

GREAT LAKES FISHERY COMMISSION

2002 Project Completion Report¹

Effect of pressure on oxygen uptake in bloater

by:

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Final completion report for the

Great Lakes Fishery Commission

Effect of pressure on oxygen uptake in bloater

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Dear Randy,

Please find enclosed our final completion report on the bloater buoyancy. At the outset I said that the deliverable would be a few tables and figures with estimates of the effect of pressure on oxygen uptake in captive bloater. We have delivered on this point.

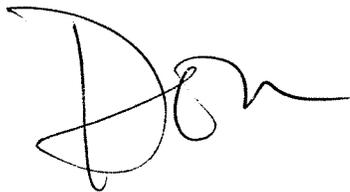
The students involved in the project are very keen to write this up and publish it. But we (they) need to do a much more complete job of looking at the literature.

We also present what I am sure are the best estimates of routine oxygen uptake for bloaters at 10 C. We plan on trying to get some measurements at 5 in the fall. We are experiencing some disease problems in aqualab so the fish are being treated with antibiotic this week, and we will not make any measurements until they have been off antibiotics for at least two weeks.

The students also have extracted the lipid from some fish and have been trying to measure the effect of temperature on its density. We will report on this separately, as it was not explicitly stated as a deliverable for this project.

I would like to take this opportunity to thank you, Ann, and the commission for your support throughout this project.

Sincerely
E. Don Stevens

A handwritten signature in black ink, appearing to read "Don", with a large, stylized initial "D" and a horizontal line extending to the right.

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Introduction

In spite of the fact that bloaters play a major role in the ecology of the great lakes, little is known about their metabolism. We attempted to learn something about their metabolism by measuring routine oxygen uptake. In addition, we attempted to learn something about the effect of pressure on their metabolism because it has been argued that bloaters often make vertical migrations. Metabolism was estimated by measuring oxygen uptake.

Materials and Methods

Animals

Bloater (*Coregonus hoyi*) were raised at the University of Wisconsin Great Lakes Water institute from fertilized eggs of Lake Michigan bloater, and in 1999, after their first year of growth, were moved to the University of Waterloo Biotelemetry Institute. In May 2002 bloater were moved to the Hagen Aqualab, University of Guelph, where they were kept in recirculating aerated freshwater tanks (183 cm diam, 41 cm deep) at $10.5 \pm 0.3^\circ\text{C}$ on a 12L:12D photoperiod. Fish were fed commercial salmon feed (Biodiet Grower food pellets, Bio Oregon Inc, Warrenton, OR) twice daily to satiety. Fish were age 3+ when tested and were tested in groups of 3 to 5 fish.

Respirometer

We measured oxygen uptake using flow-through respirometry and a purpose built respirometry chamber. The respirometer was a plexiglass cylinder with a internal chamber 63 cm long and 33 cm ID (actually 13 inches ID). The chamber volume was 55 litres. The plexiglass tube was sealed at both ends with PVC lids fitted with O-rings. The lids were bolted to the chamber (12 bolts 9/16"). Ball valves permitted control of flow into and out of the chamber. To create good turnover within the chamber, inflow water was directed to the bottom of the chamber with a tygon tube and outflow water left directly out of the top of the lid. Flow was regulated to about 1 litre/min and was measured with a graduate cylinder and a stopwatch. When in use, the respirometer was positioned at an angle of approximately 40° to provide the fish more space for horizontal movement and the chamber was immersed in a holding tank (1080 litres) maintained at $10 \pm 0.5^\circ\text{C}$.

Oxygen uptake

Oxygen uptake was calculated applying the Fick equation using the measured values of water flow through the chamber, the concentration of oxygen in the inflow water, and the concentration of oxygen leaving the chamber.

$$\begin{aligned} \text{VO}_2 &= (\text{O}_{2\text{in}} - \text{O}_{2\text{out}}) * \text{flow} \\ \text{mg O}_2/\text{min} &= (\text{mg O}_2/\text{ml water} - \text{mg O}_2/\text{ml water}) * \text{ml water}/\text{min} \end{aligned}$$

The concentration of oxygen was measured using a YSI oxygen electrode (model 5750) designed to fit into the B.O.D. bottle connected to oxygen metre (model 58, Yellow Springs, OH). The output of the metre was recorded continuously with a chart recorder.

The oxygen electrode was housed in a temperature regulated modified 300 ml B.O.D. bottle. The temperature at the electrode was the same as that of the fish in the respirometer. The inflow tube of the B.O.D. bottle projected into the bottle's interior and had an elbow on it that directed water directly onto the oxygen electrode to ensure adequate flow across the membrane of the electrode. Valves were arranged so that oxygen concentration of inflow water or outflow water could be measured. The electrode was calibrated daily with humidified air using local total pressure. Blanks were run with no fish and all values are corrected for the blank value which was less than 2% of the fish value. Oxygen concentration in the respirometer was never less than 75% saturated.

Experiments

We carried out two experiments. The first was designed to measure routine metabolic rate and the second to measure the effect of pressure on metabolic rate.

Routine oxygen uptake.

We took extraordinary precautions when measuring routine rate to avoid stressing the fish at any time during the procedure. The fish were never net dipped from one tank to another. The respirometer was immersed in the holding tank and the fish were slowly driven into the respirometer. The respirometer was left in the holding tank for the measurements. The fish had been held in the same holding tank for about one month prior to testing and were used to being driven into the respirometer. Once in the respirometer, it was sealed and water flow was initiated. Fish were fed in the chamber at the usual time (4 p.m.). We carried out 4 runs; two runs lasted 3 days and two runs lasted 2 days for a total of 10 24 hour trials. All trials were carried out at 10 ± 0.5 °C. Fish were weighed after the trial.

Effect of pressure on oxygen uptake.

In order to maintain flow through the respirometer and change pressure within it meant that we had to use a high pressure, low flow pump (Goulds model 1SVA1F4E0 vertical stainless steel multi stage pump; 1.5 HP, 230 V). Flow control was accomplished with a flow control (Dole FCGB-1) and a ball valve. Pressure was regulated with a by-pass circuit that returned water to the holding tank. Pressure was measured in the respirometer with a gauge (Omega model DPG 10L, stainless steel). Because the pump ran continuously it warmed the water in the holding tank to about 13 C. In addition it was not possible to maintain pressure and constant flow at very low pressures. Thus we measured the effect of a change in pressure. We carried out 10 trials; each trial spanned three days and consisted of VO_2 measurements during: an afternoon and overnight period; a day of simulated vertical migration; and a day of recovery.

Day 1 -- Four fish were fed 2.0 g of food in the holding tank at about 3 p.m., one hour later they driven into the chamber. The respirometer was pressurized, flow was initiated, and we ensured that all air bubbles inside the respirometer had escaped. The average baseline respirometer pressure during this overnight adaption period was 0.27 atm (4 PSI).

Day 2 -- During the day of pressure simulated vertical migrations we reproduced a bloater journey from near surface depths, to a depth of 30 metres, then back to near surface depths again. Pressure changes were made very slowly to simulate the fish slowly swimming to change depth.

-- At 10:30 a.m. pressure was increased slowly and gradually (over a 10 minute period) from the holding pressure of 0.27 atm to 1 atm (14.7 PSI);

-- At 1:30 p.m. pressure was increased slowly and gradually (over a 20 minute period) increased from 1 atm to 3 atm (44.1 PSI);

-- At 4:30 p.m. pressure was decreased slowly and gradually (over a 20 minute period) from 3 atm to 1 atm (14.7 PSI); and

-- At 7:30 p.m. pressure was decreased slowly and gradually (over a 10 minute period) from 1 atm to baseline values (0.27 psi).

Fish were held at each pressure for a period of three hours. Time zero was defined as ten minutes before the first pressure increase. All measurements were recorded directly from the digital oxygen metre. Respirometer readings were taken ten minutes before and after each pressure change along with every hour between each pressure change. Reservoir readings were made ten minutes before each change of pressure.

Day 3 -- Outflow respirometer readings during the day of recovery were collected with both chart recorder and oxygen metre. Readings were made in the morning (9:30) and in the afternoon (3:00-5:00). Bloaters were removed from the respirometer following the last measurement and then weighed.

Results

A. Routine oxygen uptake

Figure 1 shows the routine oxygen uptake of bloaters for each of the complete runs while the fish were in the respirometer. In all cases, oxygen uptake gradually decreased over the night after being placed in the respirometer. Figure 2 shows the same results of the four runs superimposed to make more clear that there was an initial decrease over the first night in the respirometer. The average value from midnight to 8 a.m. on the first day was 120.0 ± 2.3 mg O₂/kg*h and on the second day was 103.2 ± 2.8 mg O₂/kg*h.

Figure 3 shows the same data aligned with time of day to show diel trends. Fish were placed in the respirometer in the late afternoon and were fed each day in the late afternoon while in the respirometer. Thus the increase seen in the late afternoon is a combination of three factors: increased activity associated with being moved into the chamber, increased activity associated with feeding, and the heat increment of feeding.

The average value for the complete data set was 134.1 ± 2.2 mg O₂/kg*h (229 hourly observations). A reasonable estimate of routine metabolic rate of bloater chub at 10 C and feeding was about 130 mg O₂/kg*h.

B. The effect of pressure on oxygen uptake.

Baseline oxygen uptake was higher in these trials because the temperature was higher (the high pressure pump created heat) and probably because the high pressure pump created noise and vibrations that presumably acted as a stressor. Mean temperature during these pressure trials was 13.9 ± 0.05 °C. The results were normalized to the mean of the 5 values prior to the first pressure change (i.e., the mean of the oxygen uptake values at 6:30, 7:30, 8:30, 9:30, and 10:20). Raw data is given in the appendix.

Routine metabolic rate increased a trivial amount from 100% to 104% as pressure was increased from 4 to 14.7 psi, but increased about 20% when pressure was increased to 44.1 psi. This increase occurred in all 10 trials. When pressure was decreased back to 14.7 psi, oxygen uptake decreased in all 10 trials to about 112%, and decreased back to baseline levels when pressure was restored to 4 psi. The observation that the increase on going to 44.1 psi and the subsequent decrease makes us confident in concluding that there is a small but consistent increase in oxygen uptake associated with an increase in pressure. We do not know anything about the mechanisms that may account for the change.

Figure legends

Figure 1. Oxygen uptake of captive adult bloater chub during the 10 trials. Fish were fed in the holding tank immediately before the trial. Then 4 or 5 fish were placed in the respirometer (about 4:00 p.m.), and the respirometer was sealed and covered. Water was pumped continuously through the respirometer and oxygen concentration was measured in the inflow water and outflow water. The four panels depict separate trials that ran for 2 or 3 days. The fish were fed at about 4:30 p.m. when they were in the respirometer (shown by the large triangle). The room was on a controlled photoperiod: lights on at 6 a.m. and off at 6 p.m.. Feeding is indicated by the large triangles.

Figure 2. Oxygen uptake of captive adult bloater chub during the 10 trials; same data as in Figure 1 except the data are superimposed to make the trends more clear.

Figure 3. Oxygen uptake of captive adult bloater chub over a 24 h period; same data as in Figure 1. Oxygen uptake tended to be high in the late afternoon immediately fish were placed in the respirometer or after being fed. Top panel shows data for the 10 individual trials and the bottom panel shows means \pm sem. The large triangle indicates time when fish were placed in the respirometer or fed when they were in the respirometer. The room was on a controlled photoperiod: lights on at 6 a.m. and off at 6 p.m..

Figure 4. The effect of change in ambient pressure on oxygen uptake in bloaters. The fish had been left in the respirometer since late afternoon on the previous day. The record shown is for Day 2, the day of pressure change. Data are normalized to the data at 4 psi (the lowest pressure at which it is possible to accurately control both flow and pressure). Pressures were changed slowly (over a 10 or 20 minute period) to simulate the normal movement of the fish from one depth to another. Top panel shows data from 10 separate trials; bottom panel shows means \pm sem for all 10 trials. Pressures are gauge pressure (i.e., sea level = 0).

Appendix tables

A. ROUTINE OXYGEN UPTAKE IN BLOATER AT 10 °C.

Appendix Table 1. Routine oxygen uptake data --- date, time, oxygen concentration in the inflow and outflow, water temperature, water flow rate through the respirometer, oxygen uptake corrected for uptake by the empty respirometer, and weight specific oxygen uptake. There are four consecutive tables for the 4 individual runs. m indicates that the reading was verified on the oxygen metre, c indicates that the reading was taken from the chart record.

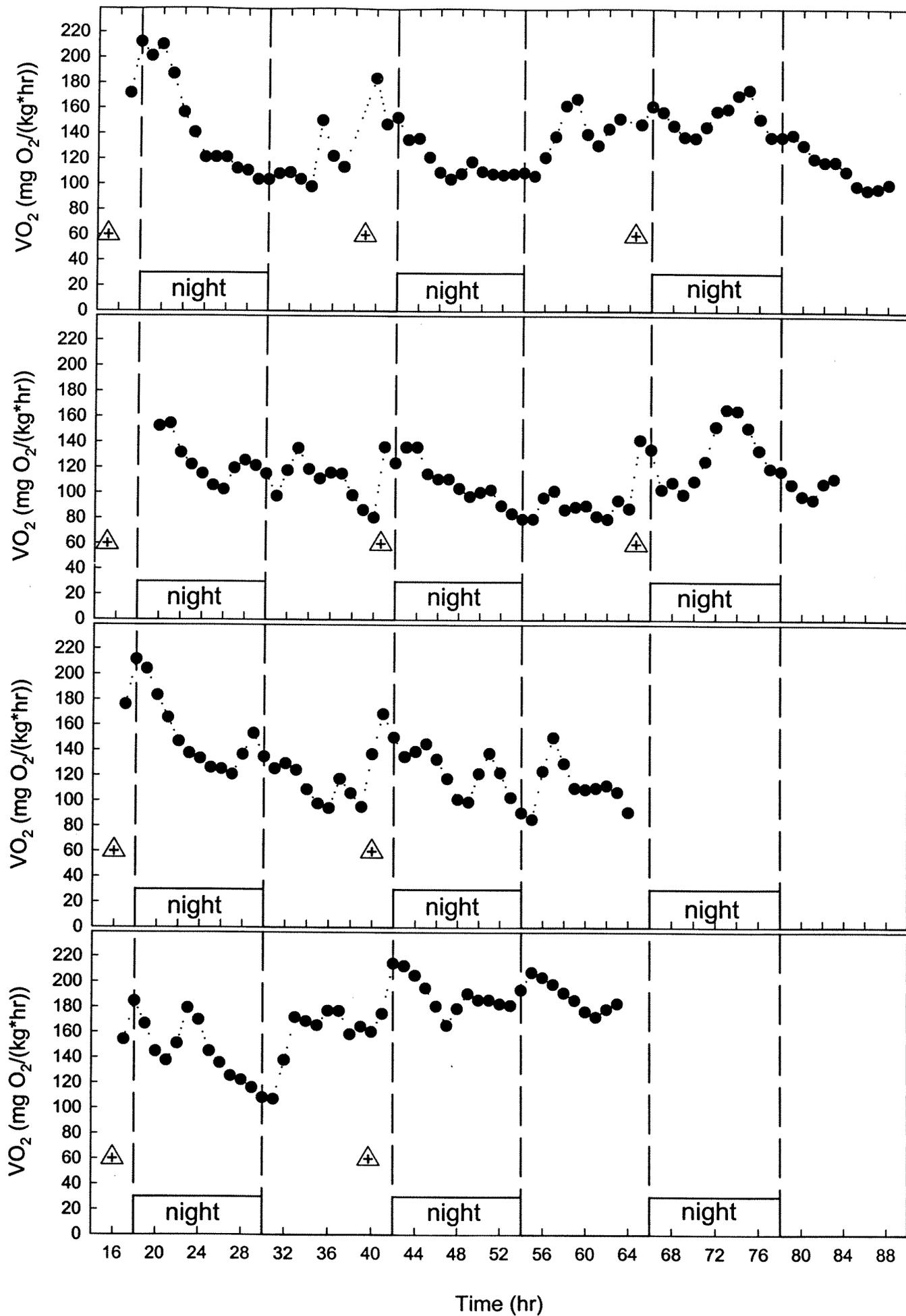
Appendix Table 2. Routine oxygen uptake data --- the same weight specific data as in Table 1 except that it is aligned by time of day. Heavy horizontal line indicates feeding and the start of a new trial.

B. EFFECT OF CHANGE IN AMBIENT PRESSURE ON OXYGEN UPTAKE IN BLOATER

Appendix Table 3. Oxygen uptake values for bloater normalized to the mean of the first 5 values at baseline pressure. First column is clock time (the fish had been driven into the respirometer in the late afternoon on the previous day. Last column is mean value at each of the pressure levels. These values were used for Figure 4.

Appendix Table 4. Raw data for oxygen uptake values of bloater used to calculate values in the appendix table 3.

Figure 1. Oxygen uptake of captive adult bloater chub during the 10 trials. Fish were fed in the holding tank immediately before the trial. Then 4 or 5 fish were placed in the respirometer (about 4:00 p.m.), and the respirometer was sealed and covered. Water was pumped continuously through the respirometer and oxygen concentration was measured in the inflow water and outflow water. The four panels depict separate trials that ran for 2 or 3 days. The fish were fed at about 4:30 p.m. when they were in the respirometer (shown by the large triangle). The room was on a controlled photoperiod: lights on at 6 a.m. and off at 6 p.m.. Feeding is indicated by the large triangles.



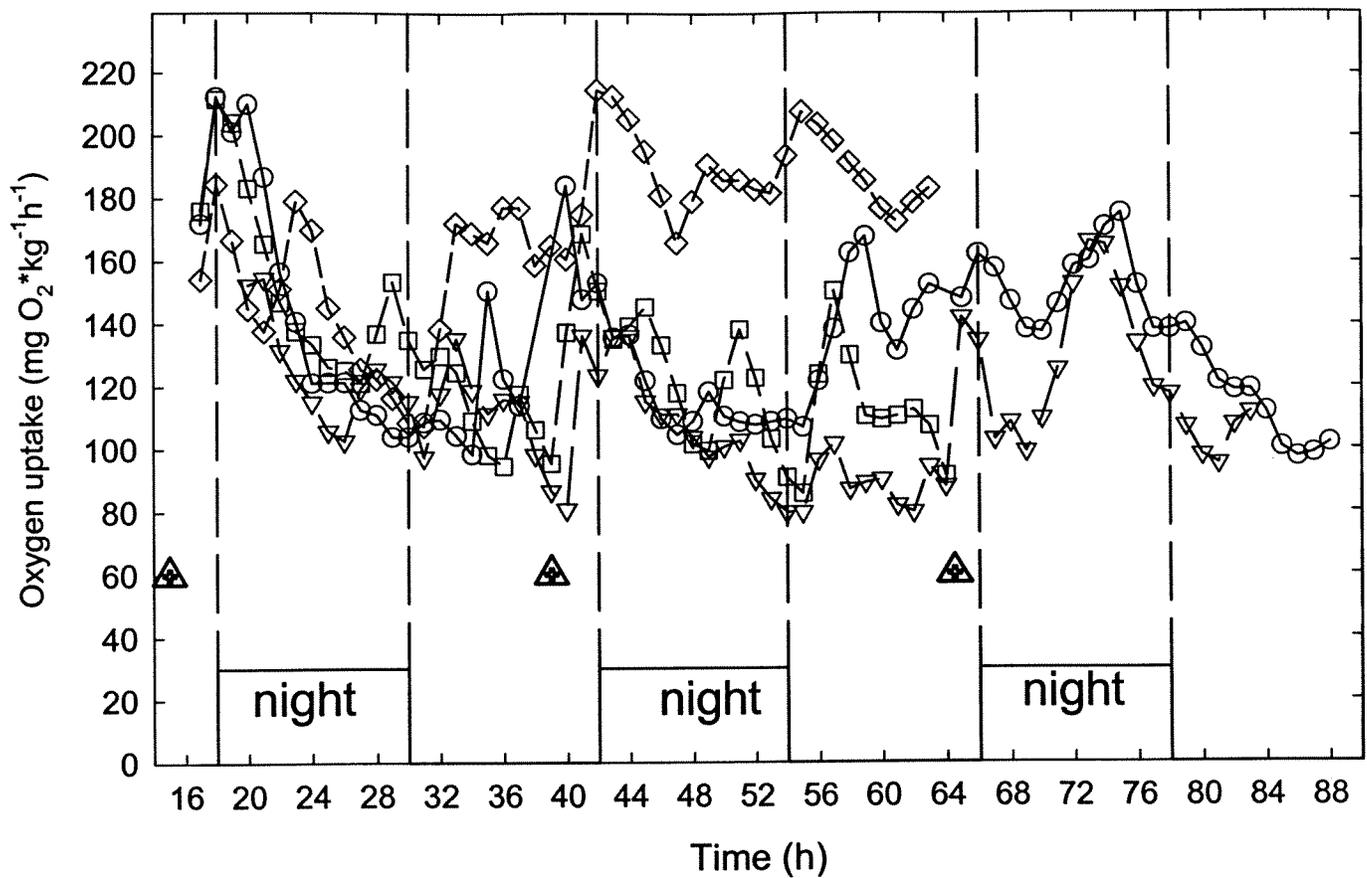


Figure 2. Oxygen uptake of captive adult bloater chub during the 10 trials. This is the same data as in Figure 1 except that the four runs are superimposed to make the trends more clear.

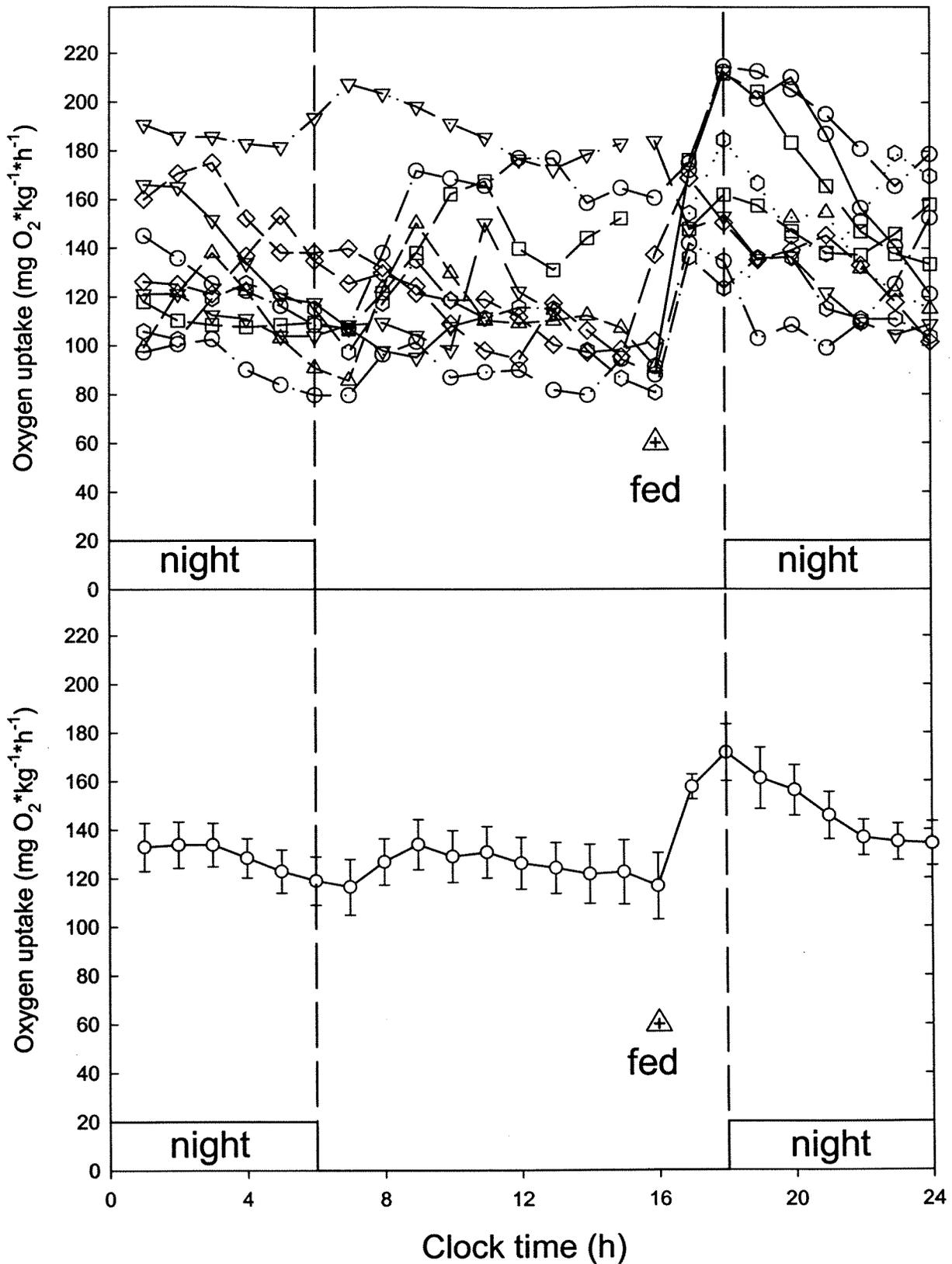


Fig 3. Oxygen uptake of adult captive bloater chub over a 24 hour period; same data as in Fig 1. Oxygen uptake tended to be high in the late afternoon after fish were placed in the respirometer or after being fed. Top panel shows data for the 10 individual trials; bottom panel shows means and sem. Triangle indicates the time when fish were placed in the respirometer or time when fed. The room was on a controlled photoperiod; on at 6 a.m. and off at 6 p.m.

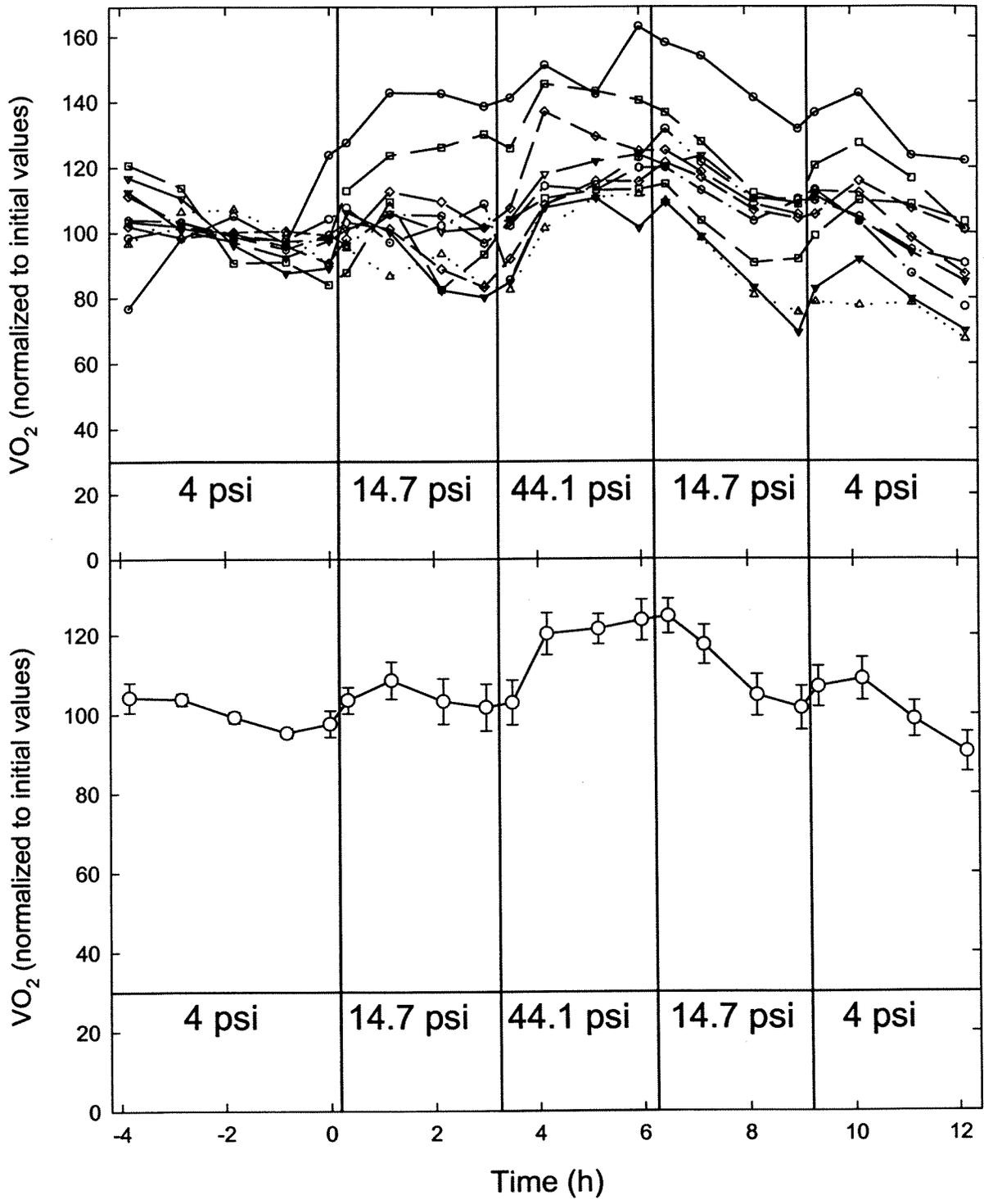


Figure 4. The effect of change in ambient pressure on oxygen uptake in captive adult bloater chub. The fish had been left in the respirometer since late afternoon the previous day. Day 2 is shown (day of pressure change). Data are normalized to the data at 4 psi (the lowest pressure at which we could control both flow and pressure). Pressures were changed slowly to simulate normal movement of fish. Top panel shows data from 10 individual trials; bottom panel shows mean and sem for all 10 trials.

Appendix Table 1. Routine oxygen uptake data --- date, time, oxygen concentration in the inflow and outflow, water temperature, water flow rate through the respirometer, oxygen uptake corrected for uptake by the empty respirometer, and weight specific oxygen uptake. There are four consecutive tables for the 4 individual runs. m indicates that the reading was verified on the oxygen metre, c indicates that the reading was taken from the chart record.

fn=final	Trials 1,2,3		mass =	682		blank =	0.025	
VO₂ calculated using Fick principal							corrected	mass
		meter					VO₂	specific
date	clock	or	O₂ in	O₂ out	temp	flow rate	mgO₂/min	VO₂
	time	chart	mgO₂/l	mgO₂/l	C	ml water/s	for all fish	mgO₂/h*kg
fed 1.8g at 3:00 pm								
May 14	4:00	m	10.33	9.25	10.6	16.4	1.038	91.3
	5:00	m	10.39	8.45	10.6	17.0	1.954	171.9
	6:00	c	10.37	7.98	10.6	17.0	2.413	212.3
	7:00	c	10.38	8.10	10.6	16.9	2.287	201.2
	8:00	c	10.38	8.00	10.6	16.9	2.388	210.1
	9:00	c	10.38	8.26	10.6	16.9	2.125	186.9
	10:00	c	10.39	8.60	10.6	16.8	1.779	156.5
	11:00	c	10.39	8.78	10.6	16.8	1.598	140.6
May 15	12:00	c	10.39	9.00	10.6	16.8	1.376	121.1
	1:00	c	10.40	9.00	10.6	16.7	1.378	121.2
	2:00	c	10.40	9.00	10.6	16.7	1.378	121.2
	3:00	c	10.40	9.10	10.6	16.7	1.278	112.4
	4:00	c	10.41	9.12	10.6	16.6	1.260	110.8
	5:00	c	10.41	9.20	10.6	16.6	1.180	103.8
	6:00	c	10.41	9.20	10.6	16.6	1.180	103.8
	7:00	c	10.42	9.15	10.6	16.5	1.232	108.4
	8:00	c	10.42	9.14	10.6	16.5	1.242	109.3
	9:00	c	10.42	9.20	10.6	16.5	1.183	104.1
	10:00	c	10.43	9.27	10.6	16.4	1.116	98.2
	11:00	m	10.47	8.71	10.5	16.4	1.707	150.2
	12:00	m	10.38	8.96	10.4	16.6	1.389	122.2
	1:00	m	10.47	9.18	10.5	17.0	1.291	113.6
fish fed 1.8g 3:00 pm								
	4:00	m	10.61	8.46	10.5	16.4	2.091	183.9
	5:00	m	10.58	8.85	10.5	16.4	1.677	147.6
	6:00	c	10.59	8.80	10.5	16.4	1.736	152.8
	7:00	c	10.59	9.00	10.5	16.4	1.540	135.4
	8:00	m	10.60	9.00	10.5	16.4	1.549	136.3
	9:00	c	10.59	9.16	10.5	16.4	1.382	121.6
	10:00	c	10.59	9.30	10.5	16.4	1.244	109.5
	11:00	c	10.58	9.35	10.5	16.4	1.185	104.3
May 16	12:00	c	10.58	9.30	10.5	16.4	1.235	108.6
	1:00	c	10.58	9.20	10.5	16.5	1.341	118.0
	2:00	c	10.58	9.29	10.5	16.5	1.252	110.2
	3:00	c	10.58	9.31	10.5	16.5	1.232	108.4
	4:00	c	10.57	9.31	10.5	16.5	1.222	107.5
	5:00	c	10.57	9.30	10.5	16.5	1.232	108.4
	6:00	c	10.57	9.29	10.5	16.5	1.242	109.3
	7:00	c	10.57	9.32	10.5	16.5	1.213	106.7
	8:00	c	10.57	9.15	10.5	16.5	1.381	121.5
	9:00	c	10.56	8.95	10.5	16.5	1.569	138.0
	10:00	m	10.52	8.62	10.5	16.4	1.845	162.3
	11:00	m	10.55	8.60	10.6	16.5	1.906	167.6
	12:00	m	10.59	8.96	10.6	16.5	1.589	139.8
	1:00	m	10.56	9.04	10.6	16.6	1.489	131.0
	2:00	m	10.56	8.89	10.7	16.6	1.638	144.1
	3:00	m	10.56	8.82	10.7	16.8	1.729	152.1
fed 1.6g at 4:30 pm								
	5:00	m	10.59	8.90	10.7	16.8	1.679	147.7
	6:00	c	10.59	8.74	10.6	16.8	1.840	161.9

	7:00	c	10.58	8.78	10.6	16.8	1.789	157.4
	8:00	c	10.58	8.90	10.6	16.8	1.668	146.8
	9:00	c	10.58	9.00	10.6	16.8	1.568	137.9
	10:00	c	10.57	8.99	10.6	16.7	1.558	137.1
	11:00	c	10.57	8.89	10.6	16.7	1.658	145.9
May 17	12:00	c	10.56	8.74	10.6	16.7	1.799	158.2
	1:00	c	10.56	8.72	10.6	16.7	1.819	160.0
	2:00	c	10.56	8.60	10.6	16.7	1.939	170.6
	3:00	c	10.55	8.54	10.6	16.7	1.989	175.0
	4:00	c	10.55	8.80	10.6	16.7	1.729	152.1
	5:00	c	10.55	8.96	10.6	16.7	1.568	138.0
	6:00	c	10.54	8.94	10.6	16.6	1.569	138.0
	7:00	c	10.54	8.92	10.6	16.6	1.589	139.8
	8:00	c	10.53	9.00	10.6	16.6	1.499	131.9
	9:00	m	10.54	9.13	10.6	16.6	1.379	121.4
	10:00	m	10.52	9.15	10.5	16.7	1.348	118.6
	11:00	m	10.52	9.13	10.5	16.5	1.351	118.9
	12:00	m	10.53	9.23	10.5	16.6	1.270	111.7
	1:00	m	10.55	9.38	10.5	16.6	1.140	100.3
	2:00	m	10.58	9.46	10.5	16.8	1.104	97.1
	3:00	m	10.54	9.40	10.6	16.7	1.117	98.3
	4:00	m	10.52	9.32	10.7	16.4	1.156	101.7

fn=final	Trials 4,5,6		mass =	574		blank =	0.025	
VO ₂ calculated using Fick principal							corrected	mass
		meter					VO ₂	specific
date	clock	or	O ₂ in	O ₂ out	temp	flow rate	mgO ₂ /min	VO ₂
	time	chart	mgO ₂ /l	mgO ₂ /l	C	ml water/s	for all fish	mgO ₂ /h*kg
fed 1.6g at 6:10 pm								
May 20	8:00	m	10.70	9.22	10.5	16.7	1.458	152.4
	9:00	c	10.70	9.20	10.4	16.7	1.478	154.5
	10:00	c	10.70	9.42	10.4	16.7	1.258	131.5
	11:00	c	10.70	9.51	10.4	16.7	1.167	122.0
May 21	12:00	c	10.70	9.57	10.4	16.6	1.100	115.0
	1:00	c	10.70	9.66	10.4	16.6	1.011	105.7
	2:00	c	10.70	9.69	10.4	16.6	0.981	102.5
	3:00	c	10.70	9.53	10.4	16.6	1.140	119.2
	4:00	c	10.69	9.46	10.4	16.6	1.200	125.4
	5:00	c	10.69	9.50	10.4	16.6	1.160	121.3
	6:00	c	10.69	9.56	10.4	16.6	1.100	115.0
	7:00	c	10.69	9.73	10.4	16.6	0.931	97.3
	8:00	c	10.69	9.53	10.4	16.5	1.123	117.4
	9:00	c	10.69	9.36	10.4	16.5	1.292	135.0
	10:00	c	10.68	9.51	10.4	16.5	1.133	118.5
	11:00	m	10.70	9.60	10.4	16.5	1.064	111.2
	12:00	m	10.70	9.57	10.4	16.7	1.107	115.7
	1:00	m	10.69	9.56	10.4	16.6	1.100	115.0
	2:00	m	10.67	9.68	10.4	16.2	0.937	98.0
	3:00	m	10.66	9.80	10.4	16.5	0.826	86.4
	4:00	m	10.66	9.87	10.4	16.8	0.771	80.6
fed 1.6g at 4:40 pm								
	5:00	m	10.64	9.34	10.4	17.0	1.301	136.0
	6:00	c	10.61	9.40	10.6	16.6	1.180	123.4
	7:00	c	10.61	9.28	10.6	16.6	1.300	135.9
	8:00	c	10.61	9.28	10.6	16.6	1.300	135.9
	9:00	c	10.61	9.48	10.6	16.6	1.100	115.0
	10:00	c	10.61	9.52	10.6	16.6	1.061	110.9
	11:00	c	10.61	9.52	10.6	16.6	1.061	110.9
May 22	12:00	c	10.61	9.59	10.6	16.6	0.991	103.6
	1:00	c	10.61	9.65	10.6	16.6	0.931	97.3
	2:00	c	10.61	9.62	10.6	16.6	0.961	100.5
	3:00	c	10.61	9.60	10.6	16.6	0.981	102.5
	4:00	c	10.61	9.72	10.6	16.6	0.861	90.0
	5:00	c	10.61	9.78	10.6	16.6	0.802	83.8
	6:00	c	10.61	9.82	10.6	16.6	0.762	79.6
	7:00	c	10.61	9.82	10.6	16.6	0.762	79.6
	8:00	c	10.61	9.66	10.6	16.6	0.921	96.3
	9:00	c	10.61	9.61	10.6	16.6	0.971	101.5
	10:00	c	10.61	9.75	10.6	16.6	0.832	86.9
	11:00	c	10.61	9.73	10.6	16.6	0.851	89.0
	12:00	c	10.61	9.72	10.6	16.6	0.861	90.0
	1:00	c	10.61	9.80	10.6	16.6	0.782	81.7
	2:00	c	10.61	9.82	10.6	16.6	0.762	79.6
	3:00	m	10.66	9.74	10.6	16.8	0.902	94.3
	4:00	m	10.56	9.68	10.5	16.4	0.841	87.9
fed 1.6g at 4:40 pm								
	5:00	m	10.58	9.21	10.4	16.8	1.356	141.7
	6:00	c	10.58	9.28	10.5	16.8	1.285	134.4
	7:00	c	10.58	9.58	10.5	16.8	0.983	102.8

	8:00	c	10.58	9.52	10.5	16.7	1.037	108.4
	9:00	c	10.57	9.60	10.5	16.7	0.947	99.0
	10:00	c	10.57	9.50	10.5	16.7	1.047	109.5
	11:00	c	10.57	9.35	10.5	16.7	1.197	125.2
May 23	12:00	c	10.57	9.08	10.5	16.6	1.459	152.5
	1:00	c	10.57	8.95	10.5	16.6	1.589	166.0
	2:00	c	10.56	8.95	10.5	16.6	1.579	165.0
	3:00	c	10.56	9.08	10.5	16.6	1.449	151.5
	4:00	c	10.56	9.25	10.5	16.6	1.280	133.8
	5:00	c	10.56	9.38	10.5	16.5	1.143	119.5
	6:00	c	10.56	9.40	10.5	16.5	1.123	117.4
	7:00	c	10.56	9.50	10.5	16.5	1.024	107.1
	8:00	c	10.55	9.58	10.5	16.5	0.935	97.8
	9:00	c	10.55	9.60	10.5	16.4	0.910	95.1
	10:00	m	10.56	9.50	10.5	16.6	1.031	107.7
	11:00	m	10.54	9.43	10.5	16.4	1.067	111.6

fn=final	Trial 7,8		mass =	572		blank =	0.025	
VO ₂ calculated using Fick principal							corrected	mass
		meter					VO ₂	specific
date	clock	or	O ₂ in	O ₂ out	temp	flow rate	mgO ₂ /min	VO ₂
	time	chart	mgO ₂ /l	mgO ₂ /l	C	ml water/s	for all fish	mgO ₂ /h*kg
fed 2.5g at 4:00 pm								
June 12	5:00	m	10.48	8.77	10.6	16.6	1.678	176.0
	6:00	c	10.47	8.42	10.6	16.6	2.017	211.6
	7:00	c	10.46	8.48	10.6	16.6	1.947	204.2
	8:00	c	10.46	8.68	10.6	16.6	1.748	183.3
	9:00	c	10.45	8.84	10.6	16.6	1.579	165.6
	10:00	c	10.44	9.00	10.6	16.5	1.401	146.9
	11:00	c	10.44	9.09	10.6	16.5	1.312	137.6
June 13	12:00	c	10.43	9.12	10.6	16.5	1.272	133.4
	1:00	c	10.42	9.18	10.6	16.5	1.203	126.1
	2:00	c	10.42	9.19	10.6	16.5	1.193	125.1
	3:00	c	10.41	9.22	10.6	16.5	1.153	121.0
	4:00	c	10.40	9.05	10.6	16.4	1.303	136.7
	5:00	c	10.40	8.89	10.6	16.4	1.461	153.2
	6:00	c	10.39	9.06	10.6	16.4	1.284	134.7
	7:00	c	10.38	9.14	10.6	16.4	1.195	125.4
	8:00	c	10.37	9.09	10.6	16.4	1.235	129.5
	9:00	c	10.37	9.14	10.6	16.4	1.185	124.3
	10:00	c	10.36	9.28	10.6	16.4	1.038	108.9
	11:00	c	10.35	9.37	10.6	16.3	0.933	97.9
	12:00	m	10.33	9.39	10.6	16.4	0.900	94.4
	1:00	c	10.33	9.16	10.6	16.3	1.119	117.4
	2:00	c	10.32	9.26	10.6	16.3	1.012	106.1
	3:00	m	10.32	9.36	10.7	16.2	0.908	95.3
fed 1.6g at 4:00 pm								
	4:00	m	10.31	8.94	10.6	16.2	1.307	137.1
	5:00	m	10.30	8.62	10.6	16.2	1.608	168.7
	6:00	c	10.30	8.80	10.6	16.2	1.433	150.3
	7:00	c	10.29	8.94	10.6	16.2	1.287	135.0
	8:00	c	10.29	8.90	10.6	16.2	1.326	139.1
	9:00	c	10.28	8.83	10.6	16.2	1.384	145.2
	10:00	c	10.27	8.94	10.6	16.2	1.268	133.0
	11:00	c	10.27	9.09	10.6	16.2	1.122	117.7
June 14	12:00	c	10.26	9.24	10.6	16.2	0.966	101.4
	1:00	c	10.26	9.26	10.6	16.2	0.947	99.3
	2:00	c	10.25	9.03	10.6	16.2	1.161	121.8
	3:00	c	10.25	8.88	10.6	16.3	1.315	137.9
	4:00	c	10.24	9.02	10.6	16.3	1.168	122.5
	5:00	c	10.24	9.21	10.6	16.3	0.982	103.0
	6:00	c	10.23	9.32	10.6	16.3	0.865	90.7
	7:00	c	10.22	9.36	10.6	16.3	0.816	85.6
	8:00	c	10.22	8.99	10.6	16.3	1.178	123.6
	9:00	c	10.21	8.72	10.6	16.3	1.432	150.2
	10:00	c	10.21	8.92	10.6	16.3	1.237	129.7
	11:00	c	10.20	9.10	10.6	16.3	1.051	110.2
	12:00	m	10.22	9.13	10.5	16.3	1.041	109.2
	1:00	c	10.19	9.09	10.6	16.3	1.051	110.2
	2:00	c	10.18	9.06	10.6	16.3	1.070	112.3
	3:00	c	10.17	9.10	10.6	16.3	1.021	107.1
	4:00	m	10.12	9.20	10.5	16.2	0.869	91.2

fn=final	Trials 9,10		mass =	572		blank =	0.025	
VO ₂ calculated using Fick principal							corrected	mass
		meter					VO ₂	specific
date	clock time	or chart	O ₂ in mgO ₂ /l	O ₂ out mgO ₂ /l	temp C	flow rate ml water/s	mgO ₂ /min for all fish	VO ₂ mgO ₂ /h*kg
fed 1.6g at 4:00 pm								
June 17	5:00	m	10.47	8.97	10.6	16.6	1.469	154.1
	6:00	c	10.47	8.68	10.6	16.6	1.758	184.4
	7:00	c	10.48	8.86	10.6	16.6	1.589	166.6
	8:00	c	10.49	9.08	10.6	16.6	1.379	144.7
	9:00	c	10.50	9.15	10.6	16.5	1.312	137.6
	10:00	c	10.50	9.02	10.6	16.5	1.440	151.1
	11:00	c	10.51	8.76	10.6	16.5	1.708	179.1
June 18	12:00	c	10.52	8.86	10.6	16.5	1.618	169.8
	1:00	c	10.52	9.09	10.6	16.4	1.382	145.0
	2:00	c	10.53	9.19	10.6	16.4	1.294	135.7
	3:00	c	10.54	9.30	10.6	16.4	1.195	125.4
	4:00	c	10.55	9.34	10.6	16.4	1.166	122.3
	5:00	c	10.56	9.41	10.6	16.4	1.107	116.1
	6:00	c	10.56	9.48	10.6	16.3	1.031	108.2
	7:00	c	10.57	9.50	10.6	16.3	1.021	107.1
	8:00	c	10.57	9.20	10.6	16.3	1.315	137.9
	9:00	c	10.58	8.88	10.6	16.3	1.638	171.8
	10:00	c	10.59	8.92	10.6	16.3	1.608	168.7
	11:00	m	10.57	8.93	10.5	16.3	1.579	165.6
	12:00	m	10.58	8.84	10.5	16.4	1.687	177.0
	1:00	c	10.60	8.85	10.6	16.3	1.687	176.9
	2:00	c	10.62	9.04	10.6	16.2	1.511	158.5
	3:00	m	10.64	9.00	10.6	16.2	1.569	164.6
fed 1.6g 3:45 pm								
	4:00	m	10.62	9.01	10.6	16.1	1.530	160.5
	5:00	m	10.58	8.84	10.6	16.2	1.666	174.8
	6:00	c	10.59	8.46	10.6	16.2	2.045	214.5
	7:00	c	10.59	8.48	10.6	16.2	2.026	212.5
	8:00	c	10.60	8.55	10.6	16.1	1.955	205.1
	9:00	c	10.60	8.65	10.6	16.1	1.859	195.0
	10:00	c	10.61	8.80	10.6	16.1	1.723	180.8
	11:00	c	10.61	8.95	10.6	16.1	1.579	165.6
June 19	12:00	c	10.61	8.82	10.6	16.1	1.704	178.8
	1:00	c	10.62	8.70	10.6	16.0	1.818	190.7
	2:00	c	10.62	8.75	10.6	16.0	1.770	185.7
	3:00	c	10.63	8.76	10.6	16.0	1.770	185.7
	4:00	c	10.64	8.80	10.6	16.0	1.741	182.7
	5:00	c	10.64	8.80	10.6	15.9	1.730	181.5
	6:00	c	10.64	8.68	10.6	15.9	1.845	193.5
	7:00	c	10.64	8.54	10.6	15.9	1.978	207.5
	8:00	c	10.65	8.59	10.6	15.9	1.940	203.5
	9:00	m	10.66	8.64	10.6	15.8	1.890	198.2
	10:00	m	10.66	8.71	10.6	15.8	1.824	191.3
	11:00	m	10.66	8.78	10.6	15.9	1.769	185.5
	12:00	m	10.66	8.87	10.6	15.9	1.683	176.5
	1:00	c	10.67	8.91	10.6	15.8	1.643	172.4
	2:00	c	10.67	8.85	10.6	15.8	1.700	178.4
	3:00	m	10.67	8.78	10.6	15.6	1.744	182.9

Appendix Table 2. Routine oxygen uptake data --- the same weight specific data as in Table 1 except that it is aligned by time of day. Heavy horizontal line indicates feeding and the start of a new trial.

Routine mass specific VO ₂ (mgO ₂ /h*kg) time in continous clock hours (16= 4pm on 1st day)									
adult captive bloater chub at 10 C									
May and June 2002									
h	Trials 1,2,3		Trials 4,5,6		Trials 7,8		Trials 9,10		avg
16	16	91.3							22.8
17	17	171.9			17	176.0	17	154.1	125.5
18	18	212.3			18	211.6	18	184.4	152.1
19	19	201.2	19	137.0	19	204.2	19	166.6	177.3
20	20	210.1	20	152.4	20	183.3	20	144.7	172.6
21	21	186.9	21	154.5	21	165.6	21	137.6	161.1
22	22	156.5	22	131.5	22	146.9	22	151.1	146.5
23	23	140.6	23	122.0	23	137.6	23	179.1	144.8
24	24	121.1	24	115.0	24	133.4	24	169.8	134.8
25	25	121.2	25	105.7	25	126.1	25	145.0	124.5
26	26	121.2	26	102.5	26	125.1	26	135.7	121.1
27	27	112.4	27	119.2	27	121.0	27	125.4	119.5
28	28	110.8	28	125.4	28	136.7	28	122.3	123.8
29	29	103.8	29	121.3	29	153.2	29	116.1	123.6
30	30	103.8	30	115.0	30	134.7	30	108.2	115.4
31	31	108.4	31	97.3	31	125.4	31	107.1	109.6
32	32	109.3	32	117.4	32	129.5	32	137.9	123.5
33	33	104.1	33	135.0	33	124.3	33	171.8	133.8
34	34	98.2	34	118.5	34	108.9	34	168.7	123.6
35	35	150.2	35	111.2	35	97.9	35	165.6	131.2
36	36	122.2	36	115.7	36	94.4	36	177.0	127.3
37	37	113.6	37	115.0	37	117.4	37	176.9	130.7
38			38	98.0	38	106.1	38	158.5	90.6
39			39	86.4	39	95.3	39	164.6	86.6
40	40	183.9	40	80.6	40	137.1	40	160.5	140.5
41	41	147.6	41	136.0	41	168.7	41	174.8	156.8
42	42	152.8	42	123.4	42	150.3	42	214.5	160.2
43	43	135.4	43	135.9	43	135.0	43	212.5	154.7
44	44	136.3	44	135.9	44	139.1	44	205.1	154.1
45	45	121.6	45	115.0	45	145.2	45	195.0	144.2
46	46	109.5	46	110.9	46	133.0	46	180.8	133.5
47	47	104.3	47	110.9	47	117.7	47	165.6	124.6
48	48	108.6	48	103.6	48	101.4	48	178.8	123.1
49	49	118.0	49	97.3	49	99.3	49	190.7	126.3
50	50	110.2	50	100.5	50	121.8	50	185.7	129.5
51	51	108.4	51	102.5	51	137.9	51	185.7	133.6
52	52	107.5	52	90.0	52	122.5	52	182.7	125.7
53	53	108.4	53	83.8	53	103.0	53	181.5	119.2
54	54	109.3	54	79.6	54	90.7	54	193.5	118.3
55	55	106.7	55	79.6	55	85.6	55	207.5	119.9
56	56	121.5	56	96.3	56	123.6	56	203.5	136.2
57	57	138.0	57	101.5	57	150.2	57	198.2	147.0
58	58	162.3	58	86.9	58	129.7	58	191.3	142.6
59	59	167.6	59	89.0	59	110.2	59	185.5	138.1
60	60	139.8	60	90.0	60	109.2	60	176.5	128.9
61	61	131.0	61	81.7	61	110.2	61	172.4	123.8
62	62	144.1	62	79.6	62	112.3	62	178.4	128.6
63	63	152.1	63	94.3	63	107.1	63	182.9	134.1
64			64	87.9	64	91.2			44.8
65	65	147.7	65	141.7					72.4
66	66	161.9	66	134.4					74.1

avg = 120.0
sem = 2.3

avg = 103.2
sem = 2.8

Appendix Table 3. Oxygen uptake values for bloater normalized to the mean of the first 5 values at baseline pressure.

clock time	TRIAL	1	2	3	4	5	6	7	8	9	10	avg	sem
6:30	-3.83	76.7	112.2	120.4	101.6	96.4	103.8	98.4	116.3	102.9	110.8	104.0	3.71
7:30	-2.83	98.1	100.4	113.5	98.1	106.1	103.2	101.4	110.0	101.4	102.5	103.5	1.51
8:30	-1.83	105.0	96.9	90.4	99.9	106.9	98.6	98.4	95.8	99.2	99.3	99.0	1.37
9:30	-0.83	96.5	92.1	90.8	100.3	99.5	94.8	97.7	87.1	96.9	95.4	95.1	1.22
10:20	0.00	123.7	98.4	84.9	100.1	91.1	99.6	104.1	90.7	99.6	92.0	98.4	3.19
10:30	0.17												100.0
10:40	0.33	127.5	101.8	88.6	104.0	96.1	96.4	107.6	107.8	114.3	99.4	104.4	3.27
11:30	1.17	142.8	106.8	110.2	102.0	87.4	105.6	96.9	101.8	125.0	113.9	109.2	4.64
12:30	2.17	142.5	101.2	83.4	89.6	94.2	105.2	102.1	83.8	127.6	110.7	104.0	5.68
1:20	3.00	138.6	102.4	94.2	84.2	84.5	96.9	108.7	81.8	131.5	102.8	102.6	5.80
1:30	3.17												105.0
1:50	3.50	141.1	105.0	104.7	92.9	83.2	102.3	85.4	86.5	127.3	108.7	103.7	5.61
2:30	4.17	151.2	118.7	111.3	109.4	102.1	114.2	108.4	109.1	146.8	138.2	120.9	5.31
3:30	5.17	142.3	122.5	113.7	116.6	111.4	112.9	114.5	112.0	144.6	130.7	122.1	3.80
4:20	6.00	163.0	124.6	113.9	116.3	112.3	119.7	122.8	102.9	141.8	126.1	124.3	5.11
4:30	6.17												117.8
4:50	6.50	158.0	121.2	115.6	122.2	110.3	119.7	131.6	110.7	138.1	126.4	125.4	4.32
5:30	7.17	153.9	124.3	104.3	117.6	98.9	112.8	121.3	100.2	129.1	119.7	118.2	4.86
6:30	8.17	141.1	111.5	91.4	107.6	81.4	103.5	110.1	84.6	113.5	109.9	105.5	5.13
7:20	9.00	131.6	109.8	92.6	105.0	76.2	109.7	110.1	71.1	110.0	106.7	102.3	5.35
7:30	9.17												112.8
7:40	9.33	136.6	112.0	99.7	106.3	79.5	109.7	113.0	84.4	121.9	113.9	107.7	5.03
8:30	10.17	142.5	104.8	110.7	116.6	78.3	104.8	103.1	93.2	128.9	113.3	109.6	5.34
9:30	11.17	123.4	94.6	109.4	108.1	79.1	94.8	87.2	81.3	118.1	99.5	99.6	4.51
10:30	12.17	122.0	85.9	104.3	101.8	68.2	90.9	77.3	71.7	102.6	88.6	91.3	4.99
													102.1

First column is clock time (the fish had been driven into the respirometer in the late afternoon on the previous day.

Last column is mean value at each of the pressure levels. These values were used for Figure 4.

Appendix Table 4. raw data for the effect of pressure on oxygen uptake in adult captive bloater chub. These values were used to calculate normalized values in Appendix Table 3.

Clock time	TRIAL	Mean										sem		
		1	2	3	4	5	6	7	8	9	10			
c	6:30	-3.83	146.2	270.4	303.8	241.1	269.8	232.2	188.8	220.4	170.8	216.8	226.0	15.32
c	7:30	-2.83	187.0	241.9	286.3	232.7	297.1	230.9	194.5	208.4	168.3	200.4	224.8	13.25
c	8:30	-1.83	200.3	233.5	228.1	236.9	299.2	220.6	188.8	181.5	164.5	194.2	214.8	12.08
c	9:30	-0.83	184.0	221.9	229.1	238.0	278.5	212.0	187.4	165.1	160.8	186.7	206.3	11.56
m	10:20	0.00	236.0	237.2	214.2	237.5	255.1	222.7	199.7	171.9	165.3	179.9	211.9	9.90
m	10:30	0.17												
m	10:40	0.33	243.1	245.3	223.5	246.7	269.1	215.7	206.5	204.2	189.7	194.4	223.8	8.29
m	11:30	1.17	272.3	257.2	278.1	242.1	244.6	236.3	185.9	192.8	207.5	222.8	234.0	9.99
m	12:30	2.17	271.6	243.8	210.3	212.6	263.7	235.2	195.8	158.9	211.8	216.4	222.0	10.48
m	1:20	3.00	264.2	246.7	237.7	199.7	236.6	216.8	208.6	155.0	218.3	201.1	218.5	9.64
m	1:30	3.17												
m	1:50	3.50	269.0	253.1	264.3	220.3	232.9	228.7	163.9	163.9	211.2	212.6	222.0	11.59
m	2:30	4.17	288.4	285.9	280.7	259.5	285.9	255.5	207.9	206.7	243.7	270.2	258.5	9.72
m	3:30	5.17	271.3	295.2	287.0	276.7	312.0	252.6	219.6	212.2	239.9	255.5	262.2	10.23
m	4:20	6.00	310.9	300.3	287.5	275.8	314.5	267.7	235.5	194.9	235.3	246.7	266.9	12.20
m	4:30	6.17												
m	4:50	6.50	301.3	292.1	291.6	289.8	308.8	267.8	252.5	209.8	229.2	247.2	269.0	10.51
m	5:30	7.17	293.5	299.4	263.2	279.0	276.8	252.4	232.7	189.8	214.3	234.0	253.5	11.26
m	6:30	8.17	269.0	268.7	230.5	255.3	227.9	231.6	211.2	160.4	188.4	214.9	225.8	10.88
m	7:20	9.00	250.9	264.6	233.7	249.0	213.3	245.5	211.2	134.6	182.6	208.8	219.4	12.27
m	7:30	9.17												
m	7:40	9.33	260.6	270.0	251.6	252.2	222.5	245.5	216.7	159.9	202.3	222.8	230.4	10.38
m	8:30	10.17	271.7	252.5	279.2	276.7	219.2	234.4	197.8	176.6	213.8	221.6	234.3	11.06
m	9:30	11.17	235.4	228.1	276.0	256.5	221.4	212.2	167.2	154.0	195.9	194.7	214.1	11.95
m	10:30	12.17	232.6	207.0	263.0	241.6	191.0	203.3	148.3	135.8	170.2	173.3	196.6	12.99