directly from the holding tanks (4·5° C) to the normoxic tank (6·4° C). Water temperature was raised to 10° C over 48 h. These fish were fed capelin *ad libitum* for 1 h on 13, 17 and 20 October, and were sampled on 24 October to obtain baseline values of morphological and physiological variables. Salinity was ~28, and dissolved O_2 was >80% of O_2 in air-saturated water.

Twelve days prior to the beginning of the experiment, 135 cod were anaesthetized (5 mg l^{-1} metomidate hydrochloride, Wildlife Laboratories Inc., Vancouver, BC, Canada), measured (fork length, L_F, in mm, and total mass, M_T, in g), tagged (Visible Implant Tags, Northwest Marine Technology, Shaw Island, WA, U.S.A.) and then placed randomly into one of the six experimental tanks, which contained water at the same temperature as the holding tanks (6° C). After a 6-day recovery period, temperature was increased progressively to 10° C ($\approx$1° C day^{-1}) whereas dissolved O_2 was reduced progressively to the desired levels.

On 30 October, 15 fish were removed and weighed to estimate mass loss during the 12-day acclimatization period. The experiment consisted of raising six groups of 20 cod at 10° C for 84 days (until 22 January 1996) at a different level of dissolved O_2 (45, 56, 65, 75, 84 or 93% saturation). Two cod died during the experiment, but not because of the treatments. The first fish died on day 33 (1 December 1995) at 65% saturation, after several days of reduced activity and feeding. The second death occurred on day 43 (11 December 1995) at 93% saturation, when a fish jumped out of the tank. These fish were excluded from the results and analyses.

On 5 November 1995, it was necessary to add an air diffuser directly into the tank with 93% saturation because of the high O_2 consumption of the fish, especially after meals. For the same reason, a diffuser was added to the tank at 84% saturation on 12 December 1995. Salinity remained at $\sim$28 for the entire duration of the experiment. Cod were fed capelin *ad libitum* for 1 h three times weekly. Meals were initiated between 1000 and 1400 hours. Food consumption was obtained by subtracting the mass of the orts (uneaten food) from that of capelin offered, and dividing by the number of fish in the tank. For each tank, this quantity was summed for all the feedings in the experiment and divided by 84 to obtain an average daily food consumption (I) per fish. Growth efficiency (E) in each treatment was calculated as the average growth in mass divided by I and multiplied by 100.

MORPHOLOGICAL AND PHYSIOLOGICAL MEASURES

Baseline fish were killed on 24 October 1995, by a blow to the head. Experimental fish were killed on 22 or 23 January 1996. The same measurements were taken on all fish. Only one fish was sampled at a time to minimize time between death and tissue collection. L_F and M_T were measured, and 5 ml of blood collected. A fraction of the blood was used to measure haemoglobin (cyanmethaemoglobin procedure, Sigma no. 525) and haematocrit. The latter was measured using preheparinized microhaematocrit capillary tubes centrifuged at 10 400 ***g*** for 5 min. The remaining blood was centrifuged at 4200 ***g*** for 5 min, and the plasma was collected into three cryovials, quickly frozen in liquid nitrogen and then stored at -80° C for determination of Na^+, K^+ and Cl^- (Ciba-Corning Model 644 $Na^+/K^+/Cl^-$ Analyser), osmotic pressure (Advance Model 3MO Micro-Osmometer), glucose (Sigma no. 16-UV), and lactate (Sigma no. 826-UV). The liver and gonads were excised and weighed (M_L and M_G, respectively). Approximately 10 g of liver and muscle (taken below the first dorsal fin) were dried 48 h at 65° C for determination of water content. Samples of liver and muscle were also frozen in liquid nitrogen and stored at -80° C for further analysis of glucose and lactate. Finally, the stomach content (M_{SC}) was weighed. Samples were handled and processed on ice.

Mean cellular haemoglobin content (MCHC) was calculated as the haemoglobin : haematocrit ratio (in %). Somatic mass ($M_S = M_T - M_G - M_{SC}$), Fulton's condition index ($K = 100 \cdot M_S \cdot L_F^{-3}$), the hepatosomatic index ($I_H = M_L \cdot 100 \cdot M_S^{-1}$) and the gonadosomatic index ($I_G = M_G \cdot 100 \cdot M_S^{-1}$) were calculated. Growth in L_F and M_T, and changes in K and I_H were calculated by comparing final and initial values of these variables. To obtain initial M_T and M_S of experimental fish, it was assumed that M_T decreased by 5·6% between 18 and 30 October (based on mass loss of 15 fish reweighed on 30 October), and that M_{SC} was zero (fish were fasted 5 days prior to the weighing of 18 October), and M_G was 0·6% of M_T (based on M_G of 20 baseline fish). Some samples were lost because of a freezer's failure. Thus no lactate or glucose data for liver or muscle of control fish were available, and the sample size was reduced for some variables in some experimental groups.

TABLE I. Temperature and O_2 levels in the six experimental tanks (mean ± S.D.)

Treatment (% O_2)	n	O_2 (% saturation)	Temperature (°C)
45	3857	45·06 ± 2·00	9·96 ± 0·28
56	3815	55·61 ± 3·13	10·09 ± 0·28
65	3843	64·94 ± 3·90	10·06 ± 0·29
75	3738	75·38 ± 2·62	9·97 ± 0·26
84	3681	84·23 ± 3·94	10·02 ± 0·28
93	3712	93·15 ± 2·53	9·89 ± 0·26

LOCOMOTOR ACTIVITY

Because only one tank could be filmed at a time, each tank was filmed in succession. Filming was initiated in the morning following a feeding day, and lasted 24 h at 1 frame s^{-1}. Each experimental tank was filmed four times during the experiment.

Upon playback of the tapes, a line was superimposed on the screen from the centre of the tank to the edge, using the sector where visibility was best. For each recording, samples lasting 1 min were examined every 15 min in the interval 1800–2400 hours. For each sample, the number of cod that crossed the line completely, in any direction, was divided by the number of cod in the tank and used as an index of spontaneous locomotor activity. It should be noted that fish could not be recognized individually, and that the activity scored could have been influenced by a few more active fish.

STATISTICAL ANALYSES

The four regressions of Winkler titrations on oxymeter readings were compared by analysis of covariance (ANCOVA). They did not differ in slope ($F_{3,21}=0{\cdot}32$, $P=0{\cdot}81$) nor in intercept ($F_{3,24}=2{\cdot}83$, $P=0{\cdot}06$), and were pooled. The resulting equation was used to correct the oxymeter readings for the entire experiment:

$$O_{2(\text{corrected})}=3{\cdot}93+1{\cdot}033\ O_{2(\text{oxymeter})},\ \cdot\ r^2=0{\cdot}99$$

Initial and final values of L_F, M_T and K for each treatment were compared with one-tailed t-tests. Comparisons of morphometric or physiological variables among groups were made by one-way analysis of variance (ANOVA). Significant ANOVAs were followed by Tukey–Kramer *post hoc* comparisons. Because of heterogeneity of variances (F_{max} test, Sokal & Rohlf, 1995), *post hoc* comparisons of growth in mass, I_G, muscle water content, liver lactate, muscle lactate, plasma lactate and osmotic pressure were made with the Games–Howell method (Sokal & Rohlf, 1995). Critical levels of dissolved O_2 for growth in length and in mass, and for change in condition, were defined as the intersection of two lines: the regression line fitted to the data for the three lowest levels of dissolved O_2, and a line representing the mean in the two highest levels of dissolved O_2 (normoxia). All statistical tests were made using SuperAnova (Abacus Concepts, 1989) or StatView 5 (SAS Institute, Inc., 1998).

RESULTS

TEMPERATURE AND OXYGEN LEVELS

Cod were maintained at the targeted values of temperature and O_2 for the entire experimental period (Table I), although O_2 levels usually dropped by 5–8% saturation for a few hours following meals. Pump failures caused two major drops in O_2 levels during the experiment. On day 44, O_2 dropped to 30%

TABLE II. Initial values of length, mass and condition factor (mean ± S.D.) in the experimental groups and in the baseline group sampled at the beginning of the experiment

Treatment (% O_2)	n	Length (mm)	Mass (g)	Condition factor (K)
45	20	441·3 ± 31·0	698·0 ± 204·1	0·79 ± 0·12
56	20	439·3 ± 27·0	717·7 ± 171·6	0·83 ± 0·09
65	19	441·4 ± 29·1	687·1 ± 155·8	0·78 ± 0·09
75	20	439·2 ± 42·7	731·0 ± 240·0	0·83 ± 0·09
84	20	441·2 ± 32·7	693·0 ± 174·6	0·79 ± 0·11
93	19	450·3 ± 24·9	766·2 ± 182·4	0·82 ± 0·09
All treatments	118	442·0 ± 31·4	715·3 ± 188·2	0·81 ± 0·10
Baseline group	20	449·0 ± 29·9	817·3 ± 175·3	0·88 ± 0·05

saturation for 16 h in the 65% saturation treatment, and on day 48 it dropped to 42% for 11 h in the treatment at 84% saturation.

Temperature differences between tanks were small (range=0·2° C, Table I) but significant ($F_{5,22641}$=254, P<0·0001), owing to large sample sizes. Temperature differed among all tanks except those at 45% and 75% saturation. These differences are negligible in the context of the experiment.

INITIAL SIZE OF FISH

At the beginning of the experiment, L_F ($F_{6,131}$=0·41, P=0·87) and M_T ($F_{6,131}$=1·24, P=0·29) were similar among all treatment groups and the baseline group (Table II). Condition did vary among groups ($F_{6,131}$=2·81, P=0·01), but this was due to the slightly higher condition in the baseline group, as the six experimental groups did not vary in condition.

GROWTH DURING THE EXPERIMENT

At the end of the experiment, L_F averaged between 481·5 ± 27·6 (S.D.) mm and 509·6 ± 23·5 mm at 45 and 93% O_2, respectively. At the same levels of O_2, final M_T averaged between 1025·2 ± 198·5 and 1479·4 ± 297·8 g, whereas K averaged between 0·89 ± 0·08 and 1·09 ± 0·09. Significant growth in L_F ($t \geq 14{\cdot}9$, P<0·0001) and M_T ($t \geq 12{\cdot}8$, P<0·0001), and significant increases in K ($t \geq 4{\cdot}5$, $P \leq 0{\cdot}0001$) occurred in all O_2 conditions. However, there were significant differences in growth (L_F: $F_{5,112}$=9·1, P<0·0001; M_T: $F_{5,112}$=13·445, P<0·0001) and increases of condition ($F_{5,112}$=8·99, P<0·0001) among treatments (Fig. 1). Growth was reduced significantly at low levels of dissolved O_2. For instance, ΔL_F was less at 45% O_2 than at ≥65% O_2, and less at 56% than at 84% O_2. The effect of hypoxia on ΔM_T was even more pronounced. ΔM_T was significantly less at 45% O_2 than at any other O_2 level. At 56%, O_2, ΔM_T was less than at 84% and 93% O_2. Changes in K were also reduced at 45% and 56% O_2 (Fig. 1). Critical values of dissolved O_2 for growth in L_F and M_T were 65% and 73%, respectively. The critical value was 73% for changes in conditions (Fig. 1).

The ANOVAs comparing I_H, liver water content and muscle water content among treatments and the baseline group were all significant ($F_{6,131}$=15·6, 28·7

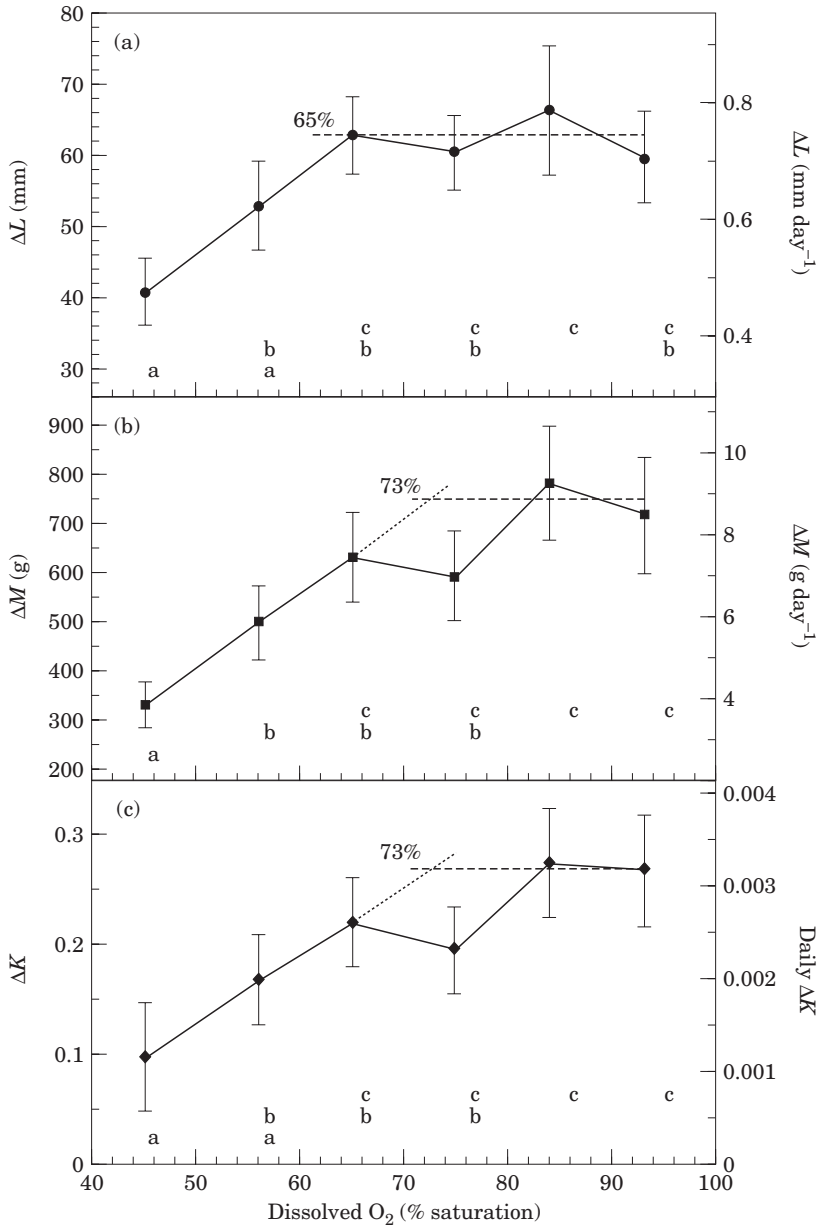

FIG. 1. Growth in (a) length, (b) mass and (c) change in condition factor (K) in Atlantic cod during an 84-day growing period at different levels of dissolved O_2. Error bars are 95% confidence intervals (CI). Means with the same letters did not differ significantly according to Tukey–Kramer [(a) and (c)] or Games–Howell (b) *post hoc* tests. – – –, Mean value in normoxia (84% and 93% O_2); · · · · the regression fitted to data for the three lowest levels of dissolved O_2. The intersection of these two lines is the critical level of dissolved O_2.

and 27·6, respectively, $P \leq 0{\cdot}0001$). I_H was significantly less at 45% O_2 than at 65, 84 or 93% O_2. Furthermore, I_H after a 84-day growing period at 45% O_2 did not differ significantly from I_H in the baseline group, which was sampled at the

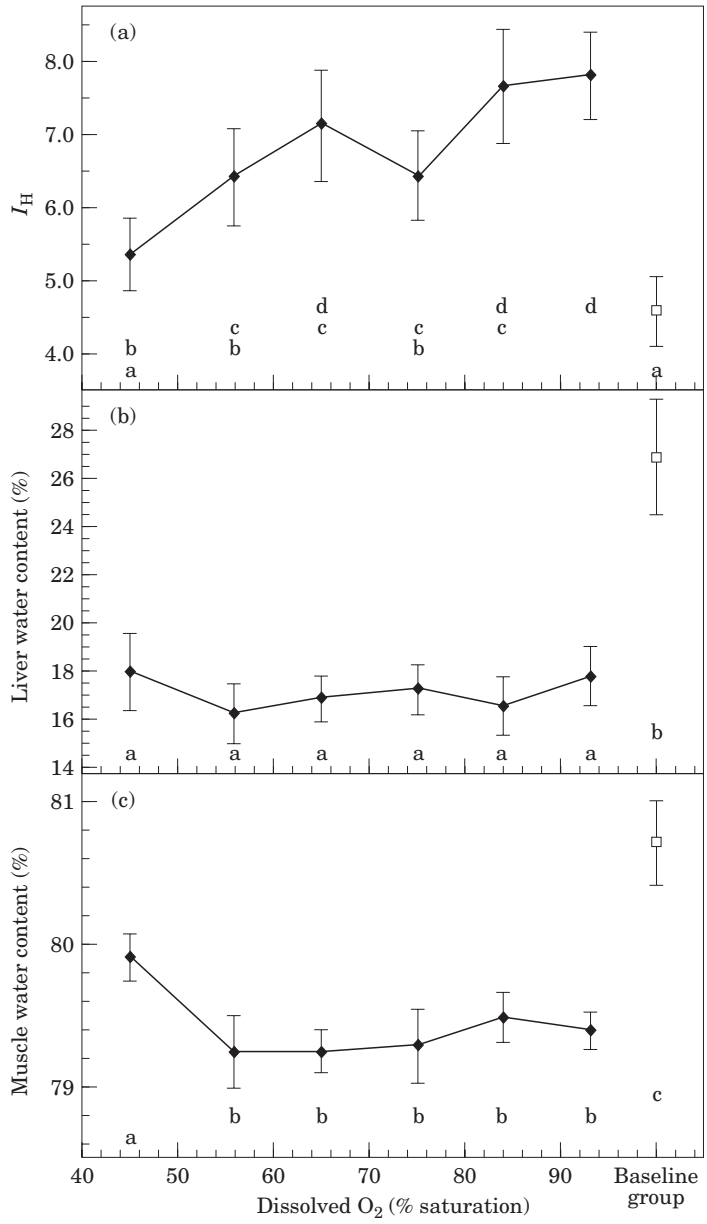

FIG. 2. Hepatosomatic index I_H (a), liver water content (b) and muscle water content (c) in Atlantic cod before (□) and after (◆) an 84-day growing period at different levels of dissolved O_2. Error bars are 95% CI. Means with the same letters did not differ significantly according to Tukey–Kramer (a) or Games–Howell [(b) and (c)] *post hoc* tests.

beginning of the growing period (Fig. 2). I_H at 56 and 75% O_2 were also lower than observed at the highest levels of dissolved O_2. Although severe hypoxia resulted in smaller livers, liver water content did not differ between treatments, and was lower in all treatments than in the baseline group (Fig. 2). Muscle

water content was also significantly lower in any treatment than in the baseline group, but it remained higher at 45% O_2 than in the other treatments (Fig. 2).

As expected for cod sampled in January, gonads had begun to grow, and I_G was higher in all treatments than in the baseline group (mean ± S.D.: 0·61 ± 0·41 g, $F_{6,131}=4{\cdot}36$, $P=0{\cdot}0005$), although the difference was significant only at 84% O_2. There was no difference among treatments, and I_G ranged from 1·24 ± 1·54 g at 65% O_2 to 5·08 ± 5·80 g at 84% O_2.

Compared with the average growth and condition at 84 and 93% O_2, chronic exposure to 45% O_2 reduced growth in L_F by 35%, growth in M_T by 56%, increase in K by 64% and final I_H by 30%. Exposure to 56% O_2 decreased these variables by 16, 33, 38 and 17%, respectively. In these comparisons, O_2 levels ≥84% were taken as normoxia because there was a definite trend for ΔM_T and ΔK to decrease below 84% O_2 (Fig. 1), and I_H was significantly higher at 84 or 93% than at 75% O_2. Growth in L_F and M_T as well as increases in condition (K, liver water content and muscle water content, but not I_H) were highest at 84% and not at 93% O_2, but these differences were not significant.

PHYSIOLOGICAL VARIABLES

Hypoxia had no clear effect on haemoglobin, haematocrit or MCHC (Table III). Haemoglobin was low in all treatments (≤4·75 g 100 ml^{-1}) relative to the baseline group sampled in October (5·13 g 100 ml^{-1}), although this was significant only for cod reared at 45, 84 and 93% O_2. There was no significant difference among the experimental groups except 56% and 84% O_2. Similarly, haematocrit was lower at 45, 75, 84 and 93% (≤22·35%) than in the baseline group (25·85%), and a significant difference was also found between 56 and 84% O_2. High values of haemoglobin accompanied large values of haematocrit, and there were no significant differences in MCHC.

The level of lactate was affected little by hypoxia (Table IV). No measures of liver and muscle lactate were available for the baseline group. The concentration of liver lactate ranged from 0·65 to 0·89 mg g^{-1}, and varied inversely with hypoxia. However significant differences were noted only for the extreme treatments, 45 and 93% O_2. The average lactate concentrations ranged from 3·25 to 4·15 mg g^{-1} in the white muscle, and there was no difference among the various levels of dissolved O_2. Plasma lactate ranged from 32 to 44 mg dl^{-1} and was not affected by the level of dissolved O_2, but all treatments had lower plasma lactate levels than did the baseline group (65 mg dl^{-1}).

Liver glucose was significantly higher at 84% O_2 than at 56% O_2 (1·45 *v.* 1·07 mg g^{-1}), but hypoxia produced no other difference: liver glucose had intermediate values in the other treatments (Table V). Muscle glucose levels varied between 0·30 and 0·42 mg g^{-1} and were not affected by hypoxia (Table V). Similarly, plasma glucose levels were unaffected by hypoxia (67–81 mg dl^{-1}) but lower than in the baseline group (117 mg dl^{-1}).

Chronic hypoxia did not produce significant differences in osmotic pressure, which varied between 338 and 346 mOsm kg^{-1} among treatments, but all experimental groups except one (84% O_2) had a lower osmotic pressure than the group sampled at the beginning of the study period (356 mOsm kg^{-1}) (Table VI). Similarly, sodium concentrations did not vary among treatments

TABLE III. Blood haemoglobin and haematocrit and mean cellular haemoglobin content (mean ± S.E.) in cod reared at various levels of dissolved O_2 and in a baseline group sampled at the beginning of the experiment

% O_2	Haemoglobin			Haematocrit			MCHC		
	g dl^{-1}	*n*	T–K	g dl^{-1}	*n*	T–K	g dl^{-1}	*n*	T–K
45	4·4 ± 0·2	20	ab	22·4 ± 0·9	20	ab	19·6 ± 0·3	20	a
56	4·8 ± 0·1	20	bc	23·3 ± 0·6	20	bc	20·5 ± 0·3	20	a
65	4·6 ± 0·1	19	abc	22·8 ± 0·6	19	abc	20·0 ± 0·3	19	a
75	4·6 ± 0·2	19	abc	22·3 ± 0·8	20	ab	20·4 ± 0·4	19	a
84	4·1 ± 0·1	20	a	20·2 ± 0·7	20	a	20·4 ± 0·3	20	a
93	4·3 ± 0·1	19	ab	21·4 ± 0·7	19	ab	20·4 ± 0·4	19	a
Baseline	5·1 ± 0·2	20	c	25·9 ± 0·7	20	c	19·8 ± 0·3	20	a

Groups with the same letter did not differ significantly according to Tukey–Kramer (T–K) tests.

TABLE IV. Lactate concentration in liver, muscle and plasma (mean ± S.E.) of Atlantic cod reared at various levels of dissolved O_2 and from a baseline group sampled at the beginning of the experiment

% O_2	Liver lactate			Muscle lactate			Plasma lactate		
	mg g^{-1}	n	G–H	mg g^{-1}	n	G–H	mg g^{-1}	n	G–H
45	0·65 ± 0·04	20	a	3·70 ± 0·22	17	a	33·6 ± 2·76	19	a
56	0·76 ± 0·07	18	ab	3·79 ± 0·50	17	a	32·1 ± 2·55	20	a
65	0·68 ± 0·04	19	ab	3·94 ± 0·27	19	a	43·6 ± 3·80	19	a
75	0·75 ± 0·05	20	ab	4·15 ± 0·32	19	a	42·1 ± 4·47	20	a
84	0·83 ± 0·08	20	ab	3·25 ± 0·27	19	a	32·4 ± 2·22	20	a
93	0·89 ± 0·07	19	b	3·63 ± 0·26	18	a	37·4 ± 4·23	18	a
Baseline							65·3 ± 5·50	20	b

Groups with the same letter did not differ significantly according to Games–Howell (G–H) tests.

TABLE V. Glucose concentration in liver, muscle and plasma (mean ± S.E.) of Atlantic cod reared at various levels of dissolved O_2 and from a control group sampled at the beginning of the experiment

% O_2	Liver glucose			Muscle glucose			Plasma glucose		
	mg g^{-1}	n	T–K	mg g^{-1}	n	T–K	mg g^{-1}	n	G–H
45	1·23 ± 0·09	19	ab	0·41 ± 0·05	19	a	67·5 ± 4·0	19	a
56	1·07 ± 0·09	18	a	0·33 ± 0·02	18	a	75·2 ± 3·0	20	a
65	1·19 ± 0·08	19	ab	0·40 ± 0·04	19	a	67·3 ± 2·8	18	a
75	1·18 ± 0·06	20	ab	0·42 ± 0·04	19	a	80·7 ± 3·3	20	a
84	1·45 ± 0·11	20	b	0·30 ± 0·02	19	a	71·0 ± 2·4	20	a
93	1·21 ± 0·07	19	ab	0·35 ± 0·04	19	a	73·1 ± 3·5	19	a
Baseline							117·4 ± 7·6	20	b

Groups with the same letter did not differ significantly according to Tukey–Kramer (T–K) or Games–Howell (G–H) tests.

TABLE VI. Blood osmotic pressure and ion concentration (Na^+, K^+ and Cl^-) (mean ± S.E.) of Atlantic cod reared at various levels of dissolved O_2 and from a baseline group sampled at the beginning of the experiment

% O_2	Osm. press			Na^+			K^+			Cl^-		
	mOsm kg^{-1}	*n*	G–H	mmol l^{-1}	*n*	T–K	mmol l^{-1}	*n*	T–K	mmol l^{-1}	*n*	T–K
45	339·7 ± 1·21	20	a	170·9 ± 1·08	20	a	4·22 ± 0·07	19	a	153·2 ± 0·82	20	ab
56	338·4 ± 1·82	20	a	171·8 ± 1·02	20	a	4·01 ± 0·07	20	ab	152·1 ± 0·85	20	a
65	340·9 ± 1·93	19	a	172·1 ± 0·83	19	a	4·18 ± 0·08	19	ab	149·9 ± 1·07	19	a
75	345·7 ± 2·01	19	a	172·3 ± 1·13	20	a	4·00 ± 0·06	20	ab	151·0 ± 1·05	20	a
84	344·4 ± 3·55	20	ab	173·1 ± 1·63	19	a	3·89 ± 0·07	19	b	151·1 ± 1·14	19	a
93	338·5 ± 3·53	18	a	173·5 ± 1·14	19	a	4·05 ± 0·07	18	ab	151·9 ± 1·13	19	a
Baseline	355·6 ± 1·66	20	b	178·7 ± 1·10	20	b	4·06 ± 0·08	20	ab	156·8 ± 0·58	20	b

Groups with the same letter did not differ significantly according to Tukey–Kramer (T–K) or Games–Howell (G–H) tests.

(170·9–173·5 mmol l^{-1}), but were significantly lower, at the end of the experiment than in the baseline group (178·7 mmol l^{-1}). Concentration of K+ differed significantly at 45 and 84% O_2 (4·22 and 3·89 mmol l^{-1}, respectively) but no other difference was found among treatments or the baseline group. With the exception of the cod reared at 45% O_2 (153·2 mmol l^{-1}), Cl^- concentration was significantly lower in the experimental groups (149·9–152·1 mmol l^{-1}) than in the baseline group (156·8 mmol l^{-1}). There was no difference in Cl^- concentration among treatments.

SWIMMING ACTIVITY

Spontaneous swimming activity was affected by the level of dissolved O_2 and by the date of recording (Fig. 3). In the first two thirds of the study period, activity was proportional to O_2 levels, and regressions of activity on O_2 level were significant. However, activity levels of cod exposed to $\geq$65% O_2 decreased in the last third of the study period, and there was no longer any relationship between activity and O_2. Activity was surprisingly high at 93% O_2 in the first half of the study, and at 56% O_2 for the entire study period.

FOOD INTAKE

Food intake was determined largely by the degree of hypoxia [Fig. 4(a)]: 93% of the variability in food intake was explained by O_2. It varied from 14·9 to 32·6 g day^{-1} $fish^{-1}$ at 45 and 93% O_2. When expressed relative to initial mass, ration varied from 2·1 to 4·6% day^{-1} at 45 and 84% O_2.

Conversion efficiency did not vary much among treatments (26–29%), and the relationship between E and O_2 was not significant [Fig. 4(b), P=0·61]. This was due to the surprisingly low E at 93% O_2, because the regression between E and O_2 was significant once this point was removed (P=0·011).

Food intake was the most important determinant of growth rate in cod subjected to chronic hypoxia, explaining 97% of its variance (Fig. 5). Only in normoxia did growth rate differ somewhat from that predicted by this relationship, as a result of the very different conversion efficiencies observed at 84 and 93% O_2 [Fig. 4(b)].

DISCUSSION

Oxygen availability influenced growth rate strongly in this study. Although positive growth rates were observed under all experimental conditions, growth rate was reduced markedly in the more extreme hypoxic conditions. The critical level for growth in length was 65% O_2, and growth was reduced significantly when levels of dissolved O_2 fell below 56%. The effects of hypoxia on growth in mass and thus on condition factor were even more pronounced. The critical level was 73%, and significant effects were observed below 65% O_2. Below 65% O_2 cod also had smaller livers. Compared with normoxia, growth in length was reduced by 36%, growth in mass by 56%, condition change by 64%, and liver index by 30% at 45% O_2.

Had growth been expressed in terms of change in energy content of the fish as recommended by Pedersen (1987), the conclusions on the impact of hypoxia would have remained the same, since body composition differed little among

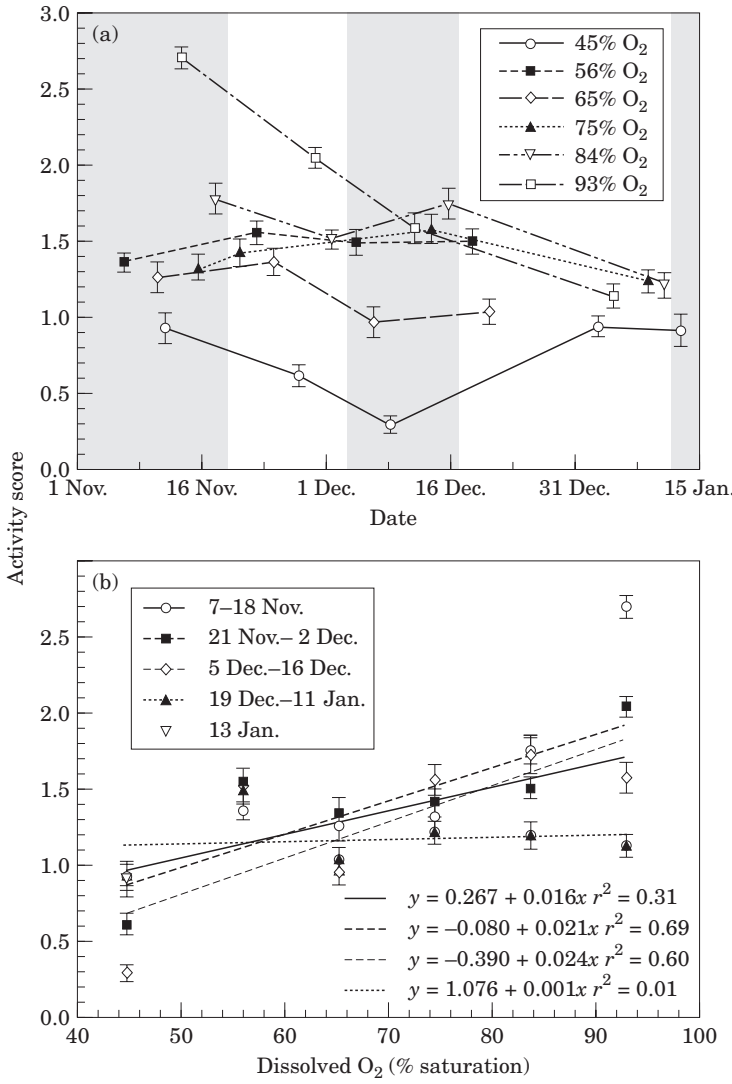

FIG. 3. Effect of dissolved O_2 on the spontaneous activity of Atlantic cod. (a) Activity level for each treatment as a function of date of recording: shading discriminates the time periods when the four series of recordings were made. (b) Regression of activity on dissolved O_2 level for each of four series of recording. Error bars are 95% CI.

treatments. Water content reflects the specific energy content in the muscle and liver of cod, and the condition factor and I_H are correlated closely with total energy content in the axial musculature and in the liver, respectively (Lambert & Dutil, 1997*a*). Therefore, in the absence of changes in the specific composition, differences in mass were proportional to differences in energy content. However, the impact of exposure to 45% O_2 would have been more pronounced if growth had been expressed in energy units, since the moisture content in muscle was significantly higher in this group. Stewart *et al.* (1967) also found an increase in water content in largemouth bass *Micropterus salmoides* (Lacépède) exposed to hypoxia.

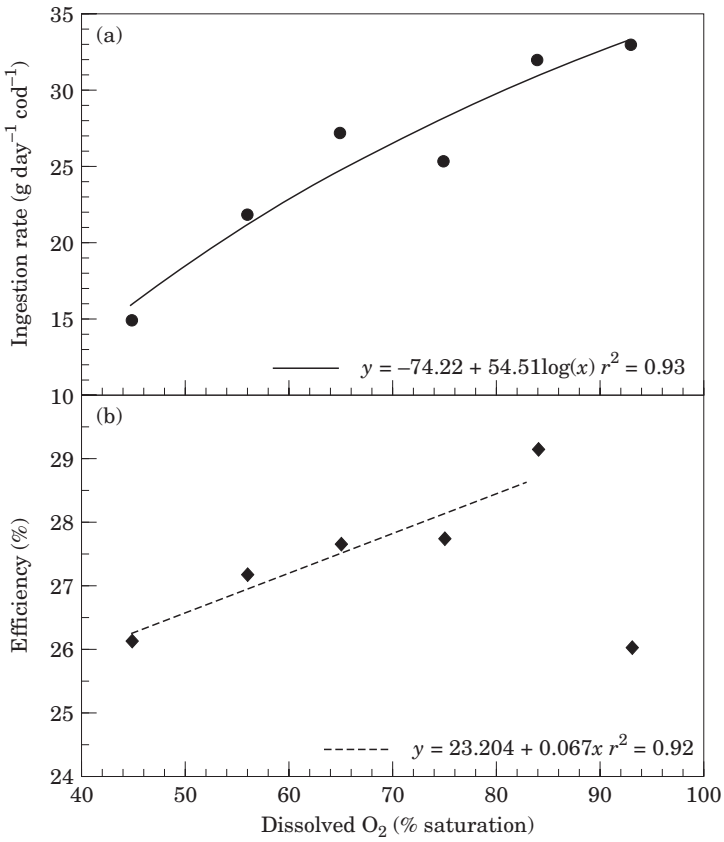

FIG. 4. Ingestion rate (a) and conversion efficiency (b) for Atlantic cod reared at various levels of dissolved O_2. In (b) the regression was calculated after excluding the point for 93% O_2.

During the first 8 weeks of the study, there appeared to be a direct relationship between spontaneous activity and the level of dissolved O_2 in the tanks. Schurmann & Steffensen (1994) also observed a reduction in swimming activity when cod were subjected to hypoxia, but only when O_2 became $<51{\cdot}7 \pm 10{\cdot}0\%$ (at 10° C), whereas activity was reduced in all tanks but the 93% O_2 tank in this study. This difference could result from the very different types of exposure to hypoxia in both studies: chronic in this study, and acute in Schurmann & Steffensen (1994). However, activity was very high at 93% O_2 in the first month of this study, compared with 84% O_2, even though growth was similar in both tanks. A tank effect, possibly related to the proximity of the tank at 93% O_2 to a source of noise (an air compressor), might be responsible for the heightened activity at 93% O_2. Removing data for this tank altered greatly the shape of the relationship between activity and dissolved O_2, so that activity no longer appeared to correlate strongly with O_2 availability, but it remained clear that activity was reduced at 45% O_2 (Fig. 3).

Space may have restricted swimming as cod grew larger, which may have obscured differences in activity, particularly in the latter part of the study. For instance, Saunders (1963) observed that crowding decreased routine

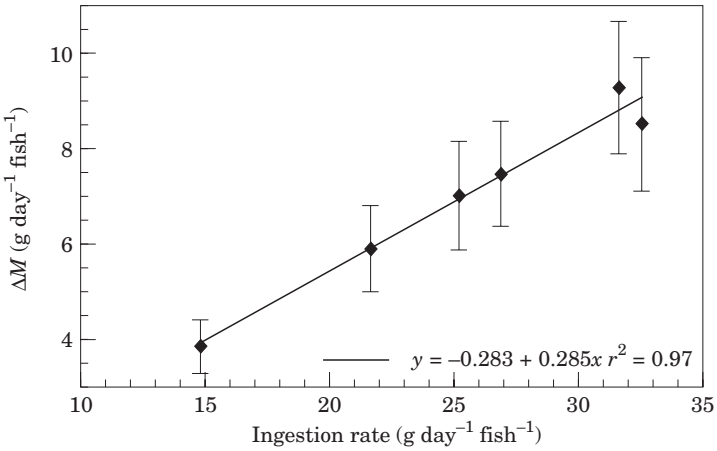

FIG. 5. Relationship between growth rate and food intake of Atlantic cod reared at various levels of dissolved O_2.

O_2 consumption in cod. He proposed that this was due to a reduction in freedom of movement, which depends on the degree of crowding and the relation between fish length and diameter of the tank. In the present experiment crowding remained constant, but the relationship between fish length and diameter of the tank changed with time, and in relation to the level of dissolved O_2.

Because individual fish were not recognized, a few individuals with extreme activity levels could have influenced the measure of mean activity in each tank. However, predicting activity level in relation to dissolved O_2 was not attempted, but rather whether differences in mean activity could have contributed to the differences in growth among tanks was assessed. Although it is difficult to assess clearly the impact of O_2 on activity in this study, increased activity can be ruled out as a potential mechanism involved in the reduced growth at low levels of dissolved O_2.

Food consumption was directly related to O_2 availability [Fig. 4(a)], as was reported in freshwater fish (Stewart *et al.*, 1967; Andrews *et al.*, 1973; Pedersen, 1987). Feeding involves an energetic cost, both to acquire and to process food. The cost of activity is negligible in the laboratory. In contrast, digestion is costly as it may increase O_2 consumption up to fivefold (Brett & Groves, 1979). In juvenile cod fed to satiation (about 4% day^{-1}), postprandial O_2 consumption, which reflects the cost of food digestion and assimilation, was about twice that of starved cod and stayed at that level as long as cod fed regularly (Saunders, 1963; Soofiani & Hawkins, 1982). Edwards (1972) even observed fivefold increases in metabolic rate of cod fed a high ration.

Because the cost of food assimilation in cod increases with food intake and is nearly as demanding as maximum activity (Edwards *et al.*, 1972; Soofiani & Hawkins, 1982), reducing food ingestion is an obvious and unavoidable way for cod to face hypoxia. A consequence of this is a reduction in growth rate: in this study, food consumption explained 97% of the variation in growth rate (Fig. 5).

Except for cod reared at 93% O_2, conversion efficiency was related directly to O_2 level [Fig. 4(b)]. This is also the case for freshwater fish species (Stewart *et al.*,

1967; Andrews *et al.*, 1973; Brett & Blackburn, 1981; Pedersen, 1987). Lower conversion efficiencies in hypoxia cannot be explained by the concomitant decreases in food consumption because in normoxia, maximum conversion efficiencies of cod are observed at intermediate ration levels (Edwards *et al.*, 1972). Since cod increase ventilation in hypoxia (Saunders, 1963; Claireaux & Dutil, 1992), the increased cost of ventilation, which can reach 10% of standard metabolic rate (Hughes, 1981), could explain the relationship between conversion efficiency and dissolved O_2 observed in this study. Increased cost of ventilation would result in a smaller proportion of the energy ingested being available for growth. The low conversion efficiency observed at 93% O_2 did not follow this pattern (Fig. 4). This could be related to the high activity level observed in this tank in the first month (Fig. 3), which could have diverted some energy away from growth.

Several observations suggest that the non-lethal O_2 levels used in this study did not cause any significant physiological disturbance. Although cod grew more slowly in hypoxia, they grew significantly in length and mass, and increased their condition, in all levels of dissolved O_2, even the lowest (45% saturation). K and I_H at the end of the experiment indicate that fish from all groups were in good condition (Lambert & Dutil, 1997*b*). Two measures of condition, the moisture content of muscle and liver, did not differ among treatments. Furthermore, no mortality occurred due to hypoxia, and very few differences were observed between treatments in the levels of the physiological parameters which were in the range of values reported for cod earlier (Claireaux & Dutil, 1992; Dutil *et al.*, 1992; Audet *et al.*, 1993). In particular, lactate levels in plasma, muscle and liver differed in only one case: liver lactate at 45 *v.* 93% O_2. These findings suggest that when being forced to live in chronic hypoxic conditions as severe as 45% O_2, cod adjust behaviour and feeding levels successfully to avoid resorting to anaerobic metabolism. This is quite impressive considering that 5 and 50% mortality rates were observed in cod from the same stock maintained at 28% and 21% O_2 for 96 h at 2–6° C (Plante *et al.*, 1998).

The negative impacts of hypoxia on cod in the wild may be even greater than the results here suggest. In this laboratory experiment, food was available *ad libitum* and the fish were not required to perform physical work to obtain food. Cod would almost certainly have to incur greater metabolic costs in the wild in order to capture food, avoid predators, and migrate. Any increase in activity would exacerbate further the effect of hypoxia on the amount of food that can be processed, and would result ultimately in slower growth rates.

This experiment was conducted at 10° C to favour fast growth and cod were fed to satiation three times a week, but hypoxic conditions may occur in colder waters and meal size and frequency may differ in wild cod. Thus hypoxia occurs in the 100–200 m zone (65–76% O_2) in the Gulf of St Lawrence, and becomes more pronounced below 200 m (23–45% O_2) (D'Amours, 1993; Gilbert *et al.*, 1997). Temperatures are usually *c.* 2–3° C at 100–200 m, and *c.* 5–6° C at 200–300 m in the Gulf of St Lawrence (Bugden, 1991; Gilbert *et al.*, 1997). The increased solubility of O_2 in water (*c.* 10% from 10 to 6° C, and *c.* 20% from 10 to 2° C, Benson & Krause, 1984), lower metabolic rate and reduced appetite in colder waters could mitigate the impact of hypoxia on growth, possibly resulting in somewhat lower critical levels than in this study. Pressure differential across

the gill epithelium is the driving force for O_2 uptake in fish (Hughes, 1981), but temperature may affect the O_2 affinity of haemoglobin and hence O_2 loading at the gills and unloading in the tissues. Behavioural adjustments in meal size and frequency may also mitigate hypoxic limitations to growth by reducing the peak postprandial oxygen demand. Oxygen demand increases rapidly following food ingestion and several hours are required for oxygen consumption to return to prefeeding level (Saunders, 1963; Edwards *et al*., 1972; Scofiani & Hawkins, 1982). More frequent but smaller meals may thus modify the critical levels reported in this study. More experiments are needed to explore the relationships among these factors under hypoxic conditions, particularly swimming activity, ration, meal frequency and temperature.

Since critical O_2 levels for growth varied between 65 and 73%, and significant decreases in growth and condition were observed below 65%, cod would be expected to avoid long exposures to waters with $<c.$ 70% O_2, especially during the main growing season (July–September, Lambert & Dutil, 1997*b*). Although a variable proportion of the population can be found in coastal waters, which are well oxygenated, many cod are fished in deeper waters during the summer period (Castonguay *et al*., 1999). How much time they spend there is not known. A better knowledge of the movements of cod in the Gulf of St Lawrence, possibly through tagging, is necessary to assess better the actual impact of hypoxia on growth.

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