GREAT LAKES FISHERY COMMISSION

2002 Project Completion Report[1]

Buoyancy Strategies of the Bloater (*Coregonus hoyi* Gill)

by:

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Final Completion Report for the Great Lakes Fishery Commission:
BUOYANCY STRATEGIES OF THE BLOATER (*Coregonus hoyi* Gill)

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1. Executive Summary

Bloater use a variety of buoyancy strategies to prevent sinking or floating at increased (bottom of depth range) or reduced hydrostatic pressure (top of depth range), respectively. A higher lipid content in larger bloater combined with a decrease in the size of their gas bladder suggests that these fish rely more on static lift and may be less active. Smaller bloater, however, may rely more on hydrodynamic lift fuelled by a high metabolic rate; this may explain why younger bloater are found throughout the water column. Secretion of gas into the bladder occurs at too slow of a rate (days to weeks) to allow neutral buoyancy at more than one depth over a period of a few hours. However, the gas bladder can be regulated at a slower rate, as evidenced the anatomy of the gas bladder and buoyancy in deeper water.

2. Studies Completed

1. Flotation Pressure and Lipid Content

INTRODUCTION

Flotation Pressure

Saunders (1953) was the first to measure gas bladder volumes of bloater to determine whether they were neutrally buoyant at the depths of capture. He noted the tendency for some

individuals to be substantially buoyant (i.e., tend to float), and concluded that they would have to expend energy to keep from being swept to the surface (Saunders, 1953). However, Saunders did not present the numerical range of values that he found, which would be interesting in light of recent research.

TeWinkel and Fleischer (1998) used indirect observations (e.g., hydroacoustics, trawls) and Boyle's Law to estimate the gas bladder volumes of bloater both at the minimum shallow depth of migration and on the lake bottom. They calculated that bloater migrated down to a minimum shallow depth that caused a 50 % reduction in gas bladder volume upon return to the lake bottom (TeWinkel & Fleischer, 1998). TeWinkel (1998) indicated the need for direct studies that would identify the depths of neutral buoyancy.

I used Boyle's Law ($P_1V_1 = P_2V_2$) to compare buoyancies of bloater to the hydrostatic pressure at the depth of capture. My goal was to measure and describe the range of buoyancies that bloater experienced at depth.

Lipid Content

Lipid contributes to buoyancy because lipids are less dense (0.86-0.93 g/ml) than the ambient freshwater (≥1.00 g/ml) and also are less dense than locomotor muscle protein (1.05-1.06 g/ml) and bone (2.04 g/ml) (Alexander, 1993; Bone *et al.*, 1995; Phleger, 1998). Fishes with a higher lipid content benefit from a greater amount of static lift. Consequently, hydrodynamic lift can be reduced at bottom depths.

My goal was to determine the extent to which lipids contribute to buoyancy in bloater with respect to size. I also was interested in gender differences and differences between wild and captive-raised bloater. I compared lipid content 1) between wild and captive-raised bloater (age-2+), 2) between males and females, and 3) with respect to mass.

METHODS

Flotation Pressure

Bloaters were collected from bottom-set index gill nets (mesh size = 38 mm) set by the Ontario Ministry of Natural Resources on July 18th and 19th of 2000 in Georgian Bay, off Lion's Head Harbor, Ontario. Each gill net was set for 19 and 22 hours, respectively at a depth range of 70-97 m. Depth-temperature profiles were measured with a bathythermograph. Undamaged bloater were carefully picked from the nets and stored on ice until measurements could be made on shore (1.5 to 3.5 hours later). The majority of bloater were collected from a depth of 70 m.

Lengths, gender, and gas bladder volumes were determined for 40 fish; 26 of these fish were kept on a block of dry ice until their mass was later measured (see below). Ten of the 26 fish were used later for fat analysis (see below).

A 50 ml graduated cylinder (1 ml gradations) was used to measure gas bladder volumes. The graduated cylinder was inverted in a bucket of lake water taken from the surface (approximately 18 ^{0}C), and the abdomens of the bloaters were individually slit to bladder gas into the graduated cylinder (a funnel was positioned at the end of the graduated cylinder to channel gas from the bladder). The 26 freeze-dried bloater (with deflated swim bladders) were thawed in running water 6-7 hours later, and the mass in water (Mw) and mass in air (Ma) were measured.

Flotation pressure (atm) of my bloater was calculated with equation (1) and the difference in pressure between the fish and the depth of capture was calculated using equation (2):

(1) Fish flotation pressure (F.P.) = x/Mw (From Saunders, 1953)

where x = gas bladder volume

Mw = mass of the fish in water

(2) % difference in buoyancy = [(Fish F.P. - total pressure)/total pressure]*100

where total pressure = hydrostatic pressure (at depth of capture) + ambient pressure

When gas bladder volume was calculated by the combined gas law ($P_1V_1/T_1 = P_2V_2/T_2$) and compared to that calculated for from Boyle's Law ($P_1V_1 = P_2V_2$), there was no difference. Therefore, temperature corrections for gas bladder volume were not necessary. I compared my results (n = 26) with Saunders' (1953) results (n = 63).

Lipid Content

Lengths, weights and gender were previously determined and all bloater were sorted by size; 10 wild (see previous) and 10 captive-raised fish of similar size were randomly selected and numbered. All 20 bloater were kept frozen in plastic bags for 1-2 months maximum before lipid contents were measured during August 2000 by the University of Guelph Laboratory Services. Whole-body lipid content was measured by solvent extraction and expressed as percentage of wet body weight.

RESULTS

Flotation Pressure

My results are in agreement with Saunders (1953). Bloater in my study were 12 % positively buoyant (i.e. adapted to pressures 12 % greater than that at which they were captured), on average (range: -29 to 51 %), and the bloater in Saunders' study (1953) were 15 % positively buoyant, on average (range -24 to 54 %) (Figure 1; Appendix I). This means that the average bloater had a tendency to float and was neutrally buoyant at a depth 12-15 % *greater* than the

depth of capture. Smaller bloater showed extreme ranges of buoyancy (-29 % and 51 %) (Table 1). The size of the bloater (mass and total length, respectively) showed a strong, direct relationship to the volume of the gas bladder both at the depth of capture ($r^2 = 0.81$, $r^2 = 0.71$) and at a depth at which the fish would have been neutrally buoyant ($r^2 = 0.91$, $r^2 = 0.75$) (Figure 2). In addition, the relative volume of the gas bladder decreased as the mass of the bloater increased (mass, $r^2 = 0.29$, $p = 0.004$; total length; $r^2 = 0.13$, $p = 0.072$) (Figure 3).

All the bloaters that were captured and measured ranged from 188–302 mm (total length), and were thus considered adults.

Lipid Content

Although some variation existed, lipid content in both captive and wild bloater generally increased with size (Tables 1 & 2). Average lipid content in captive-raised bloater was 11.9 % ±2.1 (SEM) for fish 213 mm, total length ±1.1 (SEM) and 60.0 g ±7.3 (SEM). Lipid content in Georgian Bay bloater was 11.0 % ±2.0 (SEM) for fish 211 mm, total length ±0.6 (SEM) and 66.0 g ±5.7 (SEM).

Percent lipid increased with wet weight in both captive and wild fish (ANCOVA, $p = 0.002$) but these regressions did not differ between captive and wild fish and so can be described by a single regression line (Figure 4). Total length (mm) was a much weaker predictor of lipid content (data not shown).

There was no significant difference between lipid content in captive fish compared with wild fish (ANCOVA, $p = 0.45$). There was no significant difference between lipid content in males compared with females (ANCOVA, $p = 0.11$).

DISCUSSION

Flotation Pressure

The range in buoyancy values that I found is comparable to the free vertical range of movement that has been described for other species of fish. The average (12-15 %) and maximum (50-54 %) buoyancy values that I report for bloater agrees well with previous studies on other fishes (Harden Jones, 1952; Caulton & Hill, 1973; Arnold & Greer Walker, 1992). In contrast, my minimum buoyancy values (-24- -29 %) do not agree with previous studies on cod (-50- -90 %) (Harden Jones & Scholes, 1985; Ona, 1990). However, lower limits within the free vertical range are less well-defined in fishes than upper limits (Arnold & Greer Walker, 1992) and may be affected by the maximum range of depths within which each species is found. They also may be affected by the time of capture, as I cannot be sure that the bloater in my study were not caught at the center of their vertical range.

The average buoyancy values in my study (12 %) and in Saunders (1953) (15 %) are comparable to Harden Jones' (1952) and Caulton and Hill's (1973) laboratory work on freshwater physoclistous species (i.e., closed swim bladder). Harden Jones (1952) was able to show that perch (*Perca fluviatilis*) swam uninhibited at pressure decrease of 16 % of that to which the fish were adapted, and Caulton and Hill (1973) reported that *Tilapia mossambica* swam comfortably within depths that were ± 16 % that to which the fish were adapted. Thus, it is tempting to suggest that the swim bladders of different fish species cause similar restrictions of comfortable movement between depths, at least at small pressure changes.

The extent of shallow water (50-54 %) excursions are in agreement with the calculated minimum depth of movement and may be explained in terms of the minimum depth beyond neutral buoyancy at which bloater can maintain position in the water column (Harden Jones, 1952; TeWinkel & Fleischer, 1998). Harden Jones (1952) calculated that freshwater teleosts would not ascend to depths that involved pressure reductions of greater than 50 % of the pressure to which

they were adapted. Movement beyond 50 % positive buoyancy may cause damage to the expanding gas bladder and/or cause the fish to be carried to the surface (Tytler & Blaxter, 1973– cited in Arnold & Walker, 1992). However, bloater are physostomous and should be able to expel gas from their gas bladder via the pneumatic duct. I found that it took up to 30 minutes for captive-raised bloater to expel bubbles after a rapid decompression of 50 % (see Appendix II).

The extent of deep water (-24- -29 %) excursions may be explained in terms of the maximum depth beyond neutral buoyancy at which bloater can maintain position in the water column (TeWinkel & Fleischer, 1998), or it may be an artefact of changing hydrostatic pressures experienced by the fish when the gill nets are pulled to the surface. Alternatively, the negative buoyancy values that we witnessed in bloater may represent the maximum depth to which it is energetically efficient to use hydrodynamic lift to compensate for negative buoyancy. Lastly, negative buoyancy values may be a result of bloater expelling gas bladder gas through the pneumatic duct, and cannot be ruled out. Low gas bladder volumes can occur in dead or dying fish, and is probably caused by relaxation of the pneumatic sphincter muscle (Ona, 1990).

TeWinkel and Fleischer (1998) used indirect observations (e.g., hydroacoustics, trawls) and Boyle's Law to quantify the gas bladder volumes of bloater both at the minimum shallow depth of migration and on the lake bottom. They calculated that bloater migrated to a minimum shallow depth equal to a 50 % reduction in gas bladder volume upon return to the lake bottom (TeWinkel & Fleischer, 1998). In light of my results, the 50 % reduction in gas bladder volume upon return to the lake bottom would mean that bloater would be neutrally buoyant near the lake bottom. However, it is unclear how my findings relate to Fleischer and TeWinkel (1998), who found bloater to be neutrally buoyant at mid-water depths, and negatively buoyant on the lake bottom. TeWinkel (1998) commented thus:

"Because bloater are in general neutrally buoyant at their mid-water depths and negatively buoyant on the lake bottom (Fleischer & TeWinkel, 1998), a limit described as 50 % reduction in swim bladder volume may be the swim bladder volume at neutral buoyancy. Alternatively, this limit may represent a combination of reduction and expansion around the volume at neutral buoyancy."

Saunders (1953) attributed high positive buoyancy (i.e. excessive flotation pressure) in bloater to movement inshore from deeper water (possibly to feed) without adjusting the gas bladder volume. Conversely, negative buoyancy values in some bloater may be the result of a prolonged stay at higher hydrostatic pressures. For example, resorption of gases from the bladder is driven by the partial pressure difference between the gas bladder and the ambient environment, and may exceed secretion rates at deeper depths (Blaxter & Batty, 1984; Harden Jones & Scholes, 1985).

A wide range of buoyancy values corroborates the results of TeWinkel and Fleischer (1998); they found variations in the ranges of vertical migration among individual bloater. Cavadias and Gee (1987) concluded that the extensive adjustments of gas bladder buoyancy in darters (*Percina* spp.) allowed these fish to adapt to and utilize a wide range of environments, and the same may be true with bloater.

Fleischer *et al.* (1997), Eshenroder *et al.* (1998), Eshenroder and Burnham-Curtis (1999), and Eshenroder *et al.* (1999) hypothesized that a higher lipid content in larger deepwater ciscoes, including bloater, would reduce the relative volume of the gas bladder needed for neutral buoyancy, and this seems to be the case (Figs 2.3 & 2.4) (also see below). Butler and Pearcey (1972 – cited in Bone *et al.*, 1995) also found higher lipid content and lower contributions of gas bladder volume in an adult lantern fish (*Diaphus theta* – Myctophid), and Ona (1990) reported

similar results for oceanic herring (*Clupea harengus*).

Lipid Content

Lipid content in both captive and Georgian Bay bloaters showed variation but generally increased with the size of the fish. I found no differences in lipid content with respect to origin (captive-raised versus Georgian Bay fish) or gender.

The measured lipid content in the captive and Georgian Bay bloater used in my study was within ± 2.6 % of the lipid content in a recent study by Madenjian *et al.* (2000). Conversely, Rowan and Rasmussen (1992) and Hesselberg *et al.* (1990) reported lipid contents for bloater that ranged from 12.0–22.9 % and 11.9–24.8 %, respectively–from 0 to 12.9 % greater than the lipid contents of the fish in my study (Table 2.3).

A direct relationship between size and lipid content is common among fishes and also has been reported for bloater (Madenjian *et al.*, 2000–see below). Lipid content of the bloater in my study was best expressed as a function of weight, where 36 % of the variation in lipid content was caused by variation in wet weight. However, total length of our bloater was a much weaker predictor of lipid content than wet weight. In contrast, Madenjian *et al.* (2000) demonstrated a strong relationship between lipid content and total length for Lake Michigan bloater. They reported that 50 % of the variation in lipid content of bloater sampled in 1994-1995 could be explained by total length (Madenjian *et al.*, 2000).

Related factors such as food assimilation efficiency and temperature can affect accumulation of lipid in fishes; higher temperatures may allow higher feeding and growth rates, but can also impose higher metabolic demands in coregonines (Binkowski & Rudstam, 1994; Rudstam *et al.*, 1994). Although sizes were similar among captive-raised and Georgian Bay bloater, thermal histories and diets were different. However, laboratory temperatures were within

1 ^{0}C of optimum temperatures for the size range of the captive-raised bloater in my study (Wells, 1968; Rudstam *et al.*, 1994). The Georgian Bay bloater measured in my study could have conceivably experienced lower temperatures than our captive bloater. Assimilation efficiencies may be similar among bloaters, and perhaps genetically predetermined (Eschmeyer & Phillips, 1965).

Locality within the Great Lakes may also affect size (and hence lipid content) of bloater, due to differences in bathymetry and optimal prey habitat (Koelz, 1929; Wells & Beeton, 1963) and lake productivity (Brown *et al.*, 1987). For example, Rowan and Rasmussen (1992) presented lipid values for bloaters measured in the mid-1970's that varied by greater than 10 % with respect to locality within the Great Lakes (Table 2.3). In general, lipid content values ranged from the highest in Lake Michigan to the lowest in Lake Superior; values for Lake Huron bloater were intermediate. Madenjian *et al.* (2000) found significant differences in lipid content with regards to sampling sites in Lake Michigan, and Brown *et al.* (1987) noted that Georgian Bay bloater typically have a lower condition factor (indirect measure of lipid content) as compared with bloater from the main basins of Lake Huron and Lake Michigan.

In summary, a hypothetical bloater weighing 100 g has a 5 ml gas bladder and a lipid content of 18 % (see Table 2.1 and Figure 2.4). Assuming a minimum density of 1.05 g/ml (Alexander, 1993), the fish would be neutrally buoyant at about 10 m (2 atmospheres). At this depth, the lipid accounts for 34 % of the buoyant force and the gas bladder accounts for 66 % of the buoyant force.

Figure 1. Flotation pressure of bloater at various depths, over a range of depths that I examined. Note that some of the points are superimposed. The solid line represents neutral buoyancy; fish above the line are positively buoyant (tend to float), and fish below the line are negatively buoyant (tend to sink). Triangles: Saunders (1953); Circles: my data.

Figure 2.1

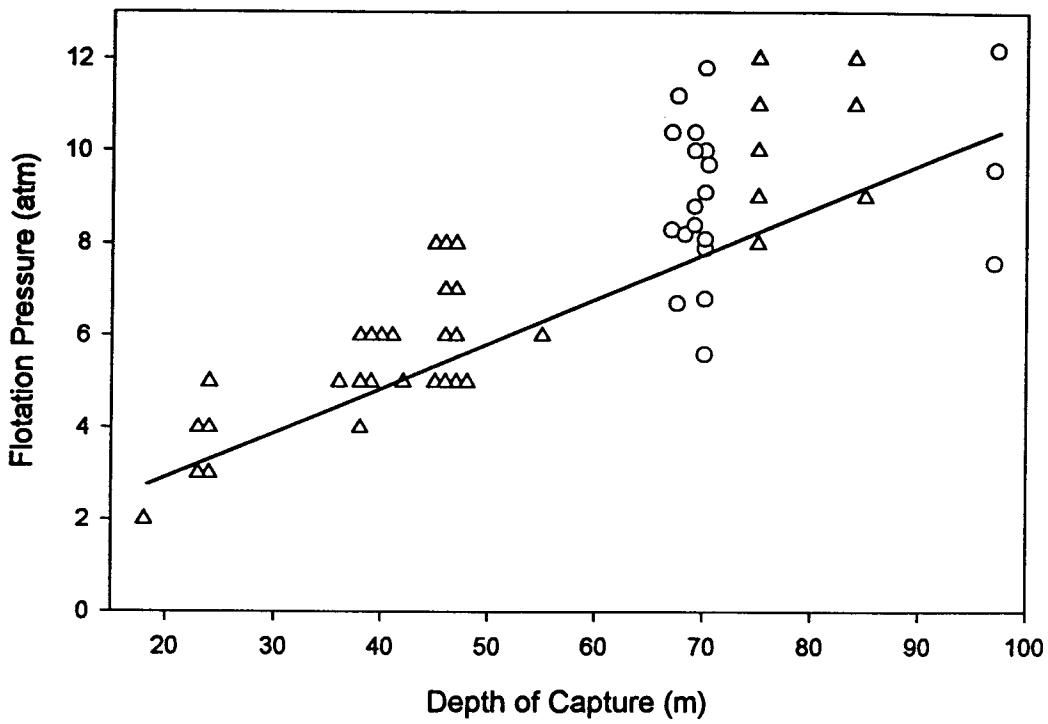

Flotation Pressure (atm)
0
2
4
6
8
10
12
20
30
40
50
60
70
80
90
100
Depth of Capture (m)

Figure 2.2. Regression analysis. Volume of the gas bladder of bloater (*Coregonus hoyi* Gill) in relation to body size of Georgian Bay fish (n = 25). The circles represent measurements from individual bloater. Note the direct relationship between gas bladder volume and body size **A.** at neutral buoyancy (mass: $r^2 = 0.91$, $Y = 0.038X + 1.128$; total length: $r^2 = 0.91$, $Y = 0.061X - 9.406$), and **B.** at the depth of capture (mass: $r^2 = 0.80$, $Y = 0.048X + 0.512$; total length: $r^2 = 0.82$, $Y = 0.079X - 13.217$).

Figure 2.2

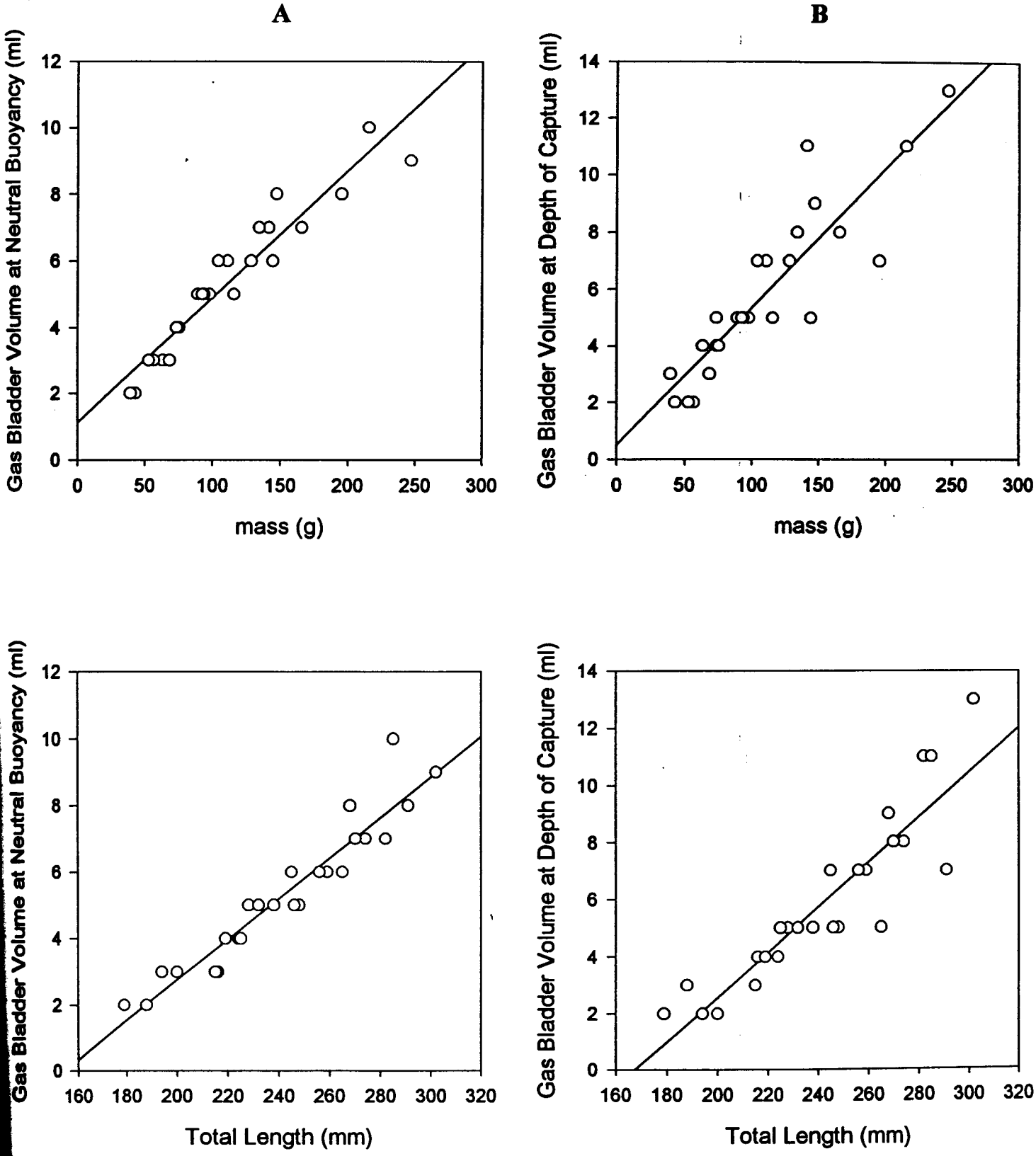
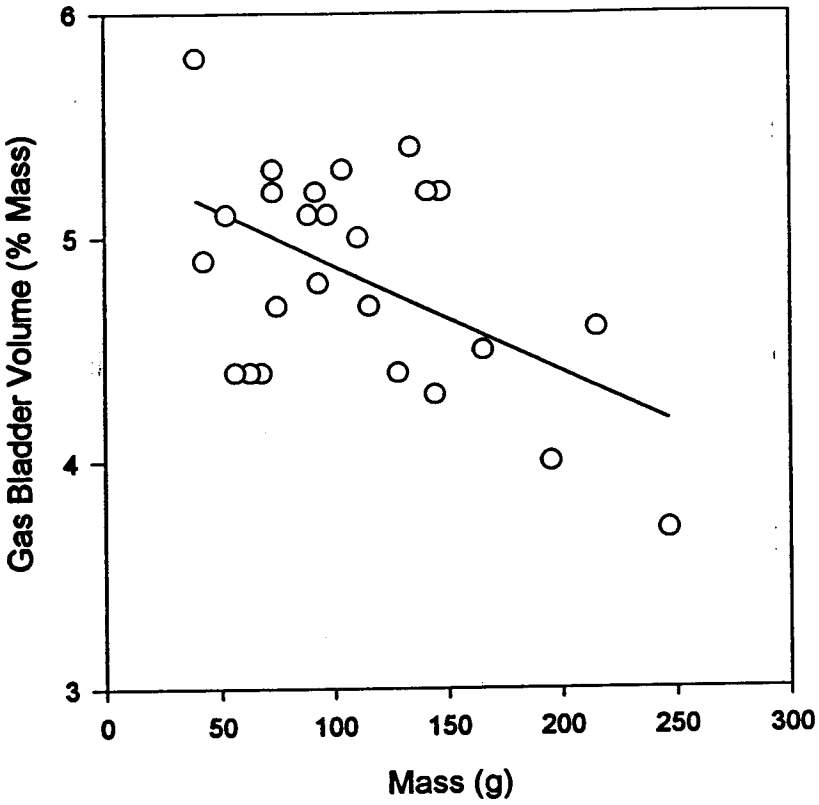

A
B
Gas Bladder Volume at Neutral Buoyancy (ml)
Gas Bladder Volume at Depth of Capture (ml)
mass (g)
mass (g)
Gas Bladder Volume at Neutral Buoyancy (ml)
Gas Bladder Volume at Depth of Capture (ml)
Total Length (mm)
Total Length (mm)

Figure 3. Relative gas bladder volume, expressed as a percentage of body mass, versus size of bloater. Mass: **$r^2 = 0.29$, $p = 0.004$**; total length: $r^2 = 0.13$, $p = 0.072$.

Figure 2.3


Gas Bladder Volume (% Mass)
6
5
4
3
0
50
100
150
200
250
300
Mass (g)

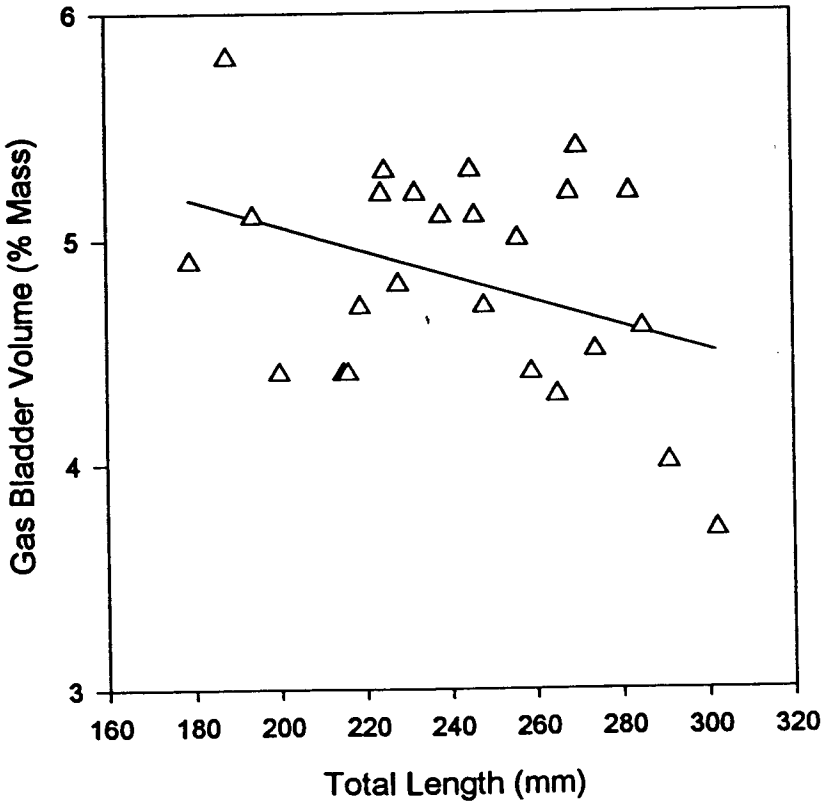

Gas Bladder Volume (% Mass)
6
5
4
3
160
180
200
220
240
260
280
300
320
Total Length (mm)

Table 1. Size and buoyancy attributes of bloater (n = 26) from Georgian Bay. Note how the neutral gas bladder volume approximates mass of the fish in water. g.b. = gas bladder

FL (mm)	TL (mm)	Gender	Mass	% buoyancy diff.	% gas bladder vol.	Neutral g.b. vol.	Mass in water	% lipid
167	188	M	39.6	+28	5.8	2	2.3	15
160	179	F	42.9	+4	4.9	2	2.1	8.5
174	194	F	52.9	-29	5.1	3	2.7	6.2
180	200	F	56.7	-28	4.4	3	2.5	14.3
193	216	F	63.3	+51	4.4	3	2.8	2.7
194	215	F	68.4	-12	4.4	3	3	x
199	224	M	73.3	+1	5.2	4	3.8	6.9
204	225	F	73.4	+17	5.3	4	3.9	7.6
195	219	F	75.1	+24	4.7	4	3.5	11.4
214	238	F	89	+17	5.1	5	4.5	12.5
208	232	M	92.2	+4	5.2	5	4.8	X
205	228	M	93.5	+17	4.8	5	4.5	25
220	246	F	97.5	+9	5.1	5	5	x
221	245	M	104.1	+28	5.3	6	5.5	x
229	256	F	110.8	+35	5.0	6	5.5	x
222	248	F	115.7	-12	4.7	5	5.4	x
232	259	F	128.4	+30	4.4	6	5.7	x

238	270	F	134.2	+14	5.4	7	7.2	x
255	282	F	141.3	+49	5.2	7	7.4	x
236	265	F	144.4	-27	4.3	6	6.2	x
243	268	F	147.1	+15	5.2	8	7.7	x
245	274	F	165.7	+10	4.5	7	7.5	x
260	291	F	195.3	-7	4.0	8	7.8	x
253	227	F	198.8	+19	4.4	9	8.7	x
259	285	F	215.3	+8	4.6	10	9.9	x
276	302	F	246.8	+39	3.7	9	9.2	x

Table 2. Comparison of the lipid contents of captive-raised and wild (Georgian Bay) bloater.

Status	Mass	TL (mm)	% lipids	Gender
Captive-raised	33.2	179	2.8	M
Captive-raised	50	199	3.9	F
Captive-raised	56.5	295	7.9	F
Captive-raised	47.2	185	9.5	M
Captive-raised	52.8	202	11	F
Captive-raised	40	188	11.4	M

Captive-raised	78.1	217	12.9	F
Captive-raised	52.6	207	15.4	M
Captive-raised	78.5	220	21.3	F
Captive-raised	111.6	240	22.5	F
Georgian Bay	63.3	216	2.7	F
Georgian Bay	52.9	194	6.2	F
Georgian Bay	73.3	224	6.9	M
Georgian Bay	73.4	225	7.6	F
Georgian Bay	42.9	179	8.5	F
Georgian Bay	75.1	219	11.4	F
Georgian Bay	89	238	12.5	F
Georgian Bay	56.7	200	14.3	F
Georgian Bay	39.6	188	15	M
Georgian Bay	93.5	228	25	M

Figure 4. Lipid content in relation to size (mass) of bloater. Lipid content was independent of gender and was the same in captive and wild fish. Lipid content increased with size.

Dark circles: captive-raised fish; open circles: wild (Georgian Bay) bloater.

Figure 2.4

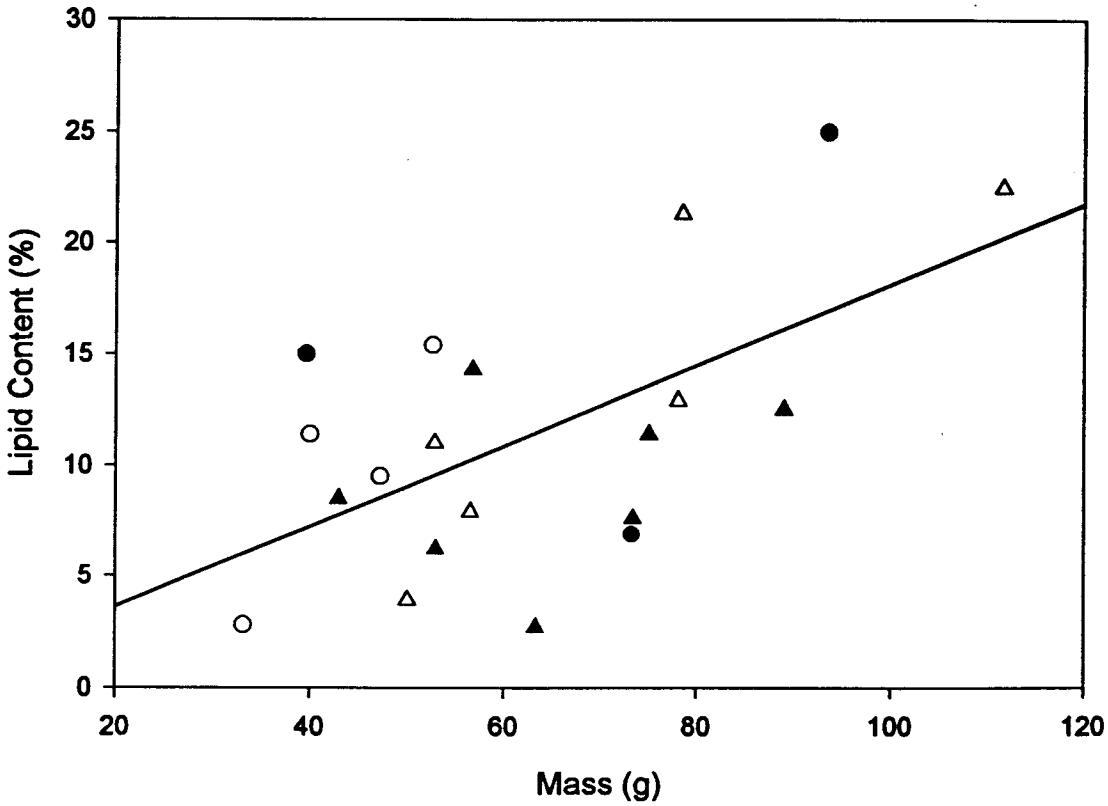


Table 3. Comparison of lipid contents of bloater in the Great Lakes. All values are means ±SEM in parentheses.

Location	year	lipid % wet wt.	TL (mm)	Mass	n	Reference
L. Michigan (Saugatuck)	1978	24.8	254	178	10	Hesselberg *et al.*, 1990
Northern Channel	1975	22.90	----	285	20	Rowan & Rasmussen, 1992
Lake Michigan	1972	21.95	278	257	860	Rowan & Rasmussen, 1992
L. Huron, Northern Basin	1974	16.36	285	292	123	Rowan & Rasmussen, 1992
Georgian Bay	1976	13.85	269	226	135	Rowan & Rasmussen, 1992
L. Michigan (Sturgeon Bay)	1994-1995	13.6 (0.4)	201 (3)	---	22	Madenjian *et al.*, 2000
L. Huron, Southern Basin	1974	12.13	349	372	113	Rowan & Rasmussen, 1992
L. Michigan (Port Washington)	1994-1995	12.8 (0.6)	211 (3)	---	20	Madenjian *et al.*, 2000
L. Superior	1974	12.06	260	182	585	Rowan & Rasmussen, 1992
L. Michigan, (Saugatuck)	1986	11.9	253	126	13	Hesselberg *et al.*, 1990
captive-raised	**2000**	**11.9 (2.1)**	**213 (11)**	**60.0 (7.3)**	**10**	**current study**
Georgian Bay	**2000**	**11.0 (2.0)**	**211 (6)**	**66.0 (5.7)**	**10**	**current study**
L. Michigan, (Saugatuck)	1994-1995	8.8 (0.5)	200 (3)	---	24	Madenjian *et al.*, 2000

2. Gross Anatomy and Histology of the Gas Bladder

INTRODUCTION

Other than the presence or absence of the pneumatic duct, the main difference in the anatomy of physoclists and physostomes is the presence of well-developed *retia mirabilia* (Harden Jones & Marshall, 1953; Fange, 1983). *Retia* are well-defined vascular bundles consisting of many thousands of alternating arterioles and venules that function to concentrate blood gases for subsequent secretion into the gas bladder (Steen, 1970; Fange, 1983; Pelster, 1997). In addition to distinct *retia*, physoclists are characterized as having a higher proportion of oxygen within the gas bladder, and a faster rate of secretion relative to the physostomes (Steen, 1970; Fange, 1983). The absence of a rete system in physostomes probably also means that regulation of the gas bladder is a balance between poor gas concentrating and secretion abilities on the one hand, and a potentially high resorptive capacity on the other (Steen, 1970).

In physoclists, the volume of the gas bladder is regulated by separate, well-developed secretory and resorptive regions that have different properties. The secretory region (usually located anteriorly) is typically supplied by thousands of arterioles and venules forming a counter-current exchanger (*retia*) (from the coeliaco-mesenteric artery). Secretive and resorptive processes include discernible areas in the gas bladder wall–typically called the gas gland (anterior portion) and oval gland (posterior portion) (Steen, 1970). The *retia* are directly connected to the thick gas gland epithelia within the secretory portion of the bladder (Harden Jones & Marshall, 1953; Steen, 1970; Fange, 1983).

Although the same sources of blood supply and drainage can exist within physostomous swim bladders, areas used for secretion or resorption of bladder gases are not easily discernible from each other (Steen, 1970), and are generally accepted as being rudimentary or non-existent in

physostomous fishes such as salmonids (Saunders, 1953). Therefore secretory (*retia*–gas gland complex) and resorptive regions typically do not exist (*Anguilla* is a notable exception) (Steen, 1970). The implication is that physostomous fishes either have inefficient gas secretion capabilities or none at all.

The capacity for bloater to inflate and deflate their gas bladder relies upon some sort of vascular mechanism that can concentrate and secrete gas into the bladder–possibly *retia*–and an opposing mechanism that allows gas bladder deflation–an open pneumatic duct. This latter point is an uncertainty in adult bloater as they are always bloated when brought to the surface, and unlike other Great Lakes physostomes (e.g., lake trout, *Salvelinus namaycush*, lake whitefish, *Coregonus clupeaformis*), they do not appear to be able to release gas when brought to the surface quickly (personal observation; Van Oosten et al., 1946; Tait, 1959). Furthermore, when bloater are squeezed, the gas bladder wall will rupture before gas is released from the bladder (personal observation).

Two questions arise: 1) If bloater do not fill their gas bladder by gulping air at the surface, and *if* they do *not* have *retia*, how do they secrete gas? 2) Is the pneumatic duct closed in adult bloater, thus preventing them from expelling gas from their bladder?

This section focuses on the presumed abilities or lack thereof for bloater and their putative ancestor, the shallow water cisco (*Coregonus artedi*), to control gas bladder volume.

The mechanisms for deflation (i.e., pneumatic duct, *retia*) and inflation (i.e., *retia*) of the gas bladders of cisco and bloater have never before been described. My approach was to examine the gross anatomy and histology of the gas bladders of both cisco and bloater in order to infer their capacity to regulate the volume of their gas bladders.

Comparisons were made with previous work on the anatomy of the gas bladder in other

Salmoniformes (i.e., salmonines and coregonines) that lack *retia* in the strictest sense, and also with the physoclistous type of gas bladder that has been described for fishes with *retia* and well-developed gas secretion and resorption abilities.

Gross anatomy

My first objective was to examine and describe the orientation and topography of the gas bladder *in situ*. My second objective was to examine and describe the blood vessels supplying and draining the gas bladder in adult cisco (*Coregonus artedi*) and adult bloater.

Histology

My objective was to examine both adult cisco and adult bloater for the presence of dense bundles of alternating arterioles and venules that could function as a counter-current gas exchanger (*retia mirabilia*). The presence of *retia* would imply that these species had adequate control over gas secretion (and perhaps resorption) and could theoretically maintain a relatively constant level of gas within the gas bladder. The blood vessel area and width of the gas bladder wall was not quantified or measured in the current study, but has been quantified and measured elsewhere, in other coregonines (Jasinski, 1963; Fahlen, 1967).

My second objective was to examine cross sections of the pneumatic duct to determine whether it was open to the esophagus. An open pneumatic duct would imply that bloater could expel excess gas from the bladder if they ascended above the depth in the water column at which they were neutrally buoyant.

METHODS

Cisco (*Coregonus artedi*) were collected with a 25 mm mesh gill net from Lake Opeongo, Algonquin Park, Ontario on May 4, 2000. A small incision was made on the ventral side of the fish prior to fixation in 10 % buffered formalin. The fish were later transferred into 100% ethanol

for 2 days, and then into 70 % ethanol prior to gross anatomical or histology observations.

Bloater were collected with 63.5–65 mm mesh gill nets from a commercial fisherman from Georgian Bay, off Meaford, Ontario on June 18th, 2002. Bloater were packed on ice on board the fishing vessel; lengths were measured 6 hours later on shore and a small incision was made on the ventral side of the fish, prior to fixation in 10 % buffered formalin. Fish were later transferred to 70 % ethanol prior to gross anatomical or histology examination.

The gas bladders of 3 captive-raised bloater (age-3+) were photographed. All captive-raised bloater were euthanized by a sharp blow to the head and were stored on ice until they were photographed a few hours later.

Gross Anatomy

All fish were carefully dissected so that the body wall was removed from the left side to reveal the gas bladder and other internal organs. The majority of fish examined were females. The gross anatomy of the gas bladder of 32 adult cisco (fork length = 128 cm ± 8.8 SD), 20 adult bloater (total length = 275 mm ±17.9 SD; fork length = 247 mm ± 17.8 SD), and 3 captive bloater (total length = 232 mm ±22.4 SD; fork length = 202 mm ±18.6 SD) were examined *in situ* with a dissecting microscope.

The length and diameter of the pneumatic duct of lake herring and bloater were measured with a 2.0 mm (0.1 ml gradations) eyepiece micrometer. The length of the pneumatic duct was measured *in situ* for 5 ciscoes and 2 wild bloater, from the esophagus to the adjoining region of the anterior gas bladder. The diameter of the pneumatic duct was measured at the juncture of the esophagus in 29 ciscoes and 9 wild bloater. The gas bladders of 16 bloater were then deflated and removed and the length was measured to the nearest millimeter with a metric ruler. The lengths of gas bladders of 5 ciscoes were measured *in situ* with a digital micrometer to the nearest 0.01

mm and rounded to the nearest millimeter.

Photographs of the gross anatomy were taken with a 35 mm ASAHI Pentax SP 1000 camera mounted on a dissecting microscope that magnified from 6-50 x. I used Kodak MAX 400 ISO (24 exp.) film.

Histology

Five to seven millimeter cross sections of cisco and bloater were cut and eviscerated (except for the gas bladder), fixed, and embedded in paraffin. Next, the tissue was sectioned into 7-10 micrometer slices with a microtome and stained with haematoxylin and eosin. At least 1 slide was prepared of each of the 5-7 mm gas bladder cross sections (anterior to posterior) for both cisco and bloater, and an additional 3 serial anterior and 2 serial posterior gas bladder sections were prepared for bloater. Additionally, longitudinal serial sections through the pneumatic duct were prepared for 1 specimen each of cisco and bloater. The histology of 13 cisco (fork length = 128 mm ±9.3 SD) and 8 bloaters (total length = 256 mm ±27.8 SD; fork length = 227.8 mm ±24.4 SD) was examined with a compound light microscope.

Histology slides were examined and photographs were taken with a 35 mm Olympus (Tokyo, Japan) camera mounted on a compound light microscope. I used Kodak MAX 400 ISO (24 exp.) film.

RESULTS AND DISCUSSION

Gross Anatomy

I. Orientation and Topography of the Gas Bladder

The gas bladder is a long and membranous, elliptical, sac-like structure that is extremely tapered anteriorly and less so posteriorly (Figures 5B & 8B). The gas bladder is located caudal to the pericardium and extends the length of the body cavity (Figure 5A). Boundaries of the gas

bladder are formed by the kidney, esophagus, and intestine at the dorsal, rostral-ventral, and ventral portions of the bladder, respectively. Gonads originate anterior to the pneumatic duct and are located laterally, on either side of the gas bladder (Fig. 6B).

The anterior gas bladder lies between the cranial portion of the kidney and the dorsal aspect of the esophagus (Figures 5A, 6A & 8C). Connective tissue attaches the anterior gas bladder to the kidney. At this location, the kidney slopes ventrally to connect to the esophagus.

The posterior-most portion of the gas bladder lies between the intestine and the body musculature anterior to the point where the intestine slants ventrally to form the anus. The main body of the bladder lies posterior to the ventrally-placed pneumatic duct (Figure 5B).

Mesentery holds the gas bladder dorsal to the kidney, and also attaches the ventral side of the bladder to the lateral body walls. The ribs and muscles of the body wall are laterally contiguous with the gas bladder, and thus somewhat restrict inflation to the dorso-ventral plane, as in the cod (*Gadus morhua*) (Arnold & Greer Walker, 1992) (Figure 5A & 5C).

My observations on the topography and orientation of the gas bladder are in general agreement with other salmonines and coregonines (*C. albula*: Jasinski, 1963; *Salvelinus namaycush*: Crawford, 1966; *C. lavaretus*: Fahlen, 1967; *Salmo salar*, *Salmo trutta*, Salvelinus *fontinalis*, and *Oncorhynchus mykiss*: Fahlen, 1971). However, differences in the gross morphology of the gas bladder are apparent between salmonines and coregonines.

According to Jasinski (1963), the gas bladder of rainbow trout (*Oncorhynchus mykiss*) appears to be more rounded in shape at the posterior end. Jasinski (1963) and Fahlen (1971) both implied that the anterior portion of the gas bladder in salmonines is more rounded. In contrast, the coregonines in the present study had an extremely narrow anterior portion of the gas bladder. Jasinski (1963) and Fahlen (1967) note also noted extremely narrow anterior portions of the gas

bladder in *C. albula* and *C. lavaretus*.

II. Dimensions of the Gas Bladder and Pneumatic Duct

The length of the gas bladder increased as the length of cisco and bloater increased. The entire gas bladder is about 40 % of the fork length of both cisco and bloater for the size ranges that I measured, indicating that the length of the gas bladder increases at a similar rate (Table 4) Eshenroder *et al.* (1999) also reported that the increase in the length of the gas bladder was the same for captive-raised juvenile cisco and bloater. Although my results show that increases in the volume and length of the gas bladder and lipid content are proportional to increases in length in bloater (Table 4; section 2: Figures 2, 3, & 4), it is apparent that lipid content may contribute more to buoyancy in larger bloater than the gas bladder (see previous section).

Like most fishes, the pneumatic duct in bloater (and probably cisco) is controlled by a muscular sphincter (also see **Histology**) (Harden Jones & Marshall, 1953; Fange, 1983). When contracted, this sphincter probably closes the duct of wild bloater and some cisco. For example, bloater with greatly distended gas bladders (as a result of large pressure drop from being brought to the surface) will not release gas from their gas bladder when they are squeezed (Figures 5C & 5D). As previously mentioned, the gas bladders of bloater will in fact burst when pressure is applied to them. I observed a similar situation with some cisco captured in gill nets.

The pneumatic duct is short and thin, and is proportional (in terms of length and outside diameter) to the size of the fish (Table 4). The lengths of the pneumatic duct are approximately 0.6 mm *in situ* for 128 mm cisco (fork length) and 1.8 mm *in situ* for 249 mm (fork length) bloater. Width measures of the pneumatic duct are approximately 0.9 mm and 1.4 mm in outside diameter for 128 mm cisco (fork length) and 246 mm bloater (fork length), respectively, as measured at the connection of the duct to the esophagus (Table 4).

There appears to be some variation in the dimensions and morphology of the pneumatic duct among and within salmonines and coregonines. However, it is difficult to make definitive conclusions because some of the information is ambiguous. Like the cisco and bloater in my study, Jasinski (1963) and Fahlen (1971) also reported short pneumatic ducts for *C. albula* and various salmonines. Jasinski (1963) reported that this duct was thick-walled in *C. albula*. Fahlen (1971) noted that the pneumatic duct was "wide open in distal parts" in *Salmo salar*, *Salmo trutta*, *Salvelinus fontinalis*, and *Oncorhynchus mykiss*. Neither Jasinski (1963) nor Fahlen (1971) provided measurements. Fahlen (1967), however, reported that the pneumatic duct of *C. lavaretus* was long and thin. His measurements were approximately 2x longer than the pneumatic duct of bloater (when the entire length was measured) (Table 4). A bend is present in the pneumatic duct in the cisco and bloater at a point slightly greater than half way from the esophagus to the gas bladder (Figs. 6A, 6B, & 6C). Fahlen (1967) also reported a bend in the pneumatic duct of *C. lavaretus*.

III. Gas Bladder Wall

On the exterior surface, a dense strip of red pigmented spots is visible on the median dorsal side of the gas bladders of both cisco and wild bloater (Figure 5B) (also see **Histology**). These spots are chromatophores, and can be found in the body cavity of salmonids, particularly near the kidney (Dr. J. Leatherland, pers. comm.). The chromatophores are particularly evident at the extreme anterior and posterior portions of the gas bladder, where it tapers and the spots are located more laterally. It is apparent that the chromatophores reside in the outer layer or tunica externa of the gas bladder (see **Histology**), but probably have no function in regulation of the volume of the gas bladder (Dr. J. Leatherland, pers. comm.).

Although I did not make detailed comparisons between captive-raised and wild bloaters, it

was obvious that the entire gas bladder of adult captive-raised bloater (age-3+) was very thin and translucent in comparison to wild bloater (Figure 7A & 7B). Crawford (1966) reported a similar finding in lake trout (*Salvelinus namaycush*). He found that wild lake trout had thicker gas bladder tissues than captive-raised fish (Crawford, 1966).

On the exterior ventral surface of the gas bladder, a layer of adipose tissue is noticeable in the captive bloater. I also observed this layer in the histology cross sections in cisco and wild bloater (see **Histology**) (Figure 8B).

IV. Vascularization of the Gas Bladder

Like most fishes, the coeliaco-mesenteric artery (CMA, which arises from the dorsal aorta) supplies blood to the gas bladders of cisco and bloater (Figures 5A, 6B, & 8A) (Harden Jones & Marshall, 1953; Steen, 1970). In physoclistous fishes and the physostomes with distinct *retia* (e.g., *Anguilla anguilla* and *Esox lucius*) the CMA branches into 2 parts: a metabolically active secretory portion with *retia*, and a metabolically passive resorbent portion (resorption is driven more by gas partial pressure) (Steen, 1970; Blaxter & Batty, 1984; Harden Jones & Scholes, 1985; Alexander, 1993). However, unlike physoclists and the more-derived physostomes (e.g., *Anguilla*, Figure 13), no *retia* were visible anywhere on the exterior surface of the gas bladder in cisco and bloater. As in most physostomes, the secretory and resorbent portions of the gas bladder, if they do exist, were not evident (Steen, 1970).

In both cisco and bloater, the CMA runs ventrally from the cranial portion of the kidney, and lies to the right side of the pneumatic duct (Figure 6B). Like other salmonines and coregonines, the main gas bladder vessels that are visible to the naked eye arise from a branch of the CMA that runs along the esophagus and up either side of the pneumatic duct (Figures 5A, 6B, & 6C) (Jasinski, 1963; Fahlen, 1967 & 1971). These blood vessels branch laterally and extend the

length of the bladder in cisco, bloater, and other coregonines (Figure 5A), but are largely confined to the pneumatic duct and anterior region of the gas bladder in salmonines (Jasinski, 1963; Fahlen, 1971). The gonads are usually observed lying against these lateral blood vessels, concealing them. Closer examination reveals that these two main blood vessels are usually comprised of 3 veins and 2 arteries that alternate (Figures 7A, 7B, & 7C). Ramifications of these main blood vessels occur at fairly regular intervals along the length of the gas bladder to form networks that enter the internal layers of the gas bladder. In some captive bloater, the lateral gas bladder vessels spread onto the dorsal side of the gas bladder, at the posterior end.

I observed an additional blood supply from the CMA to the anterior surface of the gas bladder in captive-raised bloater (Figure 8A). These blood vessels (CMA) probably are damaged when the gas bladder is removed from captive-raised bloater, resulting in a haematoma (region of broken blood vessels resulting in pooling blood) (Figure 8B).

Further examinations of the histology of the gas bladder revealed blood vessels originating from the dorsal side of the gas bladder. These blood vessels may in fact be intercostal arteries branching from the dorsal aorta and running from the mesonephros of the kidney, as has been reported for other fishes (Harden Jones & Marshall, 1953; Fahlen, 1971). Fahlen (1971) reported that intercostal arteries supplied the posterior 2/3 of the gas bladder in salmonines of the genus *Salmo, Salvelinus*, and *Oncorhynchus*. However, these connections do not appear to be prevalent in either cisco or bloater, as they were noticed on only one occasion for each species. Furthermore, it is unclear whether these blood vessels actually supplied the gas bladder. No one else has reported a similar finding for other coregonines.

I did not observe the origins or destinations of the venous blood in the cisco or bloater gas bladders. However, venous blood leaving the gas bladder usually re-enter the hepatic portal vein

in most fishes (Harden Jones & Marshall, 1953; Steen, 1970). Fahlen (1967) reported that the hepatic portal vein follows the CMA along the esophagus and up the pneumatic duct to the gas bladder in *C. lavaretus*.

Histology

I. Layers of the Gas Bladder Wall

In terms of order and content, the layers of the gas bladder in cisco and bloater are similar to each other and to other coregonines. The layers of the gas bladder in coregonines are generally similar to salmonines, but are quite different in terms of vascularization. Furthermore, the layers of the gas bladder in coregonines and salmonines are *generally* similar to the layers of the gas bladder in a distantly related marine physoclistous fish–*Argentina silus* (Fange, 1958; Jasinski, 1963; Crawford, 1966; Fahlen, 1967 & 1971). The differences between the Salmoniformes and *A. silus* exist within specializations of the different layers of the gas bladder wall (Fange, 1958).

Fange (1958 & 1983) reported 3 concentric layers of the gas bladder in physoclists that can also be found in physostomes (from outer–inner layers): tunic externa, submucosa, and mucosa. These three layers of the gas bladder are also found in Salmonids, and in particular, in the coregonines in my observations and previous studies (Jasinski, 1963; Crawford, 1966; Fahlen, 1967 & 1971) (Figure 9A, 9D, & 9F) .

The outer layer of the gas bladder, the tunica externa, is comprised of fibrous connective tissue (collagenous), guanine crystals, and a circular muscular layer that is contiguous with the body wall (this 4th layer is part of the body wall; see Fahlen, 1967) (Figures 9A & 9C). A thin longitudinal muscle layer was discernible immediately exterior to the thicker circular muscularis mucosa in some slides of the cisco and bloater. Jasinkski (1963) also reported some longitudinal muscle fibers in the tunica externa of *C. albula*.

The silver appearance of the gas bladder, as observed with the naked eye, is usually caused by guanine crystals present in the tunica externa (Blaxter & Batty, 1984) (Figures 5D & 7A). Guanine provides a relatively impermeable layer which functions to prevent diffusion of gases from the gas bladder (Fange, 1983; Blaxter & Batty, 1984). Fange (1958) reported an especially thick tunica externa with dense amounts of guanine in the distantly related marine physoclist, *A. silus*, which is usually found at depths of 220-500 m.

The tunica externa has adipose deposits on the ventral side of the bladder (see also **Gross Anatomy**) (Figure 9A). Crawford (1966) also reported adipose deposits in the tunica externa of *Salvelinus namaycush*, whereas Fahlen (1967) reported that adipose deposits existed in the submuscularis of *Coregonus lavaretus*.

A yellow-pigmented layer is present in the tunica externa, and located on the dorsal surface of the gas bladder (Figure 11). This pigmented layer corresponds to the chromatophores that I noticed in the gross anatomical observations (Figure 5B) (also see above).

The submucosa is a thick layer that is intermediate to the outer tunica externa and the inner mucosa (Figures 9B, 9C, & 9D). The submucosa is comprised of collagenous connective tissue interspersed with vascular bundles of various sizes (see **Vascularization of the Gas Bladder**, below). These vascular bundles are the branches of the coeliaco-mesenteric artery and hepatic portal vein.

The mucosa is comprised of epithelium with underlying connective tissue (termed the "lamina propria") and smooth circular muscle (Figure 9E & 9F). The epithelium faces the internal lumen of the gas bladder. Capillaries enter the lamina propria and supply the gas bladder epithelia, where secretion takes place (Fahlen, 1967) (Figure 9E).

All the layers of the gas bladder wall appear to be the thickest in cross sections anterior

and adjacent to the pneumatic duct (Figures 10A, 10B, 10C, & 10D). Particularly evident in the anterior sections of cisco and bloater is the thick, well-developed mucosa (Figures 10C & 10D). The mucosa of an anterior portion of the gas bladder in bloater is 0.25-0.40 mm (Figure 10C), and a section of mucosa at the interface of the pneumatic duct and gas bladder is 0.30-1.45 mm (Figure 10D). Judging from my observations, the mucosa in the main portion of the gas bladder appears to be thinner, but additional measurements need to be made. Fahlen (1967) also reported thickened mucosa in the anterior portions of the gas bladder in *C. lavaretus*.

A thick, well-developed mucosa in the anterior region of the gas bladder is consistent with gas secretion (Fange, 1966). In addition, the epithelium of a ventral portion of the gas bladder in the cisco is slightly greater thickness in relation to the remainder of the peripheral epithelium throughout all sections, although this might have been caused by contraction of muscles on the opposing side of the gas bladder (Figure 9F) (Fahlen, 1967).

II. Diaphragm of the Gas Bladder

Longitudinal serial sections of the cisco reveal that a portion of the gas bladder anterior to the pneumatic duct contains a "diaphragm" that appears to seal off the anterior-most portion of the gas bladder from the posterior portion (Figure 11). The anterior portion of the gas bladder in front of the diaphragm is extremely small–about 6 % of the average length of a cisco gas bladder: 2.8–3.4 mm (maximum length) by 0.9–1.0 mm (maximum height) (see Table 4). Some longitudinal sections revealed that this diaphragm contained an aperature, as it was open in some sections, but closed in most. To my knowledge, a gas bladder diaphragm has not been reported in any other Salmonids or related species.

I did not observe a diaphragm in the longitudinal serial sections of the gas bladder in bloater; therefore it probably does not exist. However, it is possible that if a diaphragm does exist

in bloater, it was not visible because it was extremely small, and/or it was in an uncontracted state and so was not readily seen.

The presence of a diaphragm implies greater specialization of secretory and resorptive regions of the gas bladder versus a simple sac-like gas bladder with no regional differentiation (Fange, 1966). For example, faster rates of gas secretion in fishes are generally correlated with differentiation of the gas bladder into separate compartments (Fange, 1966).

III. Pneumatic Duct

My observations of the longitudinal serial cross sections of the pneumatic duct in both cisco and bloater reveal that it is open to the esophagus in both species (Figure 12B). It is apparent that the pneumatic duct of bloater is very muscular (see Gross Anatomy) at both the connection to the esophagus and probably at the entry into the gas bladder (Figures 12A, 10A, & 10B). The presence of a strong sphincter muscle at the juncture of the pneumatic duct and the esophagus is common in physostomes and has been mentioned for several other species of fish (Crawford, 1966; Fahlen, 1967 & 1971; Maina *et al.*, 1996).

IV. Vascularization of the Gas Bladder

I observed no *retia* in cisco and bloater on a level comparable to physoclistous fishes or the more derived physostomes (e.g. *Anguilla rostrata*, Figure 13). However, I did observe vascular bundles within the gas bladders of cisco and bloater that are similar to what Fahlen (Fahlen, 1959 & 1967) reported in *Coregonus lavaretus* (Figures 9B, 9C, & 9D). The vascular bundles are branches of the coeliaco-mesenteric artery (CMA) that have branched from the large lateral blood vessels (Figures 5A & 7A) (see **Gross Anatomy**) of the gas bladder. These lateral blood vessels form the vascular bundles on the ventro-lateral periphery of the thickest portion of the submucosal layer (Figure 9A). This portion of the submucosal layer is contiguous with the

body musculature. Smaller vascular bundles radiate around the periphery of the gas bladder from these main lateral blood vessels, forming a variety of different lumen sizes within the submucosae (Figure 9B). Although each vascular bundle may possess gas secretion abilities, the smaller vascular bundles (diameter of one erythrocyte) will be the most efficient gas exchangers and can properly be termed *micro retia* within the gas bladder (Fahlen, 1967; Harder, 1975) (Figures 9C & 9D). The vascular bundles are set within the submucosal layer of the gas bladder in rows of 3 or more (up to 8 vessels counted in bloater) alternating capillaries and veins, and *not* within a dense package of many thousands of alternating arterioles and venules, as can be seen in *Anguilla* (Fahlen, 1967; Steen, 1970). Fahlen (1967) reported up to 10 blood vessels within the vascular bundles in *C. lavaretus*. Fahlen (1967) suggests that the *micro retia* act as counter-current gas exchangers and enable secretion of gases into the bladder.

Micro retia have not been reported in any salmonines (Crawford, 1966; Fahlen, 1971). However, Fange (1958) reported *retia* in the distantly related marine physoclist, *Argentina silus* that are equivalent to the *micro retia* reported by Fahlen (1967) in *C. lavaretus* and by me for cisco and bloater in the present study.

In captive-raised bloater, I observed blood vessels (with the naked eye) running into the gas bladder between the anterior-most portion of the gas bladder and the esophagus. These blood vessels are probably a branch of the CMA (Figure 8A) (see **Gross Anatomy**, above). However, I did not find a similar branching of the CMA in cisco or wild bloater. I did, however, observe a connection between the extreme anterior gas bladder and the kidney (see **Gross Anatomy**). At the anterio-dorsal juncture of the kidney, connective tissue of the gas bladder runs dorsally, at an angle, into the kidney (Figure 8C).

CONCLUSION

My observations on two species of coregonines (cisco and bloater) agree with previous studies on coregonines (Jasinski, 1963; Fahlen, 1967) and salmonines (Jasinski, 1963; Crawford, 1966; Fahlen, 1971) and suggest that coregonines have gas secretion abilities that are superior to salmonines.

Although coregonines do not have *retia*, they do have a less-derived form of vascularization–*micro retia*–that nevertheless enables gas secretion into the bladder. Salmonines, however, have neither *retia* nor *micro retia*. Additionally, cisco and bloater, like other coregonines and salmonines, have an open pneumatic duct that should enable them to expel excess gas from their bladders if they become too positively buoyant (see section 2).

The *micro retia* of coregonines are comprised of only a few alternating arterioles and venules (i.e., ≤ 10 blood vessels) (Fahlen, 1967). *Micro retia* are not present in the closely related salmonines (e.g., Atlantic salmon, *Salmo salar*; brown trout, *Salmo trutta*; lake trout, *Salvelinus namaycush*; brook trout, *Salvelinus fontinalis*; rainbow trout, *Oncorhynchus mykiss*) (Crawford, 1966; Fahlen, 1971). These *micro retia* probably function as less-derived and hence less efficient counter-current gas exchangers (Fahlen, 1967). In contrast, the *retia* of more derived physostomes (e.g., *Esox* and *Anguilla*) and physoclists are relatively efficient counter-current gas exchangers, and are comprised of several thousand arterioles and venules (Steen, 1970; Fange, 1983). For example, in *Anguilla*, the *retia* is comprised 88,000 venules and 116,000 arterioles (Krogh, 1929–cited in Fange, 1983) and is plainly visible in the gross anatomy (Figure 13).

Cisco and bloater have a pneumatic duct that is open to the esophagus and is controlled by a sphincter at the connection to the esophagus. This pneumatic duct also appears to be muscular at the entrance to the gas bladder. The presence of a muscular sphincter at the connection to the

esophagus implies preferential control of release of gas from the bladder. For example, cisco and bloater should be able to either expel excess gas from their bladders by relaxing the pneumatic duct sphincter or they should be able to maintain gas bladder volume by contracting (i.e., closing) the pneumatic duct sphincter. Cisco, being a shallow-water species (Scott & Crossman, 1973), could conceivably gulp atmospheric air from the surface to inflate their gas bladder. Adult bloater, however, are almost always found in the thermocline and hypolimnion (Wells, 1968; Crowder & Crawford, 1984; Brown *et al.*, 1985; Crowder & Magnuson, 1982; Brown and Eck, 1992) and are restricted in the vertical migrations they can make (TeWinkel & Fleischer, 1998). Therefore, adult bloater would seemingly be precluded from inflating their gas bladder by surface gulping. Although there have been reports of bloater in shallower waters (e.g., Koelz, 1929; Wells, 1968), the literature is not clear as to whether these individuals were adults.

It cannot be ruled out that adult bloater with empty gas bladders (and therefore no buoyancy restrictions to ascending in the water column) (TeWinkel & Fleischer, 1998) lack the capacity to occasionally gulp air at the surface in order to partially fill their gas bladder. For example, oceanic herring (*Clupea harengus*) are also physostomes which lack the organs necessary to secrete gas into their gas bladder, and are able to perform diel vertical migrations from 100 m or greater towards the surface (Blaxter & Batty, 1984). At the surface, herring rely on gulping air to inflate their gas bladders to some extent (Blaxter & Batty, 1984; Harden Jones & Scholes, 1985; Ona, 1990). Furthermore, I have witnessed that captive-raised bloater do in fact gulp air under ambient or increased pressure (see Appendix II).

Warmer surface waters during summer stratification would seemingly prevent deep-living adult bloater from swimming to the surface (Saunders, 1953). Although adult bloater are found at cooler temperatures than younger life stages (Crowder & Crawford, 1984; Edsall & Frank,

1997), there is no indication that temperature restricts vertical migrations (TeWinkel & Fleischer, 1999). Furthermore, during the spring (isothermal lake conditions), little vertical migration occurred in bloater (Brandt *et al.*, 1991; Argyle, 1992), in comparison to the summer (TeWinkel & Fleischer, 1999).

The coregonines in the present study differ from *Coregonus albula* and *Coregonus lavaretus* in three anatomical features that are either different or have not been reported for other coregonines. First, cisco and bloater have shorter pneumatic ducts than that of *Coregonus lavaretus* (Fahlen, 1967). Second, cisco and bloater have additional avenues of blood supply to the gas bladder. For example, cisco and bloater have intercostal arteries that supply a posterior portion of the bladder. Third, cisco have an additional gas bladder feature that, to my knowledge, has not previously been described in any other of the Salmoniformes or closely related species (see Appendix II). They have an anterior diaphragm that may close off a small anterior portion of the gas bladder from the posterior. This diaphragm was not observed in bloater. The presence of a diaphragm implies greater specialization of secretory and resorptive regions of the gas bladder versus a simple sac-like gas bladder with no regional differentiation (Fange, 1966). For example, faster rates of gas secretion into the gas bladder in fishes is generally correlated with differentiation of the gas bladder into separate compartments (Fange, 1966). Alternatively, the diaphragm may be used for some other as yet unknown function.

I also noticed that cisco and bloater (like other coregonines) (Jasinski, 1963; Fahlen, 1967), had a very narrow anterior portion (anterior to the pneumatic duct) of the gas bladder that might be adapted for gas secretion into the gas bladder. In contrast, there have been no reports of a narrow anterior region of the gas bladder in salmonines. In the cisco and bloater in my study, I observed thicker submucosa and mucosa within this anterior portion. A thick, well-developed

mucosa in the anterior region of the gas bladder is consistent with gas secretion (Fange, 1966). Fahlen (1967) also reported thickened mucosa in the anterior region of *Coregonus lavaretus*.

The gas content of the gas bladders and rates of secretion of salmonines, coregonines, and physoclistous fishes been examined in other studies. The oxygen percentage in the gas bladder of fishes is related to the capacity for oxygen secretion and negatively related to the capacity for resorption (Wittenberg, 1958; Steen, 1970). A low capacity for oxygen secretion is observed with slower rates of gas secretion in the Salmoniformes, while a higher capacity for oxygen secretion is observed with faster rates of gas secretion in the more derived physostomes and physoclists.

Although much confusion previously surrounded the high nitrogen content of the gas bladder in *Coregonus acronius* (99 % nitrogen), further research has demonstrated that at least some coregonines show higher oxygen contents comparable to that of the more derived physostomes (Scholander *et al.*, 1956; Sundnes *et al.*, 1958; Fahlen, 1967). For example, Fahlen (1967), Sundnes *et al.*(1958), and Sundnes (1963 – cited in Fange, 1983), all reported oxygen values of 61-69.3 % in the gas bladder of *Coregonus lavaretus*. In fact, Sundnes *et al.* (1958) found oxygen concentrations to increase with the depth of occurrence in *C. lavaretus*, and Sundnes *et al.* (1963 – cited in Fahlen, 1967) reported the highest levels (69.3 %) in *C. lavaretus* that had recently migrated into deeper water. However, Sundnes *et al.* (1963 – cited in Steen, 1970) also reported high nitrogen levels in *C. lavaretus* that had been at depth for several weeks, which probably indicated that the rate of gas secretion was slower than the rate of gas diffusion from the bladder (Wittenberg, 1958; Steen, 1970).

The length of time spent at depth may explain why the physostomes (including cisco and bloater) in Saunders' study (1953) had higher nitrogen concentrations at deeper depths, on

average. However, the trend for increasing nitrogen (and decreasing oxygen) in the cisco and bloater in Saunders study (1953) is less clear when the range of values is examined. For example, in cisco the maximum range of oxygen content in the gas bladder shows no trend from the surface down to 51 m, where it is the highest level – 47.5 %. In bloater, the maximum range of oxygen content in the gas bladder increases from shallow water down to 41-51 m where it is the highest – 38.3–35.8 % (Saunders, 1953).

The rate of gas secretion into the gas bladder typically occurs on a scale of days to weeks (and even months) for physostomes and hours to days for physoclists (Wittenberg, 1958; Fange, 1983). It appears as though salmonines have a very weak ability to secrete gas into their bladder, while the rates of gas secretion of coregonines approach that of physoclistous fishes (Sundnes *et al.*, 1958; Tait, 1959; Sundnes, 1963 – cited in Fange, 1983; Fahlen, 1971; Fange, 1983) .

Salmonines have a very weak ability to secrete gas into their bladder. Fahlen (1971) reported partial filling of an emptied gas bladder after 40 days in 4 species of salmonines (*Oncorhynchus mykiss, Salmo salar, Salvelinus fontinalis, Salmo trutta*). However, Wittenberg (1958) reported that it took 13 days for the salmonines in his study, rainbow trout (*O. mykiss*) and brown trout (*Salmo trutta*), to refill emptied gas bladders. However, Fahlen (1971) and Wittenberg (1958) do not mention whether or not they barred access to surface gulping in experiments. In contrast, Jacobs (1934 – cited in Tait, 1959) reported that when barred access to the surface, the salmonines in his study could not refill their gas bladders and in fact lost residual gas from their emptied gas bladder. Tait (1959) reported a similar finding. He reported that the 7 species of young salmonines (barred access to the surface) lost gas from their bladders when subjected to pressure. However, young lake whitefish (*Coregonus clupeaformis*) and cisco had secretion rates comparable to 2 young centrarchids (physoclists) (Tait, 1959).

The rates of gas secretion in coregonines is faster than salmonines and approaches that of the more derived physostomes (*Esox* and Cyprinids) (Tait, 1959; Jacobs, 1934 – cited by Fange, 1983). Tait (1959) reported that small cisco (10 g) and lake whitefish (*Coregonus clupeaformis*) (15 g), when subjected to pressure increase and barred access to the surface, had secretion rates comparable to the physoclistous sunfish (*Lepomis gibbosus*) and rock bass (*Ambloplites rupestris*). Gas secretion rates for cisco (age-1) were 0.17 atm/day while lake whitefish (age-1-3) were 0.12 atm/day, while the salmonines lost gas from their gas bladder. At this rate, it would take 6-8 days for these young coregonines to adjust to 1 atm of pressure. Additionally, Tait (1959) reported that the gas within the gas bladders of cisco and lake whitefish was 54 % and 50 % oxygen, respectively.

Indirect and direct research has shown that coregonines have a mechanism for gas secretion. In terms of efficiency of gas secretion, this mechanism is more efficient than that of the closely related salmonines (Fahlen, 1971), but less efficient than some physoclists (Harden Jones & Marshall, 1953; Fange, 1953; Saunders, 1953; Sundnes *et al.*, 1958; Wittenberg, 1958; Tait, 1959; Sundnes, 1963–cited in Fange, 1983; Crawford, 1966; Fahlen, 1967; Steen, 1970; Fahlen, 1971; Fange, 1983; but see Tait, 1959).

Table 4. Gas bladder dimensions (mm) of coregonines. The presented values are averages; the range of values are in parentheses (g.b. = gas bladder, p.d. = pneumatic duct, and n.a. = not available).

Measure	**cisco**	**FL**	**TL**	**n**
g.b. length	51 (48-53)	127 (118-133)	n.a.	5
p.d. length	0.6 (0.4-0.7)	128 (130-132)	n.a.	5
p.d. diameter	0.9 (0.6-1.4)	128 (118-136)	n.a.	29
p.d. lumen ∆	0.58	140	156	1
Measure	**bloater**	**FL**	**TL**	**n**
g.b. length	100 (80-115)	247 (241-288)	275 (273-313)	16
p.d. length	1.8 (1.2-2.4) ◆	249 (256-241)	279 (287-270)	2
p.d. diameter	1.4 (1.1-2.1)	246 (223-237)	275 (248-267)	9
p.d. lumen ∆	0.87	208	232	1
Measure	***C. lavaretus***	**FL**	**TL**	**n**
g.b. length	80	n.a.	n.a.	≤100
p.d. length	11-12	n.a.	n.a.	≤100

p.d. diameter	1	n.a.	n.a.	≤100
p.d. lumen ∆	0.5	n.a.	n.a.	≤100

◆Pneumatic duct length may have been influenced by distortion resulting from gas bladder inflation.
Fahlen (1967)
Measured entire length
∆Measured at the opening of the duct into the esophagus

Figure 5. Gross anatomy of the gas bladder. **A.** Dissection of a captive-raised bloater exposing internal organs; head removed (to the left). The esophagus can be seen on the left, with the coeliaco-mesenteric artery running to and along the sides of the gas bladder. The cranial portion of the kidney is positioned just dorsal to the esophagus. **B.** Isolated gas bladder from a cisco; anterior portion of the gas bladder to the left. Note the esophagus with the pneumatic duct connection (as shown by arrow) and the dark areas at the anterior and dorsal surfaces of the gas bladder. **C.** Dissection of a wild bloater, exposing the bloated gas bladder. **D.** Isolated gas bladder (bloated) from a bloater. Note the esophagus to the left and the pneumatic duct (as shown by arrow) between the esophagus and the gas bladder.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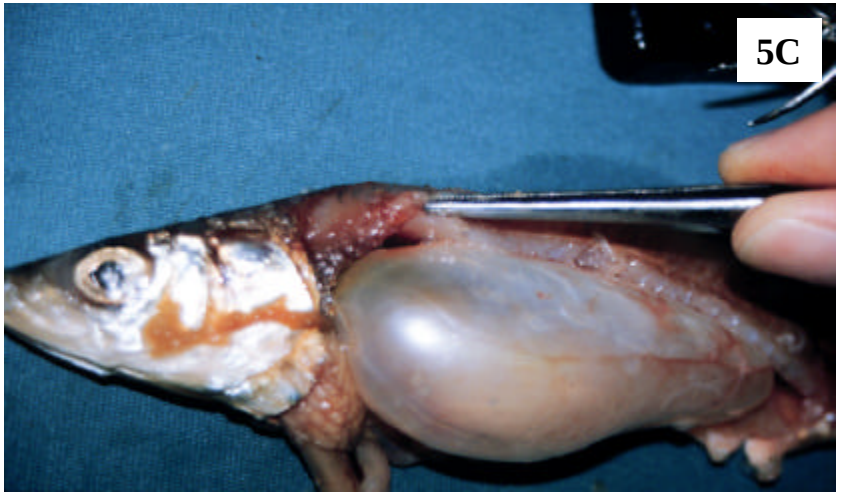
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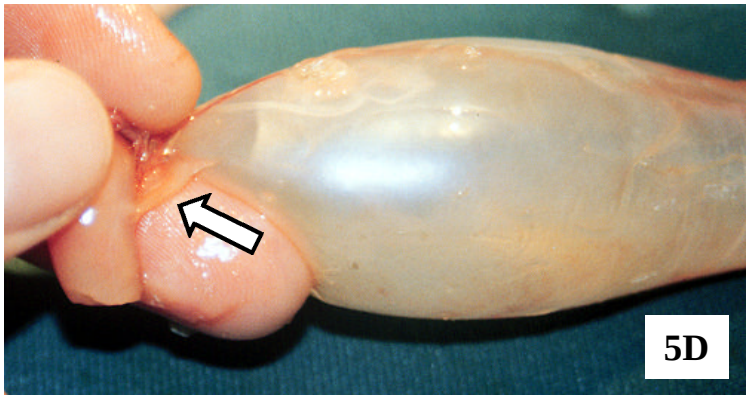
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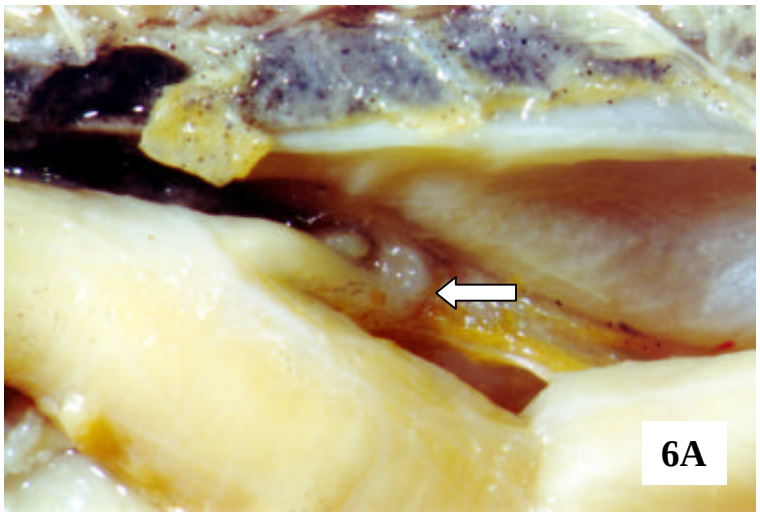
Figure 6. Pneumatic duct (indicated by arrows) *in situ*. **A.** Cisco. Esophagus at the bottom with pneumatic duct connecting to the gas bladder. Note the kidney at the top with ribs cut away to expose the gas bladder (~8X) **B.** Wild bloater. Esophagus to the left with pneumatic duct connecting to the gas bladder at the top right. Gonads (ovaries) to the right. Note the coeliaco-mesenteric artery between the esophagus and the gas bladder with a blood vessel that supplies the ovaries (6X). **C.** Captive-raised bloater. Esophagus to the left, pneumatic duct to the middle, and gas bladder to the far right. Note the coeliaco-mesenteric artery running along the esophagus and up to the gas bladder (6X).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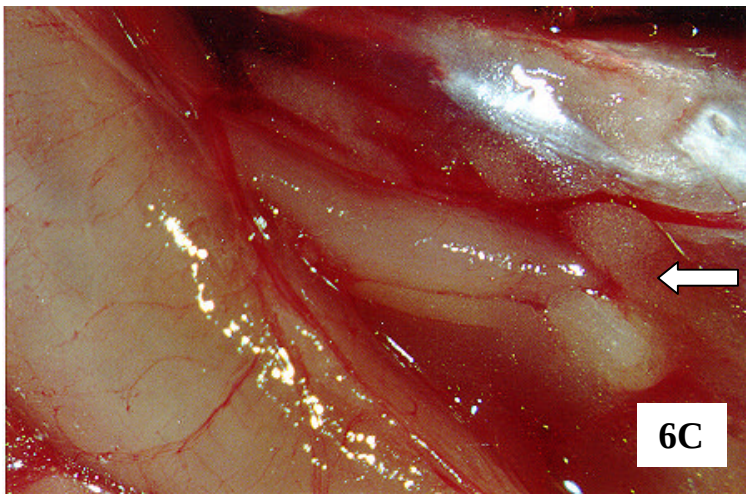
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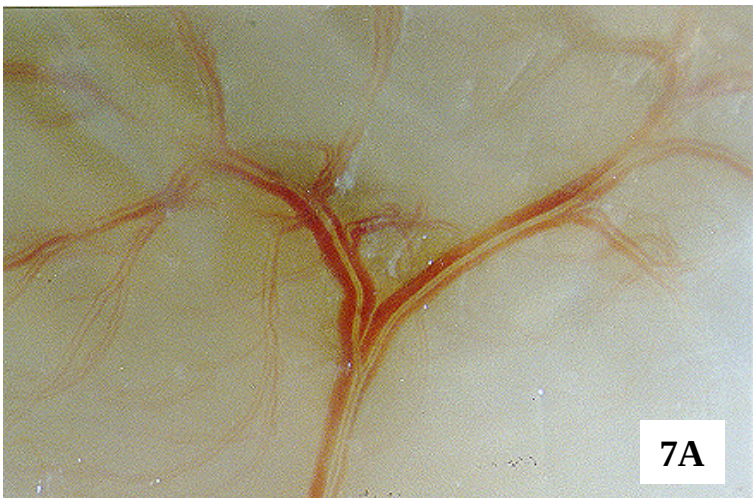
Figure 7. Coeliaco-mesenteric artery and veins. Note the alternating arteries and veins. **A.** Wild bloater (6X). **B.** Captive-raised bloater. Note the relative translucence of the bladder in relation to wild bloater (A.) (12X). **C.** Captive-raised bloater. Note what appears to be gas bubbles alongside the veins (40X).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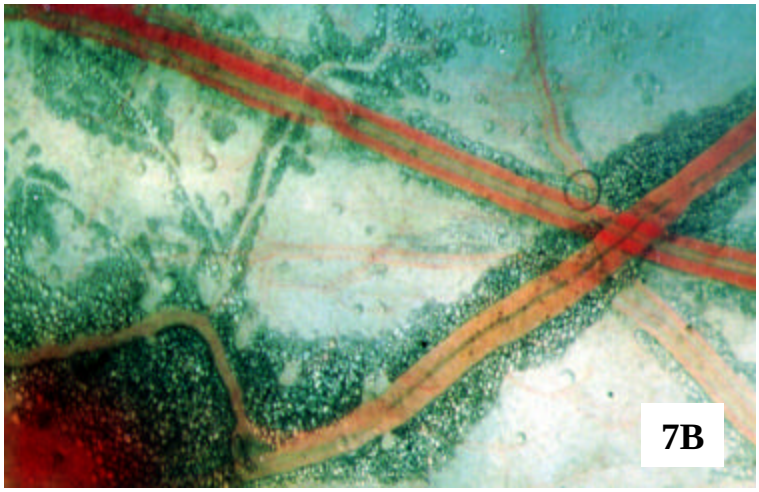
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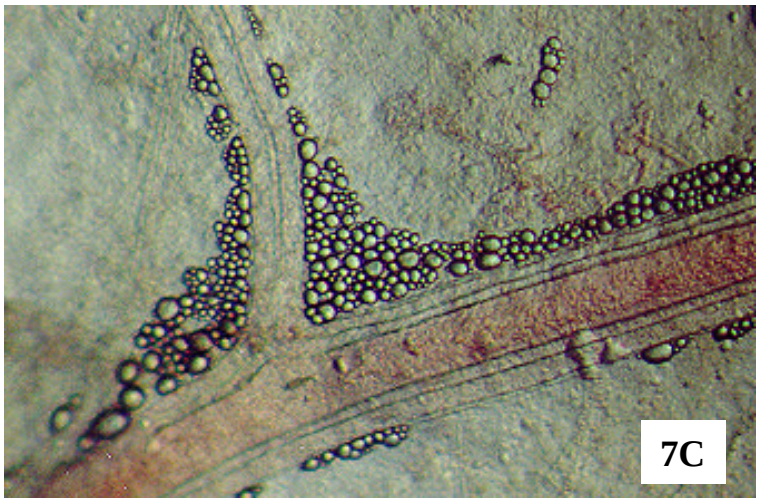
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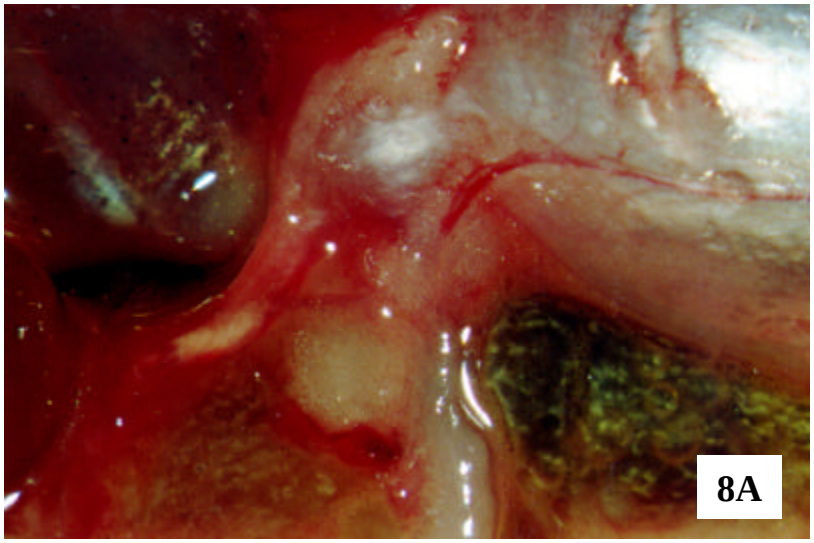
Figure 8. A. Captive-raised bloater. Pneumatic duct runs from the middle and bottom upwards into the gas bladder. Note the coeliaco-mesenteric artery running up the pneumatic duct onto the lateral gas bladder and also directly supplying the anterior gas bladder (to the left of the pneumatic duct (6X). **B.** Isolated gas bladder of a captive-raised bloater. Note the coeliaco-mesenteric artery running along the side of the gas bladder, the layer of adipose tissue on the ventral side of the gas bladder, and the haemotoma to the left (anterior gas bladder). **C.** Cisco; head to the left. Longitudinal section through the anterior gas bladder showing the connective tissue that runs dorsally towards the cranial portion of the kidney (as indicated by arrow). Esophagus to the bottom, kidney to the top, and gas bladder to the middle, right (20X).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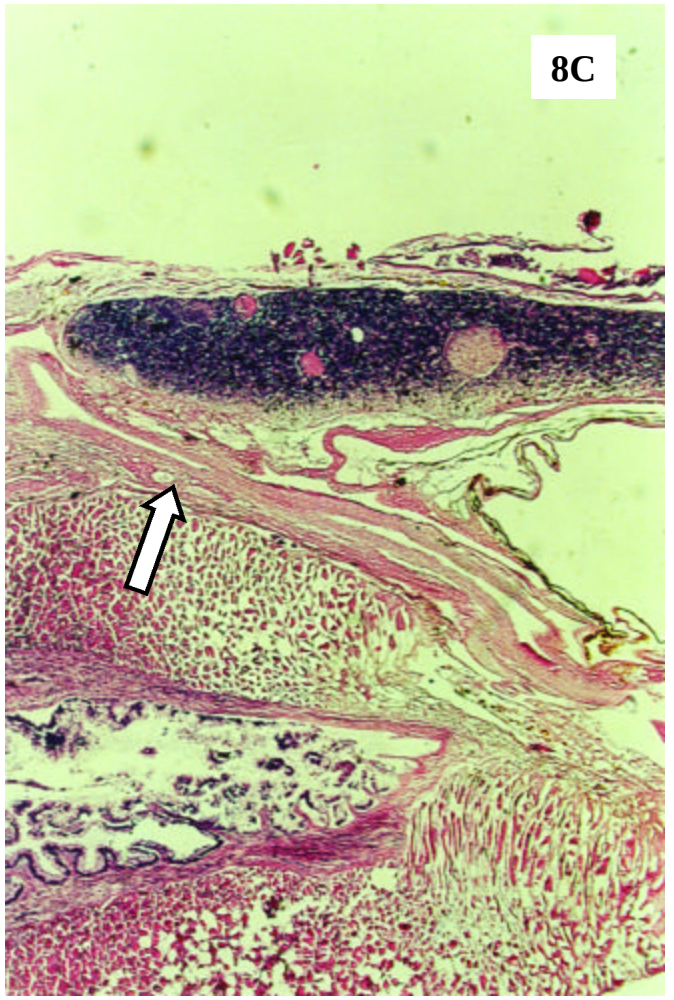
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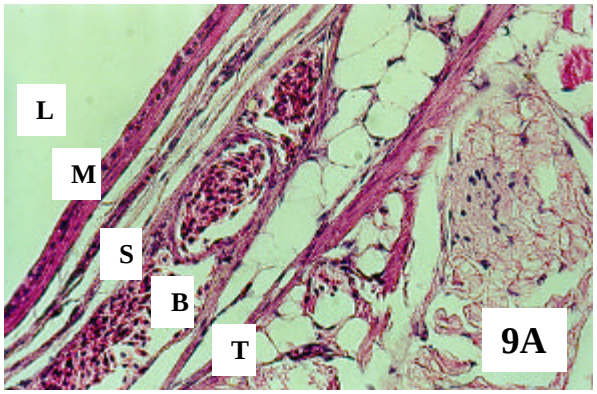
Figure 9. Cross-sections through the wall of the gas bladder of cisco. All sections are representative of what was observed in bloater. **A.** Lateral blood vessels (cross-section through coeliaco-mesenteric artery). Diagonally, from left to right: gas bladder lumen (L), mucosa (M) with epithelium, and muscular layer; submucosa (S) with connective tissue and lateral blood vessels (B) and smaller vascular bundles; tunica externa (T) with muscle; adipose tissue, and body wall (200X). **B.** Vascular bundles (V) with centrally-located artery surrounded by veins. Lumen of the gas bladder and epithelia (E) to the top (400X). **C.** *Micro retia* (R). Similar wall layers and positioning as previously mentioned (**A.**). Diagonally, from top left: lumen of the gas bladder (L), mucosa comprised of epithelium (E) and muscularis (C). (400X). **D.** *Micro retia* (R). Note the dark (yellow) layer (chromatophores) at the bottom. **E.** Mucosa. Lumen of the gas bladder (L) and epithelia (E) at the top, followed by the muscularis (C) and submucosa (S) at the bottom. Note the capillaries traversing the submucosa into the epithelia (400X). **F.** Mucosa and submucosa. Same positioning of the gas bladder layers as previously mentioned (E.). Note the thick epithelia and circular and longitudinal muscle layers of the mucosa, respectively (200X).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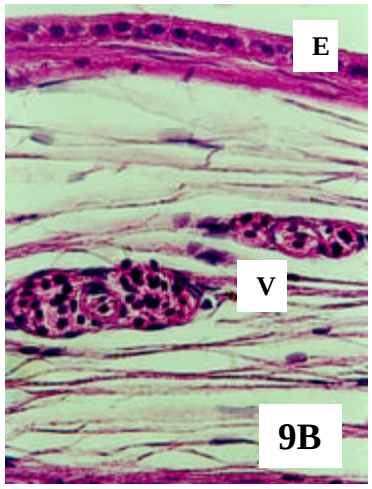
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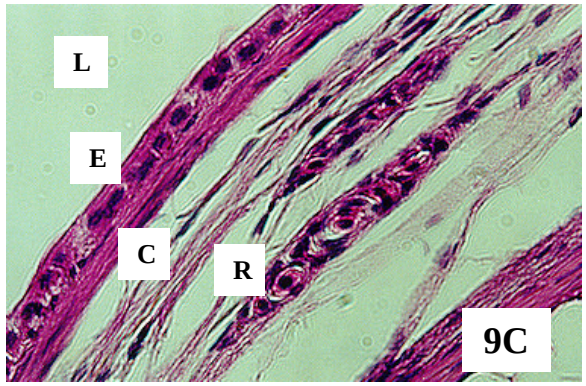
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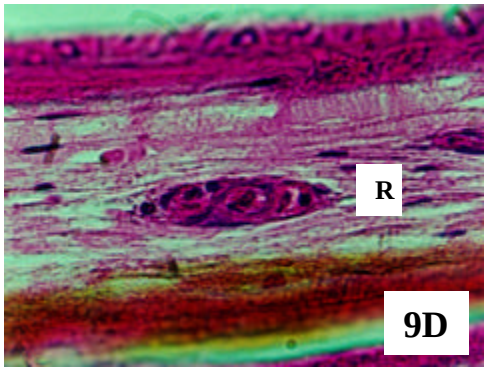
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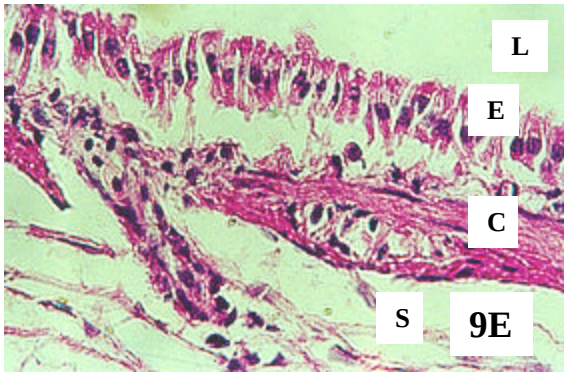
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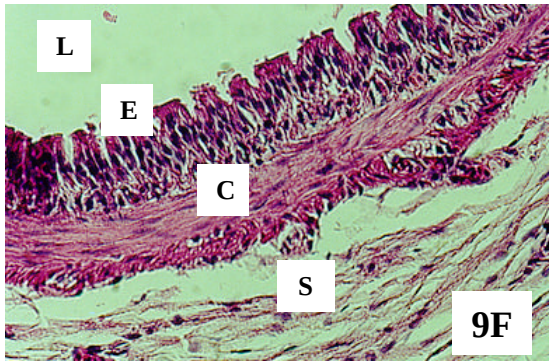
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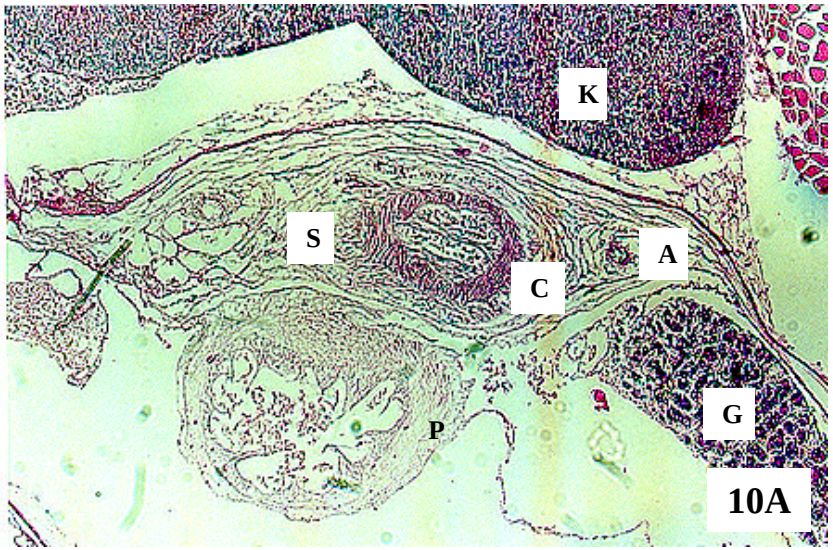
Figure 10. Cross-sections through the anterior gas bladder of cisco and bloater. **A.** Cisco. Cranial portion of the kidney at the top (K), gonads to the bottom right (G), and cross-section through the gas bladder to the middle. A cross-section through the coeliaco-mesenteric artery is visible at the right (A). Note the cross-section through the pneumatic duct (below the gas bladder) and the relatively thick layers of submucosa of the gas bladder (P). The thick muscular layer of the mucosa (C) is also visible to the middle of the gas bladder. It is not known whether this muscular layer corresponds to the diaphragm (Figure 3.7) (40X). **B.** Bloater. Diagonal, from the middle to bottom right: lumen of the gas bladder, pneumatic duct opening (O), and the connection of the pneumatic duct and gas bladder (P). Note the thick layers of the submucosa (S) and mucosa (M) (13X). **C.** Bloater. Anterior gas bladder mucosa: lumen of the gas bladder to the top (L), submucosa to the bottom (S). Note the thick epithelial (E) and muscular layer (C) of the mucosa; refer to previous figure for positioning (100X). **D.** Bloater. Similar positioning of the layers of the gas bladder as previous figure. Mucosa associated with the entrance of the pneumatic duct into the gas bladder; see Figure 3.6B for positioning (40X).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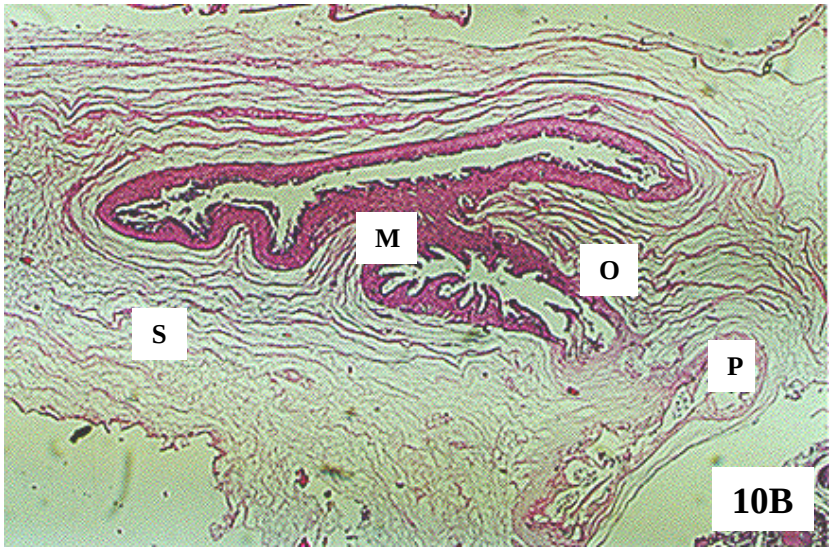
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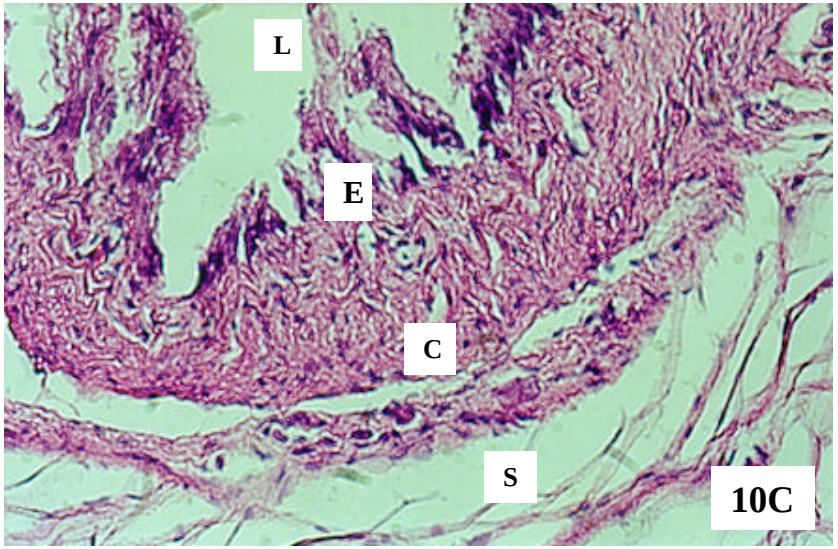
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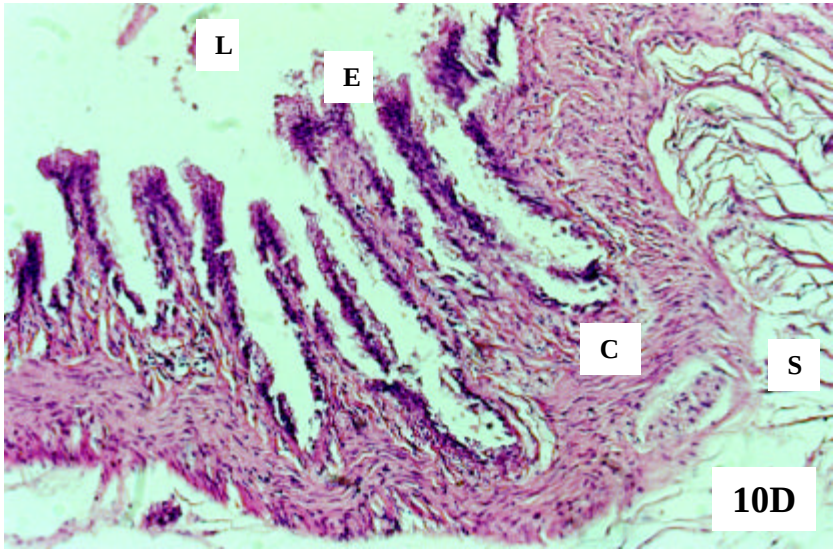
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Figure 11. Cisco. Longitudinal sectioning through the anterior gas bladder. From bottom to top: esophagus (E), gas bladder (G), kidney (K). Note the diaphragm (D), and the cross section through the pneumatic duct (P). Also note the yellow layer (chromatophores) within the walls of the gas bladder (3 frames, 20X).

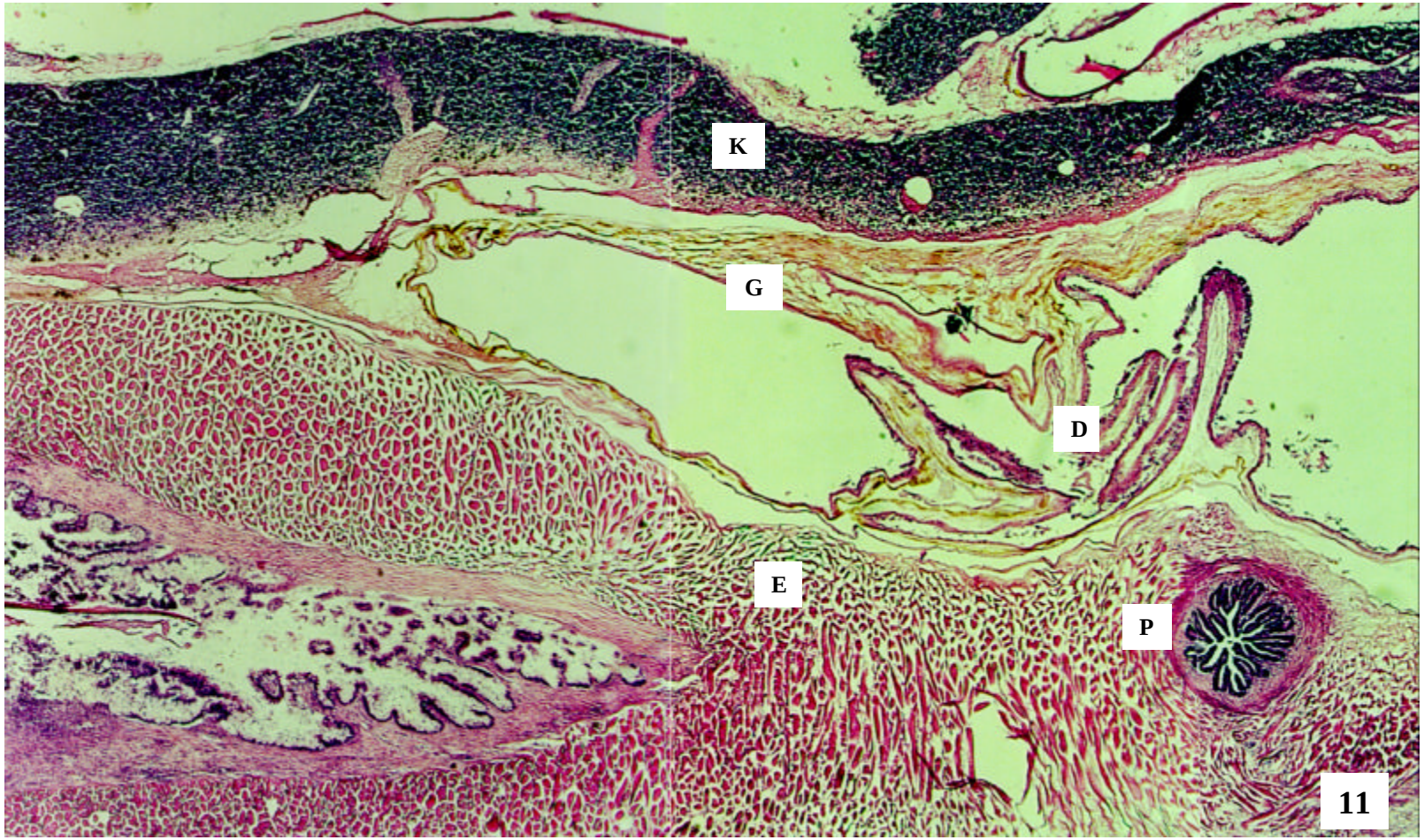
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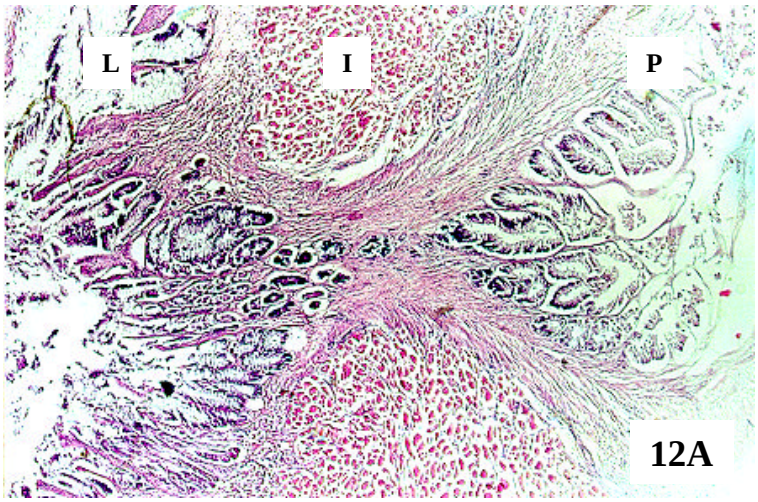
Figure 12. Pneumatic duct. **A.** Bloater. Cross section through the pneumatic duct. From left to right: lumen of the esophagus (L), intestinal wall (I), and pneumatic duct connection (P). Note the muscular layers of the intestine. This cross section either did not proceed through the pneumatic duct, or the duct was held closed by the muscles of the intestinal wall. **B.** Bloater. Longitudinal section through the pneumatic duct. From bottom to top: lumen of the intestine (L), intestinal walls (I), and pneumatic duct (P). Positioning of the fish relative to the anatomy: head to the right, and ventral surface to the bottom.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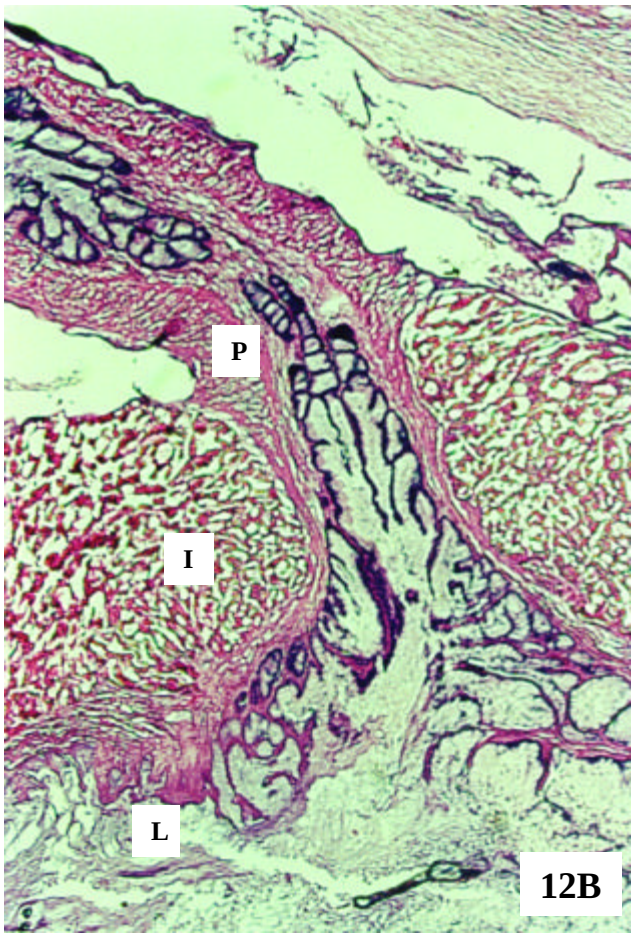
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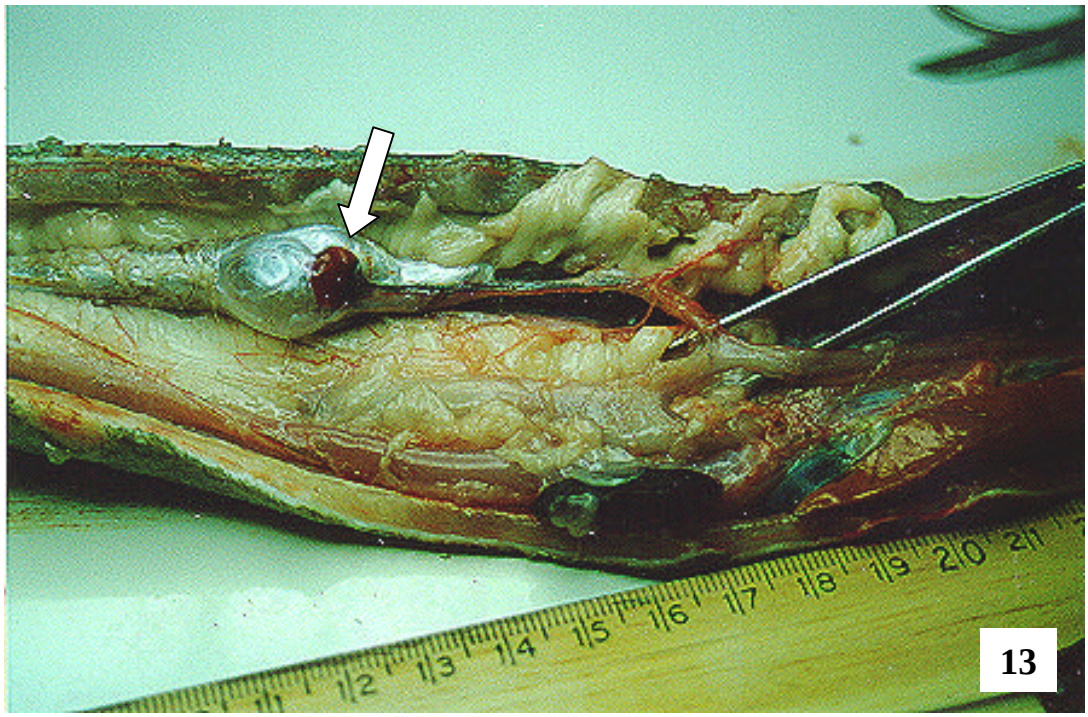
Figure 13. Dissection of a silver-phase American eel (*Anguilla rostrata*) to show the distinct retia of the gas bladder, as indicated by the arrow. Note the connection of the pneumatic duct to the esophagus (held by the foreceps), which proceeds to the gas bladder.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3. Swimming Kinematics and Capacity

INTRODUCTION

The swimming performance of a fish in a laboratory setting has often been used as a surrogate for its performance and even perhaps Darwinian fitness in the wild (Fry, 1971; Brett & Glass, 1973; Sepulveda & Dickson, 2000; Reidy *et al.*, 2000). More recently, swimming capacity and metabolic rates of fishes have proven useful as applications to bioenergetic models (e.g., Rudstam *et al.*, 1994)

There is little information available on the swimming kinematics of bloater and coregonines in general (Jones et al., 1974; Rudstam et al., 1984; Bernatchez & Dodson, 1985; Eshenroder *et al.*, 1999). A general understanding of the swimming and metabolic rates of bloater could provide insight into activity levels and the potential for horizontal and or vertical migration.

Routine Swimming Velocities

Alexander (1972) calculated that the energy needed for hydrodynamic lift is comparable to resting metabolic rates. This is not surprising, considering that routine swimming velocities can be strongly related to the rate at which a fish can extract oxygen from the water to supply its aerobic swimming musculature (Wardle, 1977).

Large fishes, including larger bloater, may rely less on hydrodynamic lift to achieve buoyancy than smaller conspecifics. For example, allometric changes in the volume of buoyancy organs (e.g., gas bladder, lipid content), may enable larger fish to be more statically buoyant than smaller fish (Eshenroder *et al.*, 1998; Eshenroder *et al.*, 1999; Eshenroder & Burnham-Curtis, 1999). If larger fish are more statically buoyant than smaller fish, it follows that they would rely less on hydrodynamic lift to achieve buoyancy. Because less movement is required for larger fish,

energy can be conserved.

Decreases in relative swimming velocity (body lengths/second) with increases in size are relatively common in fishes and are a complex interaction between many factors. Metabolic rates, thrust efficiency of the swimming muscles, respiratory abilities to supply the aerobic swimming muscles, minimization of drag, and buoyancy all influence the minimum routine swimming velocities (Magnuson, 1966; Wardle, 1977; Webb, 1977).

My main objective was to measure the routine swimming velocities of bloater because lift created by swimming contributes to buoyancy. Quantification of routine swimming velocities can be useful in understanding the energetic budget of fishes (e.g., Rudstam *et al.*, 1994), and will be integrated with metabolic rates.

My second objective was to measure the tail beat frequency and stride length of bloater in order to understand the kinematics velocity and muscle recruitment in these fish. This latter objective is not directly related to buoyancy and will not be discussed in detail.

Critical Swimming Velocities and Oxygen Uptake

Swimming capacity in fishes is a combination of the swimming endurance and metabolic rate, as measured by the critical swimming velocity and active oxygen uptake. The critical swimming velocity is the highest rate of movement that a fish can maintain for a certain period and the active oxygen uptake is a measure of the active metabolism (Fry, 1971; Beamish, 1978). The metabolic rate of a fish also is influenced by size and temperature (Fry, 1971; Beamish, Schmidt-Nielsen, 1972; Beamish, 1978).

The amount of fuel necessary for swimming in fishes is estimated by active oxygen uptake. The “scope for activity” is estimated from the active oxygen uptake minus the standard oxygen uptake, where the latter value is calculated by extrapolation. The scope for activity gives

an estimation of the aerobic capacity (Fry, 1971).

Of the Salmonids, coregonines have received relatively little attention on swimming capacity. My objectives were to measure both the critical swimming velocity and active oxygen uptake for bloater (*Coregonus hoyi*).

METHODS

Bloater were raised in captivity at the University of Wisconsin Great Lakes WATER Institute from fertilized eggs of Lake Michigan bloater. Fish were transported to the University of Waterloo Biotelemetry Institute (Waterloo, Ontario, Canada) in April 1999 following their first year of growth. They were maintained in an aerated, circular communal holding tank 3.6 m in diameter. The water depth was 39 cm at the periphery of the tank and 65 cm in the center. The holding tank had a constant supply of well water from the city of Waterloo at a temperature of 11.5 C (± 0.3 ^{0}C). Laboratory lighting was controlled by timers and set to coincide with sunrise and sunset, with a half-hour ramp-up and down time. Bloater were fed commercial salmon feed (3 mm Bio Diet Grower pellets, Bio Oregon, Inc.) once daily.

Routine Swimming Kinematics

The routine swimming kinematics of age-2+ bloater (n = 25) were recorded and analyzed. All recording took place from 1015-1115 hours, on February 10, 2000. A Sony VX 1000 digital camera was used to record bloater swimming in the holding tank; the recording was later transferred to VHS video tape, and time-stamped to the nearest fraction of a second. A ruler was within the field of view of the camera and was used to measure the length of the bloater (mm standard length), and distance travelled per unit time (cm/s). Standard lengths (SL) were then converted to total lengths (TL) by: TL = 1.18*SL (Hile, 1936 – cited in Rudstam *et al.*, 1994). In addition, the stride length (distance travelled per one tail beat) and tail beat frequency (tail

beats per second) were measured.

The holding tank contained about 300 captive-raised bloaters at the time of recording. The depth of the water was slightly deeper (47 cm at the edge) and the temperature slightly warmer (12.5 ^{0}C) than in the later studies on critical swimming speeds and oxygen uptake. There was no current in the holding tank. All swimming was voluntary and spontaneous, and was characteristic of bloater in captivity.

Critical Swimming Velocities and Oxygen Uptake

The critical swimming speed and oxygen consumption of age-3+ bloater (n = 18) were tested individually during May 2001. Prior to use in swim experiments, fish were trained by exposing them to a continuous current in the holding tank for 13 weeks. Current velocity ranged from 9 cm/s at the center to 25 cm/s at the periphery. The general behaviour of the bloater in the holding tank was to maintain station against the holding tank current. Approximately 200 fish were maintained in the holding tank during the time of the experiments.

I used a Blazka-type swimming chamber (7.2 liter volume) (Beamish 1978) for measuring critical swimming velocities. The inside diameter of the inner tube of the chamber was 8.9 cm. The swimming chamber was equipped with 2 flow-straightening baffles–one at the front of the swimming chamber, and one at the back. Before the experiments were run, small pieces of string were temporarily tied to the front baffle to aid in examining the flow of water within the swimming chamber. Water flow in general was rectilinear. An exception to this was a narrow boundary layer along periphery of the front of the tube at lower velocities.

A small voltage, 28 VAC, was applied to the electrodes on either side of the back of the swimming chamber only when the fish touched the back baffle. The practice of using small electrical currents to stimulate swimming is relatively common in swimming capacity experiments

(Beamish, 1978 & Beamish, 1980; Stillwell & Benfey, 1997; Stevens et al., 1998). Optomotor cues consisted of vertical black lines, 1 cm wide, painted on the front half of the outside of the outer chamber. A cover was placed over the front 1/3 of the swim chamber to minimize stress.

Water used for the system came from the same supply as that which supplied the holding tank, and was aerated prior to being pumped into the swimming chamber. Mean water temperature in the swim chamber was equivalent to the holding tank (11.5 ^{0}C) and was controlled by running water through a coil wrapped around the outside tube of the chamber. Water velocity was measured on two separate occasions with an Ot-meter (A Ott, Kempten) and related to speed of the propeller in the tube using an optical tachometer (Cole-Parmer Instrument Co.).

The cross-sectional area of each of the bloater in the swim trials was greater than 10 % of the cross-sectional area of the tube so correction for solid blocking was applied (Webb, 1970). Cross-sectional area of the each fish was estimated by using the measured height and width at the point where area was estimated to be the largest.

Xsec area of fish = pi*width*height

U corrected = U * xsec area of tube/(xsec area of tube - xsec area of fish)

Fish were not fed on the day prior to the test and not fed on the test day to avoid the effects of the heat increment of feeding (specific dynamic action–SDA) (Beamish, 1978; Bernatchez & Dodson, 1985). One fish was tested per day and fish were tested at the same time of day (i.e., early morning). Before the actual swim test each fish was left at a speed of 5 cm/s for 4 hours to allow the animal to become accustomed to the tube and to learn to stay off the back screen. Speeds of 10 cm/s or higher were not practical during the 4 hour initial period because electrical shocks and speed fluctuations were required to train the fish to stay off the back screen.

Therefore, I could not fully eliminate spontaneous activity at the low swimming velocities (Brett, 1964; Beamish, 1980; Bernatchez & Dodson, 1985).

During the test the velocity within the swim chamber was increased every 31 minutes in 10 cm/s increments. These time intervals and velocity increments are relatively conservative measures and are widely used in swimming capacity tests (Beamish, 1978). The speed was increased slowly over a 1 min period, and the tube was left flushing for 15 minutes. Then the tube was sealed for 16 minutes and oxygen uptake was estimated from the last 16 minutes of the 31 minute period at each new speed. Oxygen concentration never went below 8 mg/l. Critical swimming velocity (Ucrit) was calculated using the method of Bell and Terhune (1970), from the duration at the fastest speed and the speed increment:

$$Ucrit = Ui + [(Uii - Ui) * (ti/tii)]$$

where Ui = highest velocity maintained for 31 minutes

Uii = velocity (cm/s) at which bloater fatigued

ti = time (minutes) bloater swam at Uii

tii = time interval (31 minutes) for each velocity increment

To measure oxygen uptake, the slope of the decrease in oxygen concentration was calculated from the readings every 2 minutes over the 16 minute period. Oxygen was measured with an oxygen electrode (YSI model) placed in a closed circuit that removed water from the tube, passed over the electrode and then returned it to the tube. Blanks were run every day and uptake rates were subtracted from fish rates; blank rates were 6.3 ± 1.0 % of fish rates.

If a fish did not respond to two consecutive shocks within either the 15 minute recirculation or 16 minute measurement intervals, the test was ended. No shocks were needed to

induce swimming for the 3 fish with the highest swimming speeds. These fish swam continuously until the speed at which their tails touched the back screen three or more times in one minute, signifying burst swimming and the end of the trial (Beamish, 1978; Sepulveda & Dickson, 2000).

Following the termination of the experiment, the fish was removed from the swimming chamber and was weighed (to the nearest 0.1 g) and the length was measured (to the nearest millimeter).

RESULTS

Routine Swimming Kinematics

Bloater swam continuously of their own volition, despite the fact that no current was present in the holding tank. The captive bloater have long paired fins that are extended perpendicular to the body when the fish swim (Figure 14A & 14B).

Lengths and swimming kinematics of the bloater in my study are presented in Table 5 and Figures 15, 16, & 17. Over the length range analyzed, tail beat frequency (TBF) (tail beats/s), stride length (body lengths/tail beat), and relative velocity (body lengths/s) were not correlated with the length of bloater (Figure 15). However, the swimming velocity (cm/s) of bloater generally increased with length; 39 % of the variation in the swimming velocity could be explained by the length of the bloater (Figure 16).

Both tail beat frequency and stride length increased in a linear fashion with swim velocity (Figure 17). Tail beat frequency and stride length were strongly correlated with relative velocity (BL/s) and less so with dimensional velocity (cm/s). Nearly half ($r^2 = 0.45$) of the variation in swimming velocity (BL/s) could be explained by TBF and stride length. Twenty seven percent (27 %) and 38 % of the variation in dimensional swim velocity could be explained by tail beat

frequency and stride length, respectively.

Critical Swimming Velocities and Oxygen Uptake

I. Routine swimming and oxygen consumption

When oxygen uptake was plotted versus swim velocity, there was a clear discontinuity at about 1.5 body lengths per second (BL/s) (about 34 cm/s) (Figure 18). I arbitrarily divided the data at that level and refer to the data below 1.5 BL/s as routine oxygen uptake. I refer to the data above 1.5 BL/s as active oxygen uptake (see below).

At swimming velocities less than 1.5 BL/s, oxygen uptake was quite variable, ranging from 107-472 $mg*kg^{-1}* hr^{-1}$. Mean oxygen consumption was 276 mg $O_2*kg^{-1}* h^{-1}$ and was significantly related to the body mass of bloater ($r^2 = 0.35$, $p = 0.000$) and with the total length (TL) ($r^2 = 0.138$, $p = 0.015$) over the size range used (range of mass: 70-136 g; range of TL: 210-258 mm) (Figure 19; Table 6). The general trend was that larger bloater consumed less oxygen with routine swimming than smaller bloater (Figure 19). The majority of bloater used in these trials did not swim well in the chamber and so provided data for routine oxygen uptake, but not for active oxygen uptake (Figure 18).

II. Active swimming and oxygen uptake

Five of the bloater responded to the water current in the chamber by actively swimming for sufficiently long periods that oxygen uptake could be accurately measured at fixed swimming velocities (Figure 20 & Table 6). Active oxygen uptake for these 5 fish ranged from 111-555 $mg*kg^{-1}* hr^{-1}$ (mean = 253 $mg*kg^{-1}* hr^{-1}$), and increased with increasing swim velocities. Seventy five percent (75 %) of the variation in oxygen uptake by actively swimming bloater could be explained by the swimming velocity of bloater (Figures 18 & 20). This data was plotted with

swim velocity in cm/s and in body lengths/s (BL/s); it also was plotted on a linear scale and a logarithmic scale (Figure 18). The best fit (highest r^2) was achieved using body lengths/s as the measure of swim velocity and a linear scale but the differences in r^2 were very small.

The 5 bloater that actively swam had critical swimming velocities that ranged from 39-165 cm/s (1.51-6.96 BL/s). Three of the 5 actively swimming bloater had the highest overall swimming velocities. The critical swimming velocity of the 3 bloater ranged from 93–165 cm/s (4.35-6.96 BL/s). These 3 bloater required no shocks to induce swimming and they swam until they were fatigued (Figure 20).

DISCUSSION

Routine Swimming Kinematics

There is nothing unusual about the routine swimming velocity or kinematics of bloater relative to other salmonids. For example, the correlation of increased tail beat frequency with routine swimming velocity is similar to that of the goldfish (*Carassius auratus*), dace (*Leuciscus leuciscus*), and rainbow trout (*Oncorhynchus mykiss*) as originally measured by Bainbridge (1958). In addition, the stride length value (0.6 body lengths per tail beat) is also similar to values commonly reported by Bainbridge (1958).

Rudstam *et al.* (1984) also measured routine swimming velocities of bloater under laboratory conditions, and related an increase in routine swimming velocity (measured as cm/s) to an increase in length to the 0.8 power (Rudstam *et al.*, 1984). Although my results do not support the exponential model of Rudstam *et al.* (1984), the swimming velocity of their age-2+ bloater (1.22 BL/s) is similar to the swimming velocity of the slightly larger bloater in my study (1.27 BL/s) for a similar temperature range (Table 7). It appears that a small range of swimming

velocities exists in bloater that increase slightly as the fish grows in size from 1.17 BL/s (108 mm, total length) to1.22-1.27 BL/s (153-194 mm, total length), and then decrease by 21 % to 1.00 BL/s (249 mm, total length) (Table 7). However, the relative swim velocity (BL/s) did not correlate with total length in my bloater, and Rudstam *et al.* (1984) did not correlate the relative swim velocity against total length. Therefore, no conclusions can be drawn on the size of bloater and the minimum routine swimming velocity at this time.

The continuous swimming behaviour and presence of long paired fins that are held out from the body suggests that bloater, like many coregonines, probably rely on hydrodynamic lift to maintain neutral buoyancy (Dr. Bev Scott, pers. comm.). In addition, the routine swimming velocity of bloater may be equivalent to the minimum velocity at which neutral buoyancy is realized.

Magnuson (1966) cited evidence to conclude that the routine swimming velocity of scombrids was determined by the minimum velocity needed to maintain buoyancy. First, like the bloater in my study, scombrids are continuous swimmers and almost always keep their pectoral fins extended. Second, Magnuson (1966) noted an inverse relationship between the swimming velocity and the size of the gas bladder and pectoral fins in scombrids.

Because lipids can provide a relatively large contribution to buoyancy in larger bloater (Eshenroder *et al.*, 1999), an inverse relationship may also exist between the routine swimming velocity and the size of bloater (Eshenroder *et al.*, 1998; Eshenroder *et al.*, 1999; Eshenroder & Burnham-Curtis, 1999). However, I did not find an obvious inverse relationship between size and relative swim velocity over the length range (and same age) of bloater that I used. An ideal test would be to measure the routine swim velocities of bloater from very different age groups (e.g.,

age-0 to age-8), raised under the same conditions.

The extent to which bloater rely on hydrodynamic lift in nature (whether negatively or positively buoyant) is not known. For example, no one has ever witnessed the swimming behaviour of bloater or any other deepwater cisco in their natural environment (Eshenroder *et al.*, 1999). Nevertheless, my results on the upper limit to the free vertical range of bloater (see section 1. Flotation Pressure and Lipid Content) suggest that at the very least, bloater would have to use hydrodynamic lift to oppose the buoyant force created by the excess gas in their gas bladder. For example, I previously reported an upper limit of 50 % positive buoyancy (i.e., tendency to float) for bloater (section 1). Without continuous swimming with a head-down posture, these positively buoyant bloater would have floated uncontrollably to the surface.

Critical swimming velocities and oxygen uptake

I. Routine Swimming and Oxygen Uptake

The high variability in oxygen uptake at routine swimming velocities by the bloater in my study is common in fishes in general. All reports of oxygen consumption in fishes are characterized by large variation at low swim speeds (Brett, 1964; Fry, 1971; Stevens, 1973). Stevens (1973) summarized this concept for a number of data sets of oxygen uptake in fish and showed that the large variation at low swim speeds is a general trend in metabolism in fishes.

Oxygen uptake for bloater at routine swimming velocities was negatively correlated with the mass of the fish. A dependence of metabolic rates (i.e., oxygen uptake) on the weight of the fish has been commonly reported among fishes (Fry, 1971; Brett & Glass, 1973; Beamish, 1980). Besides my study, only Bernatchez and Dodson (1985) have measured oxygen uptake and critical swimming velocities on coregonines. Although too few bloater swam in my experiment to permit

useful predictions, Bernatchez and Dodson (1985) reported weight-slope exponents for lake whitefish (*Coregonus clupeaformis*) that were similar to salmonines (at velocities 20-40 cm/s; wt. slope range 0.86-0.91; average 0.88). Therefore, like salmonines, the weight specific oxygen uptake of coregonines seems slightly dependent upon weight.

It seems possible that the smaller bloater may have been more negatively affected at the slower routine swimming velocities within the swim chamber (Figure 19), as evidenced by their apparent need to swim continuously and my own observations on their behaviour in the swimming chamber (i.e., very active). Therefore, high oxygen consumption levels of smaller individuals at routine swimming velocities should be interpreted with caution. Indeed, Reidy *et al.* (2000) reported that some of the variation in oxygen uptake at low velocities in their study may have been attributed to variable reactions of individuals to confinement within the swim chamber. Therefore, high oxygen uptake levels in smaller bloater may be a result of confinement within the swimming chamber and cannot be ruled out.

However, recent evidence suggests that larger coregonines do in fact have a lower mass specific metabolism than smaller coregonines (Trudel *et al.*, 2001). In addition, Binkowski and Rudstam (1994) reported decreased feeding rates for larger size classes of captive-raised bloater. Binkowski and Rudstam (1994) hypothesized that the high consumption rates of food for bloater at small sizes was an adaptation to zooplanktivory. Trudel *et al.* (2001) hypothesized that dwarf lake whitefish and cisco would have higher metabolic rates to satisfy the energy requirements of a higher standard metabolic rate. Another hypothesis might be that the higher metabolic rate and continuous swimming behaviour of the smaller sizes of bloater facilitates encounter rates with prey, and thus feeding.

Larger bloater may be able to achieve neutral buoyancy at slower swimming velocities because their higher lipid content generates lift (Eshenroder *et al.*, 1998; Eshenroder *et al.*, 1999; Eshenroder & Burnham-Curtis, 1999) (but see **Routine Swimming Kinematics**, above). Using the swimming capacity data for cisco obtained by Bernatchez and Dodson (1985), Rudstam *et al.* (1994) calculated that 40 % of the standard metabolic rate was needed for swimming in small bloater (10 g), while 110 % of the metabolic rate was needed for swimming in larger bloater (300 g). Larger fishes may necessarily be less motile because of increased drag (i.e., more surface area in contact with water) (Schmidt-Nielsen, 1972; Wardle, 1977). Alexander (1972, Alexander, 1990, & 1993) argued that larger fishes with a comparatively larger buoyancy organ (gas bladder or lipids) required more energy to overcome drag for swimming than smaller fishes.

Because larger bloater are found at deeper depths near the bottom of their vertical range (Eshenroder *et al.*, 1998), they would experience comparatively dense water at hypolimnetic temperatures of 4 ^{0}C (i.e., water is the most dense at 4 ^{0}C). At these bottom depths, the effects of drag on a swimming fish would be maximal (and would require more energy for swimming), while the buoyant effect of the medium is at maximal levels as well. Thus, one might expect that larger bloater at bottom depths might be less active and have a lower metabolism. Furthermore, metabolic rates are generally lower in fishes caught at deeper depths (higher pressure) (Sebert, 1997).

Lower activity levels at deeper depths cannot necessarily be separated from the effects of low temperatures, which have been shown to decrease metabolic rates in fishes, including Salmonids (Brett, 1964; Beamish, 1978; Bernatchez & Dodson, 1985). However, Rudstam *et al.* (1994) observed that bloater were active at 4 ^{0}C. The level of activity of the bloater that Rudstam

et al. (1994) observed was not measured and it is not known how comparable their activity was to that at higher temperatures.

Jones *et al.* (1974) found no effect on the critical swimming velocity of 6 species of coregonines (Table 4.4) through a temperature range of 7-20 ^{0}C. In contrast, Bernatchez and Dodson (1985) reported increased metabolic rate and swim performance in lake whitefish at temperatures of 12 ^{0}C in comparison to 5 ^{0}C (Table 8). They suggested that the optimal temperature for swimming was lower in coregonines than salmonines (Bernatchez & Dodson, 1985), but their data for lake whitefish oxygen uptake and swimming velocity at 17 ^{0}C is not necessarily different from 12 ^{0}C (Trudel *et al.*, 2001). Therefore, no definitive conclusions can be made about an optimal temperature for swimming capacity in coregonines.

II. Active Swimming and Oxygen Consumption

The fact that only 28 % (5/18) of the bloater used in my experiments actively swam is not surprising. For example, critical swimming tests are characterized by large individual variation and may reflect trade-offs in performance between endurance (as measured by critical swimming velocity tests) and burst or sprint performances (Reidy *et al.*, 2000). Of the 5 fish that actively swam, the fastest bloater swam nearly 4.5x faster than the slowest fish. In addition, active oxygen uptake differed by nearly 2x in these actively swimming fish. Similarly, Reidy *et al.* (2000) reported that the fastest fish in their study, cod (*Gadus morhua*), swam nearly 2x faster than the slowest fish, and active oxygen consumption values for an individual could vary by as much as 2x between separate trials.

Critical swimming velocities for the 5 actively swimming bloater ranged between 39-165 cm/s (1.51-6.96 BL/s), with an average of 93.6 cm/s (4.00 BL/s). Beamish (1978 & 1980)

reported the median values of critical velocity trials, as determined by probit analysis. My median value, 93 cm/s (4.35 BL/s), is quite similar to my average value (93.6 cm/s; 4.00 BL/s).

The critical swimming velocities for 3 of the 5 bloater that actively swam without being shocked (93-165 cm/s; 4.35-6.96 BL/s) seem particularly high in relation to other coregonines (1.4-3.4 BL/s) (Table 4.4 & Figure 4.8) (Jones *et al.*, 1974; Bernatchez & Dodson, 1985). Furthermore, the high swimming velocities that I measured for bloater are higher than that of Pacific and Atlantic salmon (3-5 BL/s, occasionally to 7.3 BL/s), which are known to be good swimmers, and charr, which are comparatively poor swimmers (1.91-6.6 BL/s) (Jones *et al.*, 1974; Beamish, 1978; Beamish, 1980; Stevens *et al.*, 1998). In addition, Bernatchez and Dodson (1985) reported that the coregonines in their study (cisco and lake whitefish) had a lower swimming capacity and efficiency in comparison to the salmonines. They calculated that the coregonines in their study (cisco and lake whitefish) had a lower scope of activity than most salmonids and that cisco were one of the least energetically efficient swimmers (Table 9) (Bernatchez & Dodson, 1985). The same may be said for lake whitefish because their active oxygen consumption is not different from cisco (see below) (Trudel *et al.*, 2001).

It seems possible, therefore, that the high critical swimming velocities that I have recorded for bloater can be explained by an overestimation of the critical swimming velocity. In other words, the high critical swimming velocities in the 3 bloater could have resulted from the fish swimming in areas of low current velocity in the swim chamber. Low current velocity can be caused by the formation of a boundary layer along the sides or front of the swimming chamber (Beamish, 1978). If a boundary layer of low current velocity did exist within the chamber, it is possible that the areas became more pronounced with increasing speeds. However, all necessary

precautions were taken to ensure a rectilinear flow pattern existed within the swimming chamber (see **METHODS**).

Alternatively, the high critical swimming velocities of the bloater in my study may be a relatively accurate measure of critical swimming velocities. For example, anadromous populations of cisco effectively ascend high-velocity rapids of approximately 152 cm/s (5.2 BL/s) (Guderley *et al.*, 1986). Therefore, it is not implausible that at least some coregonines have the capability of swimming at higher velocities. Nevertheless, my results should be interpreted cautiously, and further examinations are needed to determine whether they are repeatable at high velocities (Reidy *et al.*, 2000).

Farlinger and Beamish (1977) demonstrated that changes in the test parameters, time increments and velocity intervals affected the outcome of the tests in large mouth bass. Additional differences among tests, including different objectives, protocols, equipment, physiological states, stocks, and seasons further confound direct comparisons (Beamish, 1978; Stillwell & Benfey, 1997). Another factor is that I have reported the critical swimming velocities of the best swimmers in my results, whereas other researchers report the average or median values. Averaging or even taking the median value for critical swim velocity measurements can dilute and thus lower the critical swim velocities of the top swimmers.

Besides my data, only two other studies exist on the swimming capacity of coregonines (e.g., Jones *et al.*, 1974; Bernatchez & Dodson, 1985) (Table 8). Due to a number of inconsistencies, these studies should be interpreted cautiously.

Jones *et al.* (1974) assessed the critical swimming velocities of several species of fishes, including 6 species of coregonines (Table 8). The 10 minute time intervals that were used by

Jones *et al.* (1974) seem to be a particularly short time interval (Beamish, 1978; Bernatchez & Dodson, 1985) and may preclude direct comparisons with most trials that typically use 30-60 minute time intervals (Brett, 1964; Beamish, 1978). In addition, the critical swimming velocity data for lake whitefish (Bernatchez & Dodson, 1985) and arctic charr (Beamish, 1980) differs from the results obtained by Jones *et al.* (1974).

A first glance at the data set of Jones *et al.* (1974) shows that many of the fish species have seemingly low critical swimming velocities in comparison to my bloater (Table 8 & Figure 21). A reason for this may be that the fish that they used were severely stressed by the capture techniques used in the field, while laboratory fish experienced a combination of long transport times inside of bags and comparatively fast acclimation to the holding tanks. The capture techniques used in the field included seining, gill netting, and hook and line–all activities that can be potentially very stressful. In addition, fishes captured in the field were experimented on only 24 hours after capture. Laboratory fish were treated with nitrofurazone, which may have unknown affects on their physiology (Jones *et al.*, 1974).

Problems also exist for the data set of Bernatchez and Dodson (1985). For example, because too few small lake whitefish swam at higher velocities, they used a rainbow trout (*Oncorhynchus mykiss*) data set (from Rao, 1968) in their lake whitefish model and extrapolated for velocities greater than 40 cm/s (Bernatchez & Dodson, 1985). Trudel *et al.* (2001) removed the rainbow trout data set and applied a logarithmic transformation to the oxygen consumption data. They found the slope of the oxygen consumption data for lake whitefish at 17 ^{0}C was lower than at 12 ^{0}C, with some data points coinciding with the oxygen consumption at 12 ^{0}C (Trudel *et al.*, 2001). This could be an indication that the cost of swimming was less for lake whitefish at 17

^{0}C in relation to 12 ^{0}C, but more data is needed. Trudel *et al.* (2001) reported that there was no difference in the oxygen uptake between cisco and lake whitefish.

Given that few bloater actively swam, I was unable to generate a predictive model with which I could directly compare my findings to other studies. Thus far my data on active oxygen uptake for bloater are within the range of other salmonid species that have been studied and are comparable to cisco and lake whitefish (Beamish, 1980; Bernatchez & Dodson, 1985) (Table 9).

The current information available on the critical swimming velocities and oxygen uptake of coregonines is particularly sparse and questionable and deserves further attention (e.g., Jones *et al.*, 1974; Bernatchez & Dodson, 1985). Many variables may dictate the performance of fishes, including physiological status and inter-individual variability of the species in question (Beamish, 1978; Reidy *et al.*, 2000). In addition, differences in experimental protocols may make further comparisons to other fish species questionable (Beamish, 1978; Stillwell & Benfey, 1997).

Nevertheless, my results indicate that bloater are generally continuous swimmers and are probably quite reliant on hydrodynamic lift to maintain neutral buoyancy. In addition, bloater have a metabolic rate comparable to that of other salmonines. Furthermore, there seems to be a size effect in bloater in regards to metabolic rate. However, further rigorous experimentation is necessary in order to fully understand the swimming capacity of bloater and other coregonines in general. Because of the paucity of good data, no further generalizations can be made on the swimming capacity of coregonines in comparison to the well-studied salmonines.

Figure 14. A. Top-down view of captive-raised bloater. Note the long pectoral and pelvic fins. **B.** Holding tank of captive-raised bloater showing the fish in movement with the paired fins held out from the body.


14A


14B

Table 5. Descriptive statistics on the routine swimming velocities and kinematics of bloater.

	Total Length (cm)	Velocity (cm/s)	Velocity (BL/s)	TBF (tail beats/sec)	Stride Length (BL/tail beat)
Mean	**19.4**	**24.8**	**1.27**	**2.6**	**0.6**
Range	16.5-24.8	16.9-40	0.96-1.75	1.88-3.33	0.43-0.71
±SD	2.11	5.39	0.22	0.33	0.07

Table 6. Mass, total length, oxygen uptake of bloater (Means ± SEM).

	Routine oxygen uptake	Active oxygen uptake
N	42 observations on 18 fish	31 observations on 5 fish
mass (g)	103 ± 2.82 (range 70 - 136)	99 ± 2.74 (range 79 - 136)
total length (mm)	234 ± 2.1 (range 210 - 258)	234 ± 2.1 (range 214 - 258)
Oxygen uptake ($mg\ O_2 * kg^{-1} * h^{-1}$)	276 ± 15.2	253 ± 18.5

Figure 15. Swimming kinematics in relation to length of bloater. None of the kinematics (tail beat frequency, stride length, or relative velocity) correlated with the length of the bloater.

Figure 4.2

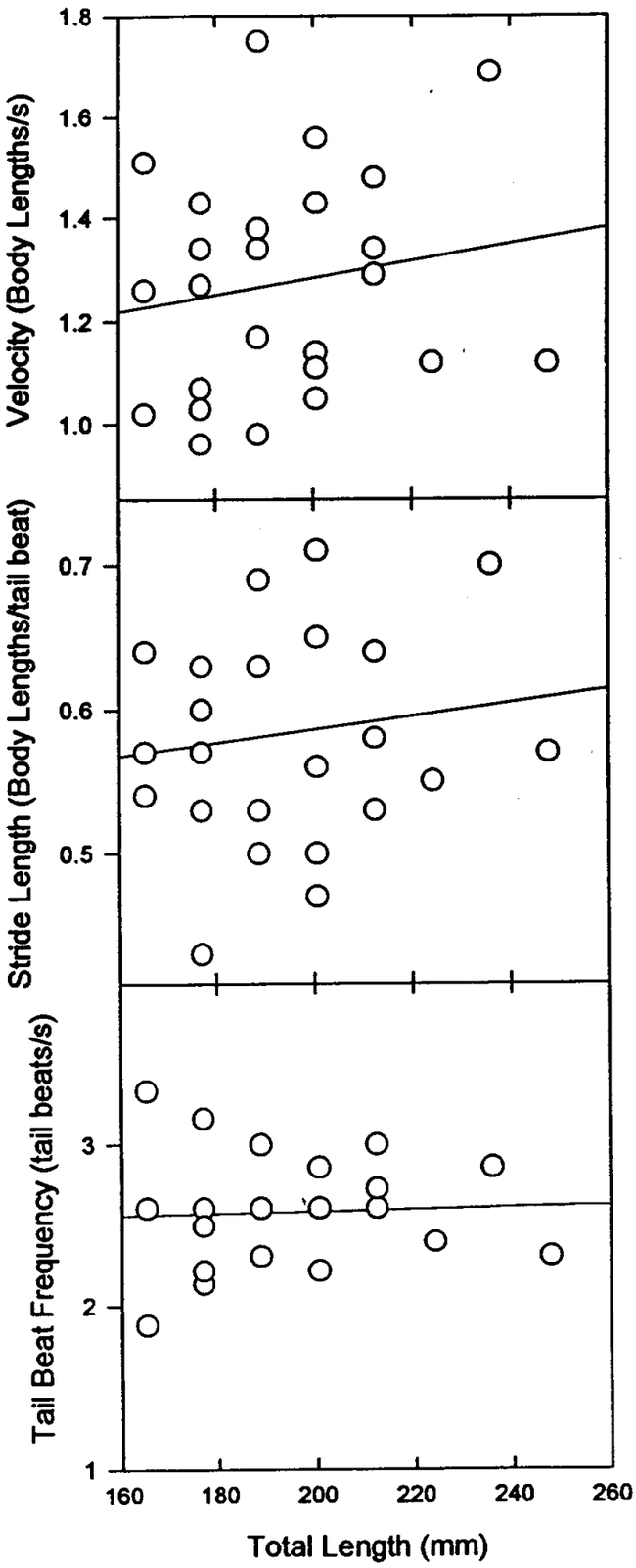

Velocity (Body Lengths/s)
1.8
1.6
1.4
1.2
1.0
Stride Length (Body Lengths/tail beat)
0.7
0.6
0.5
Tail Beat Frequency (tail beats/s)
3
2
1
160
180
200
220
240
260
Total Length (mm)

Figure 16. Swimming velocity (cm/s) in relation to length of bloater.

Figure 4.3

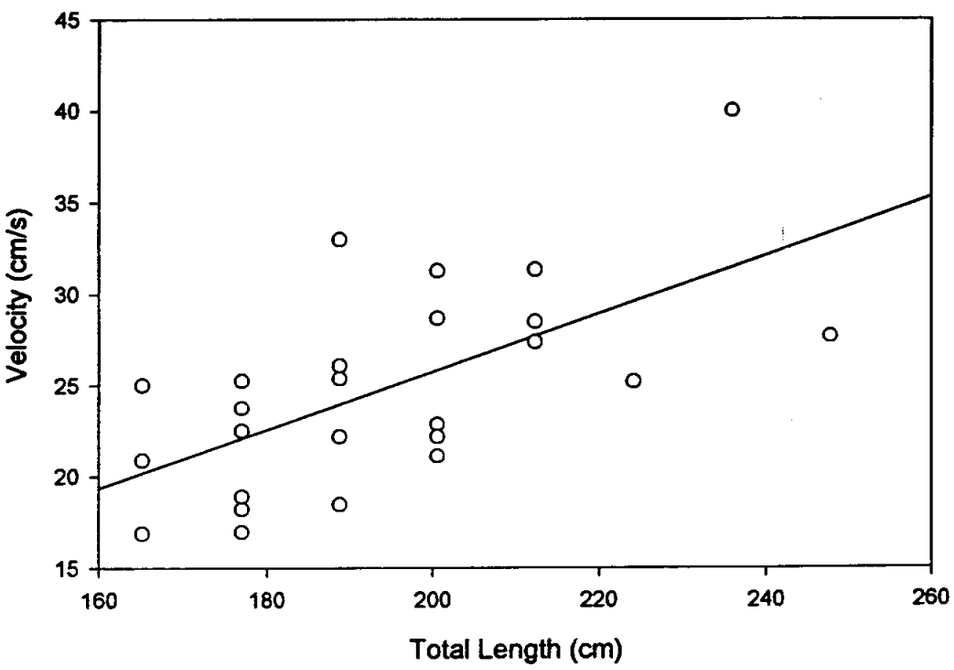
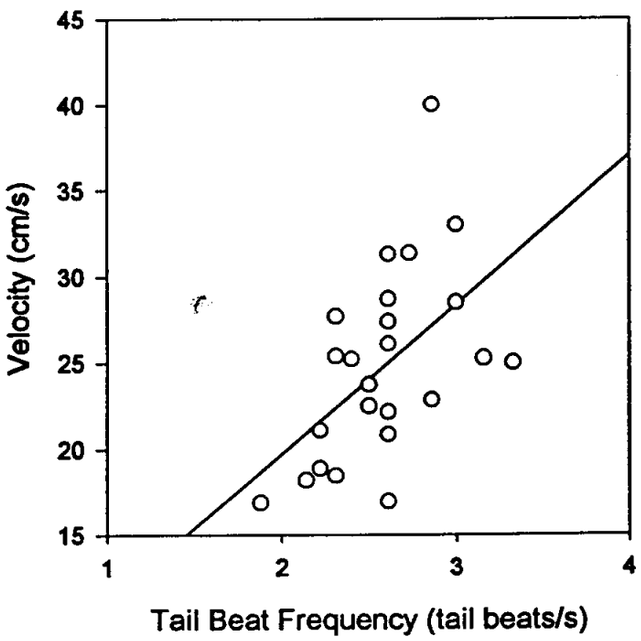
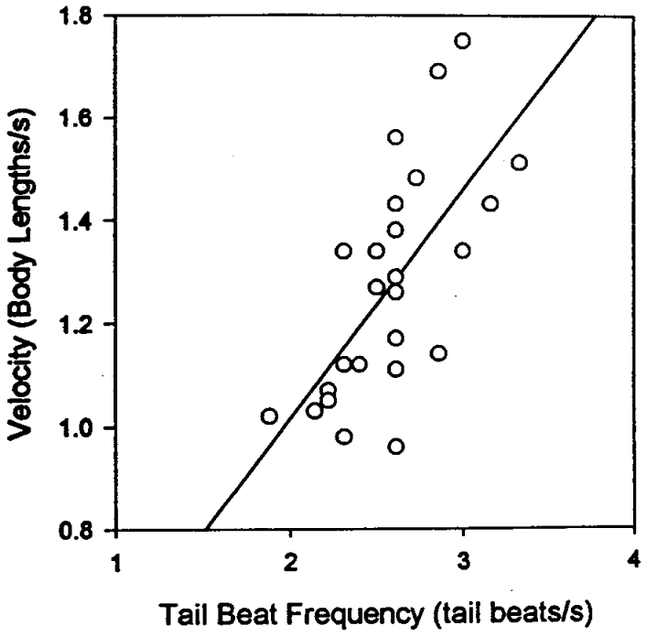
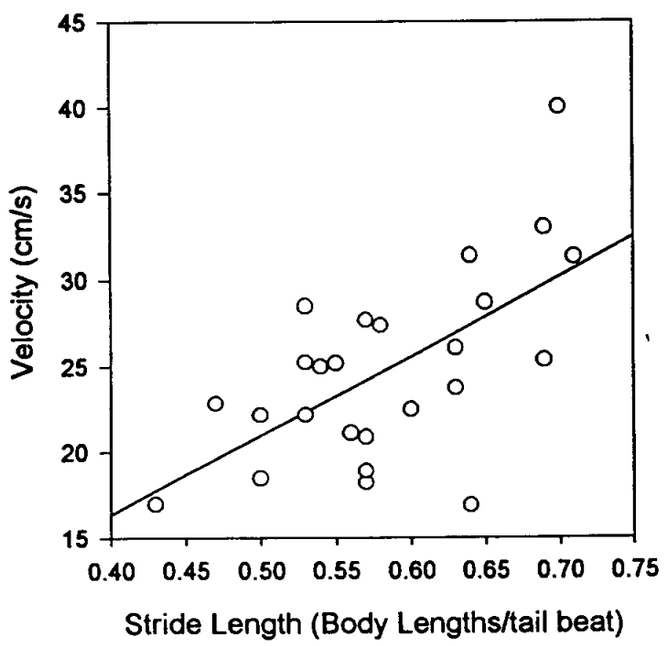

45
40
35
30
25
20
15
Velocity (cm/s)
160
180
200
220
240
260
Total Length (cm)

Figure 17. Routine swim velocity in relation to kinematics. Both tail beat frequency and stride length increased with the length of bloater.

Figure 4.4


Velocity (cm/s)
45
40
35
30
25
20
15
1
2
3
4
Tail Beat Frequency (tail beats/s)


Velocity (Body Lengths/s)
1.8
1.6
1.4
1.2
1.0
0.8
1
2
3
4
Tail Beat Frequency (tail beats/s)


Velocity (cm/s)
45
40
35
30
25
20
15
0.40
0.45
0.50
0.55
0.60
0.65
0.70
0.75
Stride Length (Body Lengths/tail beat)

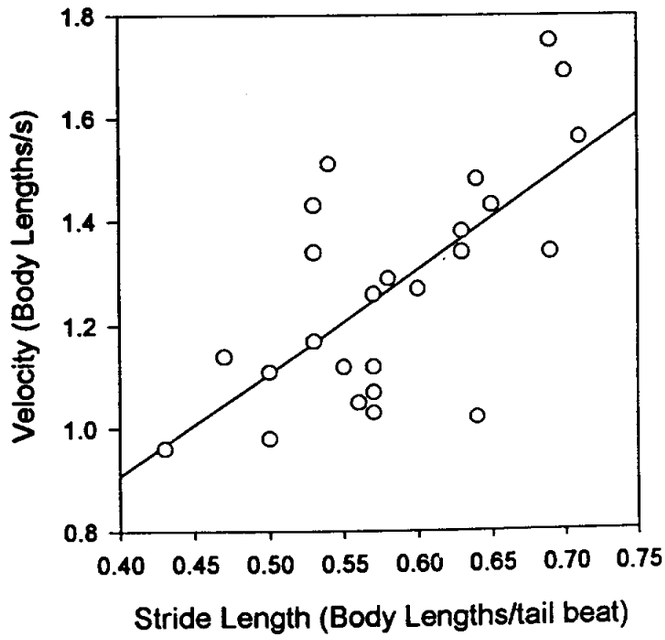

Velocity (Body Lengths/s)
1.8
1.6
1.4
1.2
1.0
0.8
0.40
0.45
0.50
0.55
0.60
0.65
0.70
0.75
Stride Length (Body Lengths/tail beat)

Figure 18. Oxygen uptake in relation to swim velocity. **A.** Relative swim velocity (BL/s), linear scaling. **B.** Relative swim velocity (BL/s); oxygen uptake on log scale. **C.** Dimensional swim velocity (cm/s), linear scaling. **D.** Dimensional swim velocity (cm/s); oxygen uptake on log scale. Correlation coefficients and graph equations relating oxygen uptake (mg O_2*kg^{-1}* h^{-1}) to swim velocity in cm/s and body lengths/s are presented below.

	Linear		Logarithmic	
cm/s	$r^2 = 0.746$	$y = 78.45 + 2.141*V$	$r^2 = 0.706$	$y = 10^{2.097+0.003396*V}$
body lengths/s	$r^2 = 0.751$	$y = 74.20 + 51.45*V$	$r^2 = 0.711$	$y = 10^{2.090+0.0816*V}$

Figure 4.5

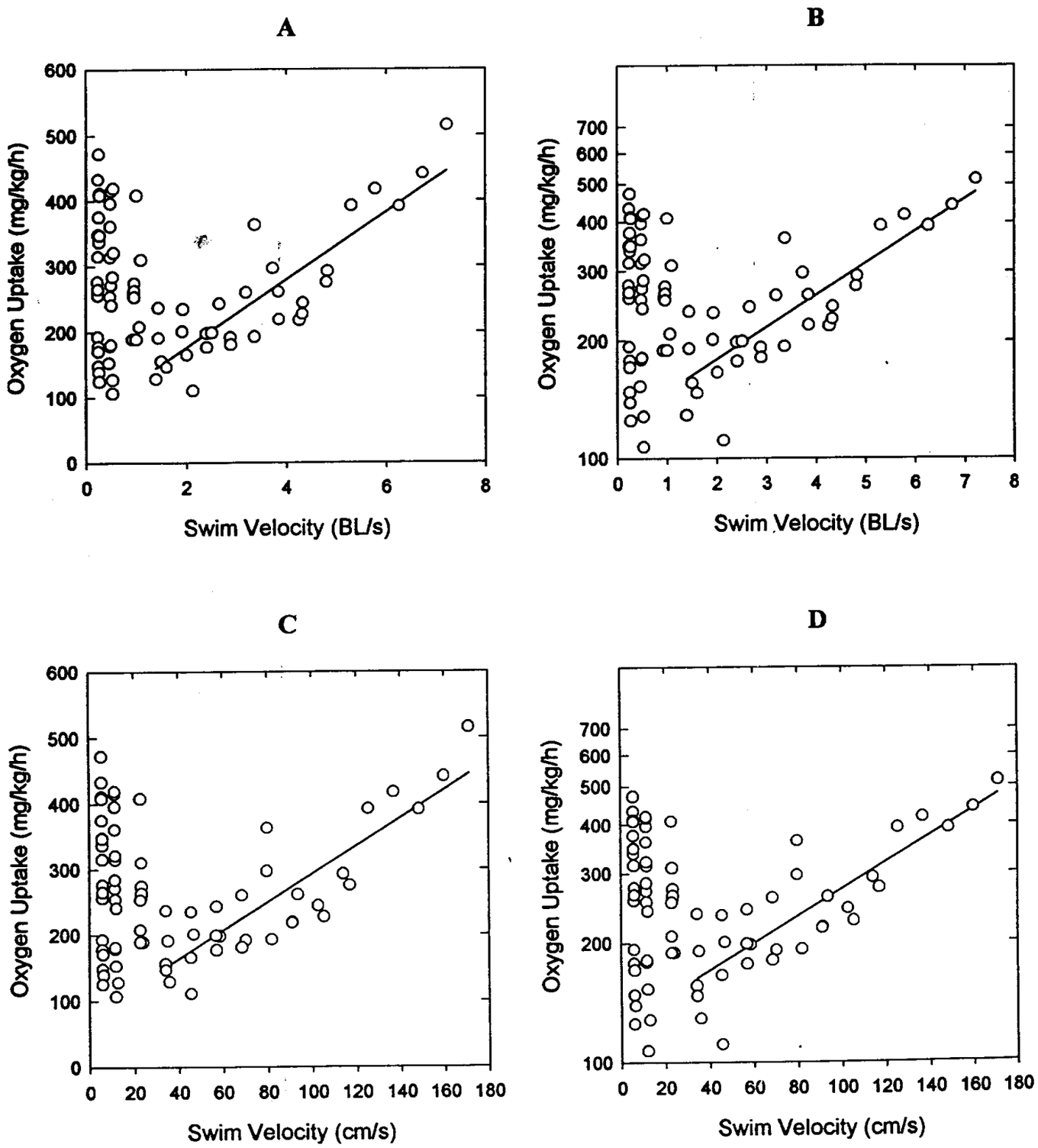

A
B
C
D
Oxygen Uptake (mg/kg/h)
Swim Velocity (BL/s)
Swim Velocity (cm/s)
0
100
200
300
400
500
600
700
0
1
2
3
4
5
6
7
8
0
20
40
60
80
100
120
140
160
180

Figure 19. Oxygen uptake in relation to size of bloater. Refer to Table 4.2 for descriptive statistics on size and oxygen uptake. **A.** Length relation for all data (routine and active oxygen uptake; n = 73); $r^2 = 0.037$, p = 0.102. **B.** Length relation for the routine swimming data (n = 42); **$r^2 = 0.138$, p = 0.015**. **C.** Mass relation for all data (n = 73); **$r^2 = 0.140$, p = 0.001**. **D.** Mass relation for the routine swimming data (n = 42); **$r^2 = 0.353$, p = 0.000**.

Figure 4.6

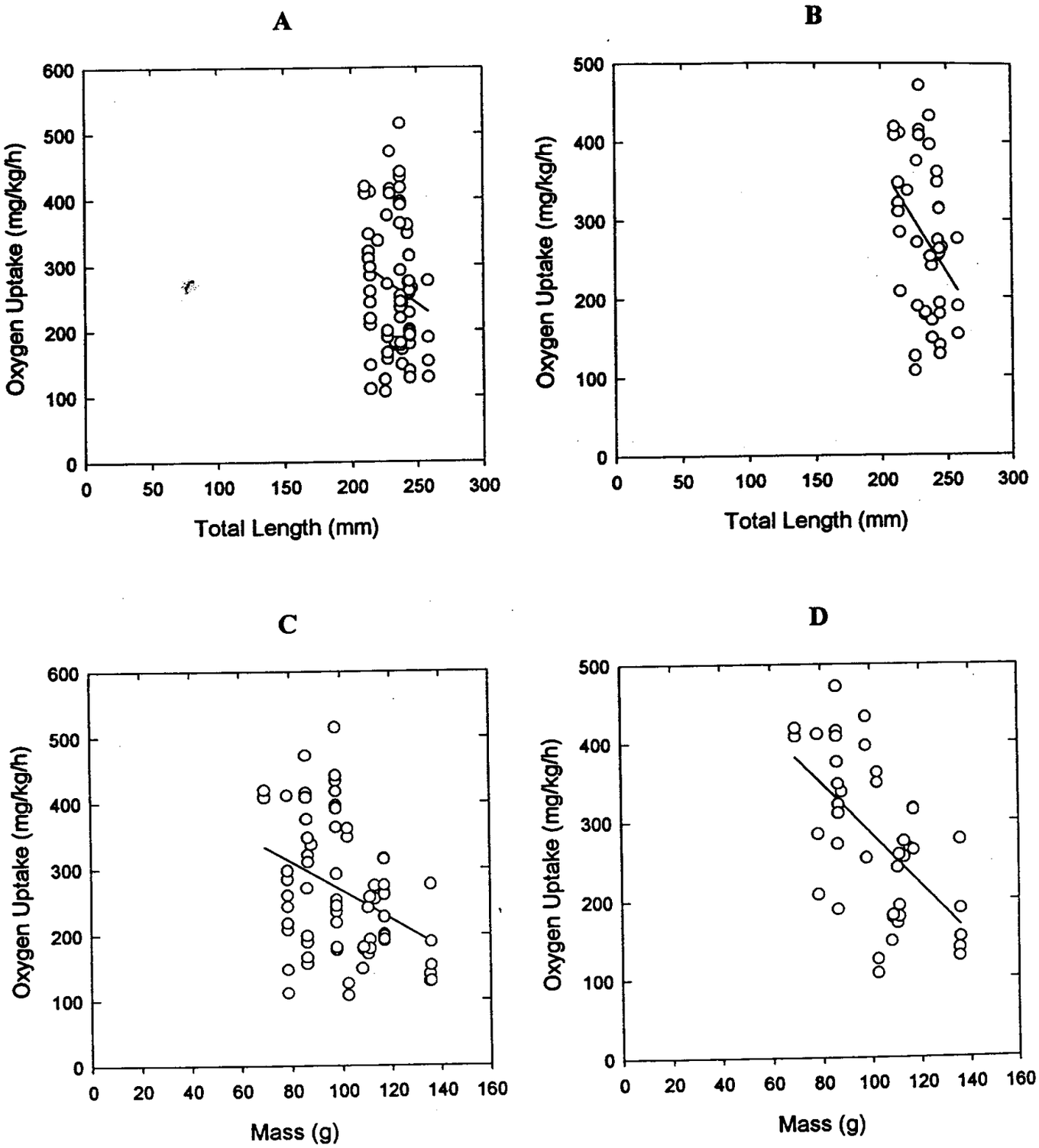

A
B
C
D
Oxygen Uptake (mg/kg/h)
Total Length (mm)
Mass (g)
0
50
100
150
200
250
300
20
40
60
80
120
140
160
100
200
300
400
500
600

Figure 20. Oxygen uptake in relation to swim velocity for the bloater (n = 5) that actively swam in the swimming capacity trials.

shocks required? (Y/N)	TL (mm)	Mass (g)	Ucrit (cm/s)	Ucrit (BL/s)
Y	258	136	39	1.51
Y	227	86.3	51	2.25
N	214	78.6	93	4.35
N	244	117.1	120	4.92
N	237	98	165	6.96

Figure 4.7

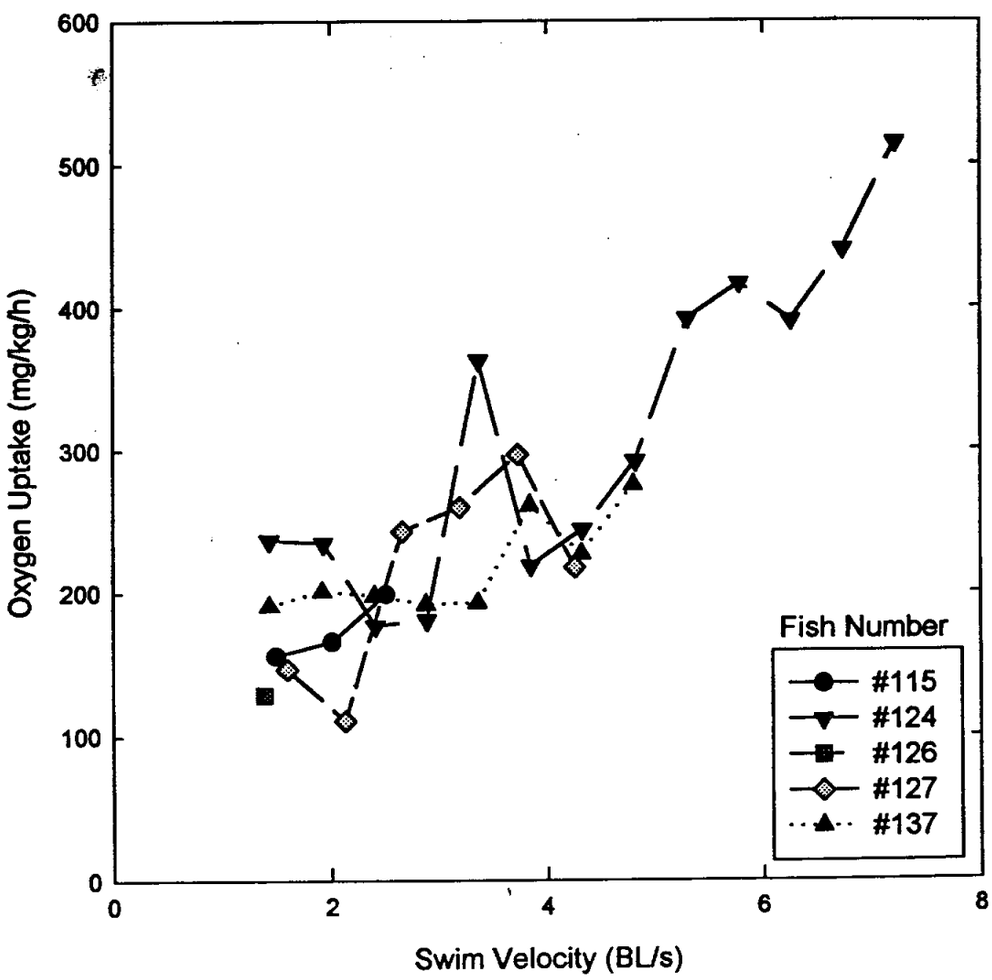
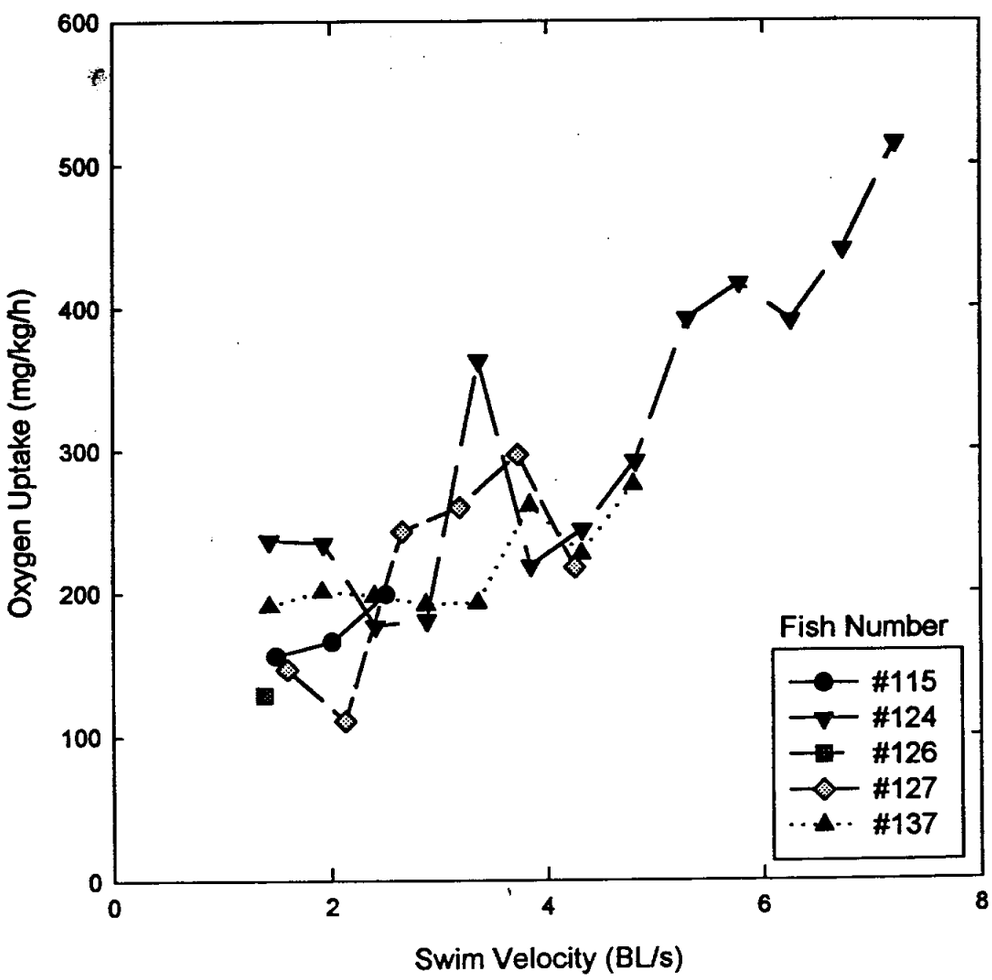

600
500
400
300
200
100
0
0
2
4
6
8
Oxygen Uptake (mg/kg/h)
Swim Velocity (BL/s)
Fish Number
#115
#124
#126
#127
#137

Table 7. Comparison of routine swim velocities in my study with that of Rudstam *et al.*(1984).

TL = total length (mm); means±SD.

Age	TL (±SD)	N	Temp. (^{0}C)	U (cm/s) ±SD	U (l/s) ±SD	N	study
1+	108 (6)	10	12	12.6 (2.7)	1.17	50	Rudstam *et al.* (1994)
2+	153 (6)	10	12	18.6 (4.3)	1.22	50	Rudstam *et al.* (1994)
2+	**194 (21.1)**	**25**	**12.5**	**24.8 (5.39)**	**1.27 (0.22)**	**25**	**current**
4-6+	249 (31)	32	12	25.0 (3.5)	1.00	50	Rudstam *et al.* (1994)

Figure 4.7


600
500
400
300
200
100
0
0
2
4
6
8
Oxygen Uptake (mg/kg/h)
Swim Velocity (BL/s)
Fish Number
#115
#124
#126
#127
#137

(Basu, 1959)

TABLE 4.4.

Figure 21. Critical swim velocity in relation to length for coregonines. Refer to Table 4.4 for descriptive statistics. Data from Jones *et al.* (1974) for broad whitefish (squares), inconnu (clear circles), lake whitefish (diamonds). The five bloater that actively swam from my study (dark circles) are presented for comparison. **A.** Dimensional swim velocity (cm/s) data over a range of lengths. **B.** Dimensional swim velocity (cm/s) data over a length range comparable to the bloater in my study. **C.** Relative swim velocity (BL/s) data over a range of lengths. **D.** Relative swim velocity (BL/s) data over a length range comparable to the bloater in my study.

	Swim velocity (cm/s)	Swim velocity (BL/s)
Broad whitefish	$r^2 = 0.35$	$r^2 = 0.64$
Inconnu	$r^2 = 0.42$	$r^2 = 0.42$
Lake whitefish	$r^2 = 0.29$	$r^2 = 0.35$

Figure 4.8

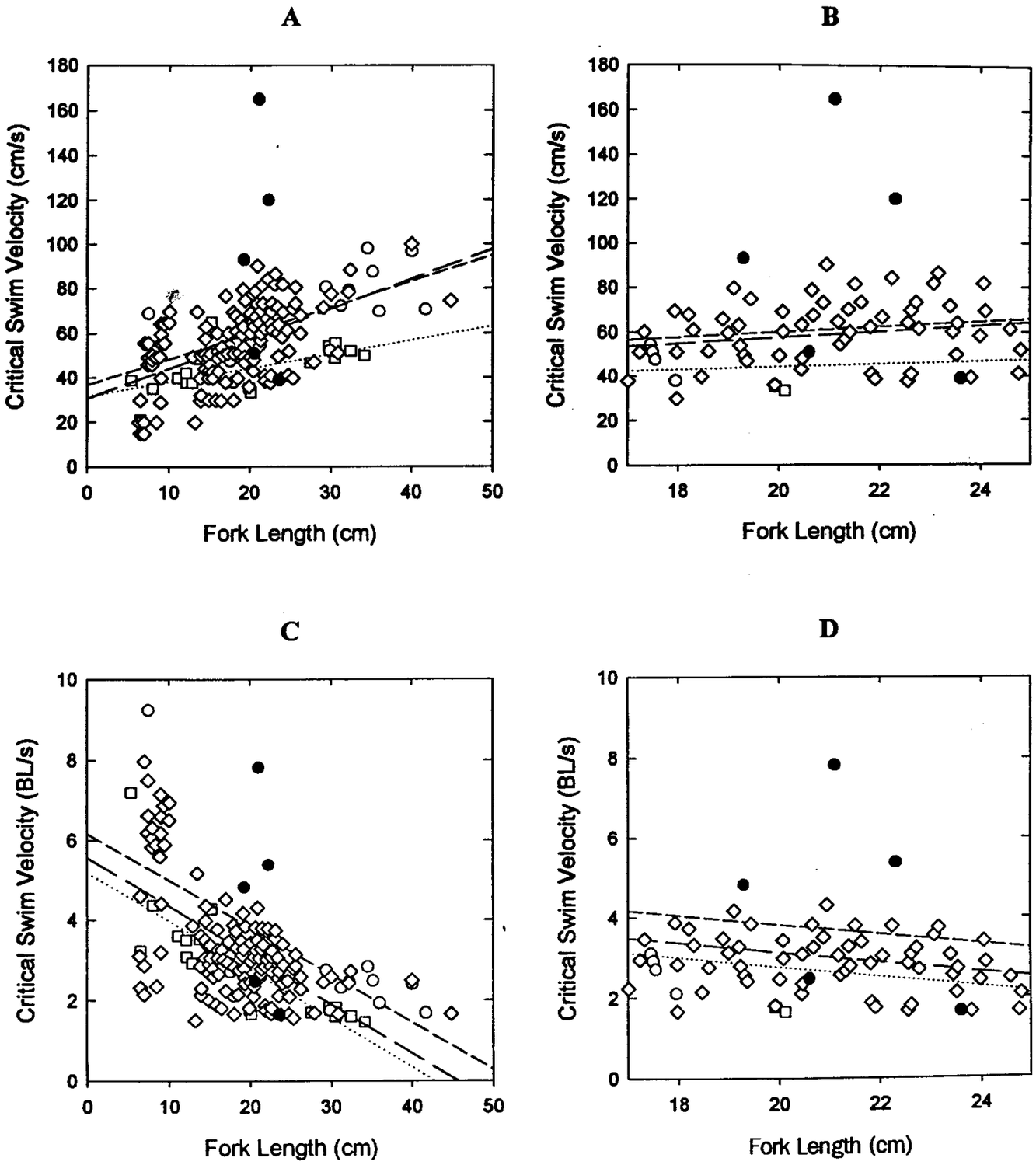

A
B
C
D
Critical Swim Velocity (cm/s)
Critical Swim Velocity (BL/s)
Fork Length (cm)
0
10
20
30
40
50
18
22
24
2
4
6
8
60
80
100
120
140
160
180

Table 4.5. Comparison of critical swimming velocities in coregonines, arranged in descending order of body lengths per second (BL/s). Values in parentheses were calculated by multiplying the average Ucrit (BL/s) by the average length of the fish and are shown for comparison. Source: 1 = My study; 2 = Bernatchez & Dodson (1985); 3 = Jones *et al.* (1974).

▲All Ucrit less than 1.50 BL/s were removed from the data set.

Common Name	species	Field /Lab	Temp (°C)	FL (mm)	TL (mm)	N	Ucrit±SD (cm/s)	Ucrit±SD (FL/s)	Ucrit±SD (TL/s)	Source
bloater	***Coregonus hoyi***	**L**	**12**	**214±16.4**	**236±16.7**	**5**	**93.6±51.5 (94.8)**	**4.43±2.5**	**4.00±2.2**	**1**
cisco	*Coregonus artedi*	L	12	----------	135±5	8	45.8±8.1	----------	3.39	2
lake whitefish	*Coregonus clupeaformis*	F/L	7-8; 12-13; 18-20	182±69	----------	126	55.2±17.0 (60.8)	3.34±1.42	----------	3 ▲
lake whitefish	*Coregonus clupeaformis*	L	12	----------	342±11	16	75.0±9.5	----------	2.19	2
inconnu	*Stenodus leucichthys*	F	12-13; 18-20	277±103	----------	14	69±18.5 (80.6)	2.91±1.87	----------	3 ▲
broad whitefish	*Coregonus nasus*	L	7-8; 12-13	189±99	----------	17	43.6±10.5 (54.6)	2.89±1.50	----------	3 ▲
least cisco	*Coregonus sardinella*	F	12-13; 18-20	295	----------	2	60	2.03	----------	3
Arctic cisco	*Coregonus autumnalis*	L	7-8; 12-13	421	----------	4	80	1.90	----------	3
mountain whitefish	*Prosopium williamsoni*	L	7-8; 12-13	30.4±1.5 (SEM)	----------	9	42.5±6.5 (SEM)	1.4	----------	3

4. Behaviour at Pressure

INTRODUCTION

Few laboratory studies have used simulated pressure regimes on fishes to estimate gas secretion rates or behaviour (Harden Jones, 1952; Tait, 1959; Bishai, 1961 & 1963–cited in Gordon, 1970; Caulton & Hill, 1973 & Caulton & Hill, 1975; Ribbink & Hill, 1979; Harden Jones & Scholes, 1985). Only two of these studies has focused on the behaviour of the fish in reaction to pressure changes (Harden Jones, 1952; Harden Jones & Scholes, 1985). However, Harden Jones (1952) used relatively small pressure changes of 33 % decompression from the pressure to which the fish were adapted. Other studies have focused on the gas secretion or resorption rates of fishes subjected to compression and decompression, respectively (Harden Jones, 1952; Tait, 1959; Caulton & Hill, 1973 & 1975; Ribbink & Hill, 1979; Harden Jones & Scholes, 1985).

Simulated pressure regimes under the controlled environment of the laboratory are important as a means of studying fish behaviour. When synthesized with other aspects of data collection, such as remote or direct sampling in the field, studying the behaviour of fish at pressure can provide insight into the biology of the fish in question and elucidate rates and extents of vertical migration (e.g., Caulton & Hill, 1973; Harden Jones & Scholes, 1985; Arnold & Greer Walker, 1992). Current interest exists in modelling the bioenergetic demands of vertical migration in fishes (Alexander, 1972; Rudstam & Magnuson, 1985; Bevelhimer & Adams, 1993; TeWinkel, 1998), and the behaviour of fishes subjected to pressure (Arnold & Greer Walker, 1992; TeWinkel & Fleischer, 1998; Eshenroder *et al.*, 1999), and both aims can be achieved by using a pressurized system in the laboratory.

Knowledge of bloater swimming behaviour at increased pressures is of interest (Eshenroder *et al.*, 1999). In addition, changes in swimming behaviours (i.e., body tilt-angles)

resulting from negative buoyancy (He & Wardle, 1986; Eshenroder *et al.*, 1999) can reduce the aspect ratio and hence target strength for hydroacoustic surveys are of interest in estimating forage fish (in this case, bloater) biomass (Blaxter & Batty, 1984; Ona, 1990; Brandt *et al.*, 1991; Argyle, 1992; Arnold & Greer Walker, 1992; Fleischer *et al.*, 1997).

My objective was to analyze the behavioural reactions of bloater subjected to a simulated vertical migration. I tested the hypotheses that a) bloater would not be able to secrete gas and would rely upon compensatory swimming movements and that b) changes in pressure would cause a patterned response in the number and duration of swim-ups, indicating a stress response.

The inability for bloater to secrete gas into their gas bladder during an imposed vertical migration would provide evidence that gas secretion is a slow process in bloater, which is to be expected by observations on the anatomy of their gas bladder (see section 2). Furthermore, if bloater are able to secrete gas into their gas bladder, it will necessarily lag behind the vertical movements (and hence pressures) of the fish (Harden Jones & Scholes, 1985; Arnold & Greer Walker, 1992; Bone *et al.*, 1995).

The extent, number and duration of vertical trips within the chamber could provide insight into swimming and general reactions to different pressures. A detectable stress response in swimming behaviour would enable predictions on the vertical restrictions of individual bloater, assuming all things equal and that wild bloater would not undergo vertical migrations that would induce stress (TeWinkel & Fleischer, 1998).

METHODS

I analyzed bloater behaviour during two consecutive days of pressure changes (1 day for compression; 1 day for decompression) using a pressurized system (for description, see below). I

simulated depth changes to 57 m above ambient (6.5 atm), similar to what adult bloater of a similar age and size would encounter in the Great Lakes if they remained near the bottom during the day and migrated upward into the water column at night, as has been shown with hydroacoustic and trawl data (Eshenroder *et al.*, 1998; Fleischer & TeWinkel, 1998; TeWinkel & Fleischer, 1998 & 1999).

All trials were recorded with a VHS video camera and the video tapes were later analyzed to determine a) whether secretion of gas into the gas bladder took place, and b) the number and duration of vertical trips to the top of the chamber by each fish. Gas secretion into the gas bladder would be identified by minimal amounts of swimming to maintain vertical station because neutral buoyancy occurs only at a specific depth (Steen, 1970).

Experimental Fish

Age-3+ fish were raised in captivity at the University of Waterloo, at a depth of 39-65 cm (1.04-1.06 atm). In the Great Lakes, bloater would normally inhabit depths of approximately 36-110 m in the Great Lakes at this age (3+) (Brown *et al.*, 1985).

Pressure System

I used a pressurized flow-through system with which I could increase or decrease pressure while maintaining both constant temperature and oxygen levels over time (Figures 22 & 23). The set-up that I used included a) cylindrical plexiglass pressure chamber (ht = 60.5 cm; diam. = 33.0 cm; vol. = 48 L), seated inside a cooling reservoir of water (Figure 23C), b) an aerated water reservoir (11.9 ^{0}C, approximately 95 % oxygen saturation) consisting of a constant supply of Waterloo well water (Figure 23A), and c) a 15 VA high pressure, low flow pump (Goulds model) that constantly replenished the pressure chamber with water (225 L/hr) from the oxygenated

reservoir (Figure 23B). A small fan was set on the floor in front of the system to prevent condensation from forming on the viewing glass of the cooling reservoir. The pressure chamber lid was fitted with analog and digital pressure gauges (Figure 22B).

Partitions were placed on either side of the pressure chamber to prevent outside visual disturbance to the fish. The VHS video camera was positioned directly across from the pressure chamber, behind a barrier made of dark plastic bags, with a small opening for the camera lens. Video cables between the camera and a monitor outside of the barrier permitted viewing of the fish behaviour and adjustment of the camera angle during the experiment.

System Parameters

Temperature and oxygen were relatively steady, as determined with a YSI oxygen probe connected to the outflow system (Figure 22B). System temperatures rose 1.5 ^{0}C above reservoir levels (11.9 ^{0}C) within the first 2 hours of the experiment, and then stayed constant at approximately 13.4 ^{0}C. Oxygen levels were high throughout the experiment at 93 % saturation and varied by only 10 %.

Protocol

Seven (7) trials were carried out from August 2nd through August 20th. The protocol of 3 trials (Trial 1, Trial 2, and Trial 6) differed significantly from the remaining 4, and were not used for comparisons. The pressure chamber was disinfected on two occasions with Wescodyne Iodine solution (according to the directions) and rinsed thoroughly before starting a new trial.

Prior to acclimation in the pressure chamber, 3 fish were randomly captured from the holding tank with a net and anaesthetized with 6 ppm clove oil (G. Wagner, pers. comm., 2000). Physical measurements were taken (mass, total length and fork length), and 2-1 X 2.5 mm Visible

Implant (VI) tags (Northwest Marine Technology) were inserted, one into each gill operculum for later identification. The combined processing time of physical measurements and tag insertion took 15 minutes. The anaesthetized bloater were kept moist with a wet towel during measurements and tag insertion, and were never out of water for more than about 30 seconds. The bloater were then placed inside the pressure chamber for overnight acclimation at ambient pressure (i.e., 1 atm + 0.06 atm from the water column). All bloater recovered from the effects of anaesthesia within 15 minutes.

Preliminary observations revealed that my captive-raised bloater would occasionally gulp air within the holding tank and during acclimation inside the pressure chamber. In addition, I have previously witnessed bloater gulping from an air bubble that was present on the pressure chamber lid during pressure increases (see Appendix II). Therefore, I inserted a plastic retainer into the top of the chamber to prevent the fish from gulping surface air during acclimation and bubbles during the experimental pressure changes. The retainer rested about 15 cm inside the pressure chamber, restricting the amount of vertical space available to the bloater to about 45 cm. The retainer was porous and permitted circulation and turn-over of water within the chamber during both acclimation and experimental pressure changes, as revealed by injecting dye into the reservoir and observing the flow through the pressure chamber. A 115 V, 1.1 amp Little Giant pump provide a constant inflow of aerated water from the reservoir at 11.9 ^{0}C overnight.

For each trial, 3 bloaters were observed in the pressure chamber. The decision to use 3 bloater was based on the rationale that the bloater have been raised in close proximity with other conspecifics in the holding tank. Bloater were held in the pressure chamber at ambient pressure for approximately 19 hours of acclimation to the chamber environment At this point, the fish had

been starved for 41 hours to prevent fecal matter from fouling water in the pressure chamber.

For all trials, the pressure system was sealed at the end of the acclimation period (0700 hours). Within a few minutes of sealing the pressure chamber, the video camera was turned on, and then the pressure was increased in a stepwise fashion in increments of 0.5 atm (simulated depth increase of 5 m) over a period of 30 to 150 seconds, every 30 minutes. A maximum pressure of 5.5 atmospheres (57 m) was achieved 5 hours later. The fish were held at maximum pressure for 24 hours. At this point (next day, 1200 hours), pressure was decreased in the same increments and intervals until ambient pressure was achieved 5 hours later.

Because more adult bloater are at their deepest depths in the Great Lakes (increased pressure) during daytime hours, and a portion of the bloater population moves upward into the water column during night-time hours, I subjected the experimental bloater to a similar schedule of compression and decompression coinciding with the time of day (Brandt *et al.*, 1991; Argyle, 1992; Eshenroder *et al*., 1998; TeWinkel & Fleischer, 1999). I subjected the experimental bloater to increasing pressure during the morning hours and decreasing pressure during the afternoon hours of the second day, respectively.

There is no published information on the rate or initiation times at which bloater vertically migrate. Therefore, I based the times for the initiation and duration of pressure changes upon a combination of Beeton's (1960 – cited in TeWinkel, 1998) results for vertical migration of *Mysis relicta* in the Great Lakes (apparent negative phototaxis), the extent of pressurization (5.5 atm above ambient) and the laboratory lighting schedule at the University of Waterloo. The duration of the intervals ensured adequate time within which I could analyze bloater behaviour and coincide the pressure changes with the laboratory lighting schemes. The extent of pressurization

that I used also coincides with what Eshenroder *et al.* (1998) reported for bloater in the Great Lakes. Using hydroacoustics, they found that bloater traversed pressures of up to 5 atmospheres (Eshenroder *et al.*, 1998).

Beeton (1960 – cited in TeWinkel, 1998) reported that *Mysis relicta* initiated their downward migration at 0300-0500 hours, and upward migration at 1700-2200 hours. The laboratory lights dimmed on by 0632 hours and I subjected the bloater to their first pressure increase by 0700 hours. Full pressurization was achieved by 1200 hours and was kept that way for 24 hours, whereupon I decreased the pressure to ambient by 1700 hours. Therefore, my simulated compression and decompression cycles each spanned 5 hours, which is comparable to the maximum time fishes would spend during vertical migration (Harden Jones, 1952).

Behaviour Observations

The extent and duration of vertical movements by individual bloater within the pressure chamber were recorded in relation to station markers (at 13 and 25 cm) on the pressure chamber. An elapsed time imprint on the video tapes (hours to hundredths of a second) was used as a reference for both the initiation and termination of a swim-up.

In the analyses, initiation of a "swim-up" was defined as the time at which the bloater left contact with the bottom (including other fish that were on the bottom) and moved upward to a set marker on the pressure chamber. The termination of a "swim-up" occurred when the bloater regained contact with bottom of the pressure chamber (including other fish that were on the bottom). Swim-ups were recorded only for those fish in which the whole body passed the "middle", or the snout and/or back passed the "top". The extent of the movement was ascertained by recording the vertical extent of the swim-up in reference to a 13 cm mark

(designated as “middle”), and a 25 cm mark (designated as “top”). Movements that occurred during and within the initial 2 minutes of a pressure change were thought to have been influenced by the change in pressure and were arbitrarily discarded. A profile of the number, extent (“middle” or “top”), and duration of swim-ups in relation to pressure changes was constructed for each fish.

Data Analysis

Of the initial 12 fish (3 per trial), 10 lived throughout the compression events and were used for statistical analysis. I conducted a one-way nested ANOVA and nested ANCOVA (using the length and mass of the bloater as a covariate) to determine whether compression had an effect on each of 5 variables within each trial. The five variables were: 1) elapsed time between a pressure change and the first swim-up (“time start”), 2) duration of the first swim-up (“duration of first”), 3) average duration of time spent off the bottom (“average duration”), 4) the number of swim-ups that occurred (“number of swim-ups”), and 5) the sum of the extent of scored movements (“total movements”).

Only 4 of the 10 bloater survived through the entire pressure cycle (compression and decompression). I conducted a separate ANOVA on both the compression and decompression cycles for these 4 fish.

In order to determine the cause of death in the bloater that were subjected to pressure, (3) of the fish that died during the experiments were placed on ice and delivered to the University of Guelph Fish Pathology Laboratory.

RESULTS

Behaviour

The bloater performed a number of short duration swim-ups (Appendix III), often times near the top of the retainer. Sometimes the bloater would nudge the retainer. However, with the exception of bloater number 9 and possibly number 10, the majority of the trial time was spent with the ventral side of the fish in contact with the bottom. The bloater adopted positive tilt angles that were sometimes near vertical when they swam to the top of the pressure chamber. Time was spent less frequently by the bloater lying on their side or readjusting their position along the bottom in relation to the other fish. However, during the decompression stage of the experiment, most of the bloater spent the majority of the time on the bottom, lying on their side.

Sometimes a "nosing" behaviour was noticed in which the bloater adopted a negative tilt angle and repeatedly touched its nose along the bottom as it swam in circles in an excited manner. This latter behaviour occurred during both the compression and decompression stages of the experiment, although all of the bloater were negatively buoyant throughout all the ranges of pressure.

Necropsy

Although 1 bloater was found dead the morning after the overnight acclimation, there was no other indication that the combined effects of anaesthetizing, inserting identification tags, and overnight acclimation inside the chamber caused undue stress to the bloater in any way.

The number of mortalities in relation to pressure changes is presented in Figure 24. One (1) bloater died during an increase to 2.0 atm and 10 bloater lived through the full compression cycle to 5.5 atm above ambient. Six (6) of these 10 bloater died during the 24 hours at maximum pressure. The remaining 4 bloater survived through the entire experiment; of these, 2 died within 1-2 days of the trial, and the remaining 2 are still alive, as of 5 months post-experimentation.

The necropsy examination by the pathologist was unable to discover gross or microscopic lesions that might explain the cause of death. There were no histological or gross anatomical symptoms that pointed to the failure of the gills, intestines, heart, liver, spleen, kidney, pancreas, stomach, or muscle.

However, small haemorrhages were found around the optic nerve and in the choroid plexus. These haemorrhages could indicate terminal hypoxia (possibly caused by the formation of gas bubbles from reduced pressure), but this is not absolute.

Compression (10 fish)

With an increase in pressure, the volume of the gas bladder should decrease in accordance with Boyle's Law (Alexander, 1993). For a typical 100 g fish with a gas bladder of 5 ml, the volume should be compressed to 0.9 ml at the maximum pressure of 5.5 atm.

Considerable variation existed both among bloater in the same trial and between fish of different trials with respect to each of the 5 variables (Figures 25-29). While some bloater repeatedly swam up over variable periods and durations of time, others remained on the bottom (Appendix III). A notable exception was bloater number 9, which spent a majority of the compression time in the water column (Appendix III).

A summary of results for pressure effects is presented in Table 10. There was no consistent change in any of the 5 measures of behaviour (e.g., "Time Start", "Duration of First", "Average Duration", "Number of Swim-ups", and "Total Movements") with respect to pressure, even when size was used as a covariate (Figures 25-29).

A summary of the results for trial effects is presented in Table 11. When used as a covariate, size (i.e., mass) accounted for all of the variation between trials in the lapsed time

between the pressure change and the first swim-up ("time start") (ANOVA, p = 0.018). In the remaining four variables, trial effects accounted for all of the variation, regardless of whether size was used as a covariate or not: "duration of first" (ANOVA, p = 0.000), "average duration" (ANOVA, p = 0.000), "number of swim-ups" (ANOVA, p = 0.027), "total movements" (ANOVA, p = 0.039) (Figures 25-29).

Compression and Decompression (4 fish)

A summary of the results for both compression and decompression is presented in Table 12. If we consider only 4 of the 12 fish that survived both compression and decompression, then compression affected three of the five measured variables, while decompression affected only one variable. No variable was affected in both compression and decompression cycles (Figures 30-34).

Compression significantly increased the duration of the first swim-up ("duration of first") (ANOVA, p = 0.020), and decreased both the number of swim-ups (ANOVA, p = 0.006) and the total number of scored movements ("total movements") (ANOVA, p = 0.006) (Figure 31). It is apparent that the number of swim-ups and the total number of scored movements were essentially the same measure (Figures 33 & 34). None of the significant changes was consistent for all 4 fish.

Decompression significantly decreased the average duration of time spent off the bottom ("average duration") (ANOVA, p = 0.026) (Figure 32). This was attributable solely to bloater number 9, which decreased the amount of time spent above the bottom as pressure was decreased (Appendix III).

In summary, changes in ambient pressure (either increases or decreases) did not result in

any systematic change in behaviour. Some bloater died as a result of the pressure changes, but the cause of death was not obvious.

DISCUSSION

The inability of bloater to secrete gas was suggested by the prolonged residence times on the bottom of the pressure chamber (indicating negative buoyancy) throughout all pressures (i.e., both compression and decompression cycles). Most of the bloater spent the majority of the experiment on the bottom of the pressure chamber, and almost all of the bloater rested on their side during decompression.

The overall large variation in the number and duration of swim-ups indicated a lack of a patterned response, and perhaps different reactions to stress induced by pressure changes that were experienced by individual bloater. The lack of a patterned response indicated that no common reactions to pressure could be shown in the 10 bloater that were examined. However, it should be noted that the effects of pressure induce excitement and abnormal activity in fishes (Sebert, 1997). Six (6) of the 10 bloater died when held at pressure for 24 hours, indicating that the amplitude and possibly rate of compression had severely negative effects.

However, 4 of the 10 bloater survived through the entire compression and decompression cycles (Figure 24), and did show changes in the number and duration of swim-ups. In these 4 fish, it appears as though changes in the duration of the first swim-up and the number of swim-ups were apparent within the first two-three pressure increases, indicating a response within the initial 50 – 100 % (0.5-1.0 atm above ambient pressure) compression (Appendix III). Further pressure changes beyond the initial pressure increases show a varied response in bloater swimming behaviours.

In analyzing the behaviour of bloater during compression and decompression, three questions come to mind. One, can bloater compensate for pressure changes of large amplitude (5.5 atm above ambient) and the rate (within 5 hours) of a simulated pressure change by gas secretion into their gas bladder and/or swimming? Two, are captive-raised bloater that have not previously been subjected to a depth greater than 65 cm within their 3+ years of life appropriate as test organisms in compressions of large amplitude and duration? Three, does the experimental protocol that I used need to be adjusted?

Can bloater compensate for pressure changes of the amplitude (5.5 atm above ambient) and the rate (within 5 hours) of a simulated pressure change that I used? It appears as though bloater can compensate for pressure changes of the amplitude and rate that I used to a limited extent by intermittent swimming and resting, and in some cases, prolonged swimming (e.g., fish no. 9; Appendix III). All compensation occurred in the form of an acute response to compression by effecting a number of swim-ups to the top of the pressure chamber. As previously noted, very little to no gas secretion took place, as evidenced by the negative buoyancy of the bloater both throughout and following the experiments.

The repeated swim-ups of variable duration near the top of the chamber were probably escape responses, and in a natural environment would have succeeded in moving the bloater to a shallower depth and lowered pressure. For example, the 4 bloater that survived the entire pressure cycle showed an increase in the duration of the first swim-up as pressure was increased. Furthermore, the time spent above the bottom of the pressure chamber was directly related to pressure changes in bloater number 9 (Figure 24 & Appendix III).

The repeated “nosing” behaviour along the bottom by the bloater may have also been an

escape response. Interestingly, Harden Jones (1952) reported a similar behaviour in perch (*Perca fluviatilis*) that was effected when the pressure was decreased by approximately 30 % of that to which the fish were adapted (i.e., neutrally buoyant). However, the "nosing" behaviour occurred during both compression and decompression cycles, and unlike the perch in Harden Jones' study (1952), all of the bloater were negatively buoyant.

The inverse relationship between the number of swim-ups and scored vertical movements in the 4 bloater that survived throughout the whole experiment is more difficult to understand, but it may be that these fish were allocating their energy to adjusting their physiology versus swimming. However, this is uncertain, and it is evident that the decreased number of swim-ups with increasing pressure may have been caused in part by the response of increasing pressure, which was suggested in bloater number 2 and number 9 (Appendix III). These two fish effected the majority of their swim-ups within the first two to three pressure increases (50-40 % compression), and then either remained on the bottom (fish no. 2), or in the water column (fish no. 9). The duration and number of swim-ups in bloater number 6 were quite variable, and are indicative of the reactions of the 10 bloater as a whole. It is interesting to note that bloater number 11 responded to compression quite differently from bloater number 9 by remaining on the bottom for almost the entirety of the pressure cycle, and that these two fish were the only ones to survive for longer than a day (5 months) the effects of the pressure cycle (Figure 24). It appeared as though some of the swim-ups were influenced by interactions with other fish within the chamber, and more work needs to be done to determine whether there is a difference in running the experiment with individual or groups of bloater.

The considerable variation in both the number and duration of swim-ups shows a general

lack of uniform reaction to pressure changes among bloater, and may be indicative of individual variation in physiological fitness (Harden Jones & Scholes, 1985; Reidy *et al.*, 2000). It is interesting to note that only a few of the plaice (*Pleuronectes platessa*) that Gibson (Gibson, 1982) subjected to pressure showed behavioural responses. Furthermore, these behavioural responses lagged behind the pressure change (Gibson, 1982). Therefore, it would seem that the excitability and abnormal behaviours of fishes subjected to pressure (Sebert, 1997), combined with a time lag in the behavioural (Gibson, 1982) and physiological response (i.e., gas secretion) (Harden Jones & Scholes, 1985; Arnold & Greer Walker, 1992; Bone *et al.*, 1995) could mask detectable patterns to stress at specific pressure intervals.

Further laboratory and field work is needed to determine whether the reactions of individual fish to pressure changes can explain the substantial variation in swimming activity of bloater. For example, TeWinkel and Fleischer (1999) reported that vertical migration in Lake Michigan varied by individual bloater, with some fish staying on the bottom while others were dispersed throughout 30 m of the water column.

Hydrodynamic lift generated by continuous swimming with a positive tilt angle was evident in the bloater, particularly in the swim-ups that lasted for several seconds to several minutes. In some cases, bloater remained in the water column of the pressure chamber, regardless of continued pressure increases (e.g., bloater no. 9; see Appendix III). Gallepp & Magnuson, 1972 (1972) summarized earlier experiments on the behaviour of negatively buoyant (i.e., tendency to sink) physostomous fishes. They noted that physostomous fishes similarly reacted to increased density by adopting a positive tilt angle and using their caudal fin to propel them to the surface, where they could gulp air to inflate their gas bladder (Gallepp & Magnuson, 1972).

Similarly, Sebert (1997) notes that pressure increases up to 20 atmospheres caused few reactions other than swimming upward.

The rate and efficiency with which bloater can compensate for increased hydrostatic pressure by secreting gas into their gas bladder is uncertain, but probably occurs on a longer scale of time than I used. Because regulation of the gas bladder volume is slow, it will necessarily lag behind the vertical movements of a fish (Harden Jones & Scholes, 1985; Arnold & Greer Walker, 1992; Bone *et al.*, 1995). A scale of days to weeks for small pressure changes may be more effective in studying the gas secretion abilities of bloater (see section 2 and below). Therefore, bloater are probably quite reliant on the lift obtained from lipids (section 1) and swimming (section 3), and longer times at smaller pressure changes are necessary to be able to adjust their gas bladder volume. More work is needed to determine the rate at which bloater can secrete gas into their gas bladder and the external cues necessary to stimulate the fish to do so.

Are captive-raised bloater useful in compression tests of large amplitude? It is questionable whether captive-raised bloater are useful as a surrogate of the behaviour of wild bloater, at least within the amplitude and rates that I used. For example, Todd *et al.* (1981) reported that a number (9) of morphological characters in bloater were influenced by being raised in captivity. Furthermore, morphological differences between parents and progeny in bloater were greater than differences between species of other deepwater ciscoes. Therefore, if the gas bladder anatomy and physiology of captive-raised bloater is similarly affected in captive-raised bloater, extrapolations of my findings to wild bloater should be done with caution.

In fact, it does appear as though the gas bladder anatomy (and probably physiology) of captive-raised bloater was different from that of wild fish. For example, in bloater of similar

length, it was obvious that the gas bladder of captive-raised individuals was translucent in comparison with Great Lakes bloater caught at a depth of 80 m (9 atm) (see section 2). The silvery, opaque appearance of the gas bladder in wild bloater is ostensibly caused by the presence of guanine in the external layer of the gas bladder (Fange, 1983; Blaxter & Batty, 1984) (see section 2). The presence of guanine is directly related to preventing gases from diffusing outward from the gas bladder, a process which is driven by gas partial pressures (Blaxter & Batty, 1984). Therefore, the ability of captive-raised bloater to retain gas within their bladder or to replace losses by diffusion while subjected to pressure increases may be different from that in wild bloater. However, more work is needed to determine the ability of bloater to both secrete and retain gas in their gas bladder.

Nevertheless, continued work on the adaptability of captive-raised bloater to pressure changes is important, as evidenced by the interest in the reintroduction of the species to Lake Ontario (Baldwin, 1999). In addition, it is interesting to note that although pressure changes had deleterious effects on the majority of the bloater in my study, 2 individuals survived a simulated compression down to 57 m and back to ambient (Figure 24).

Does the experimental protocol that I used need to be redesigned? In light of the two foregoing points, and other studies, yes. Sebert (1997) reported that the reactions of fishes to pressure is dependent on the temperature, species, compression rate, and protocol.

Caulton and Hill (1973 & 1975), Ribbink and Hill (1979), and Harden Jones and Scholes (1985) used preliminary observations on their experimental fish to determine the optimal rate of pressure change without causing visible fatigue, stress or abnormal behaviour. However, their objectives were different–they wanted to estimate the secretion and resorption rates of gas to and

from the gas bladder, and not to impose a hypothetical vertical migration scheme, as I have done for bloater. In addition, the fish that they used were all physoclists (Caulton & Hill, 1973 & 1975; Ribbink & Hill, 1979; Harden Jones & Scholes, 1985). Bishai (1961 & 1963 – cited in Gordon, 1970) reported that physoclists took longer to adjust to compression and decompression than physostomes. However, Sebert (1997) reported that rainbow trout (*Oncorhynchus mykiss*) and goldfish (*Carassius auratus*) (both shallow water physostomes) could survive maximum compression amplitudes of over 1,000 m (100 atm), provided that compression and decompression rates were slow (e.g., 21 days).

Caulton and Hill (1973 & 1975), Ribbink and Hill (1979), and Harden Jones and Scholes (1985) all used lower amplitudes for pressure increase and decrease and also increased the pressure in smaller steps over a longer period of time. However, Bishai (1961 & 1963 – cited in Gordon, 1970) subjected both physoclists and physostomes to compressions of 2 atm within 1-2 minutes. Five (5) days later, Bishai (1961 & 1963 – cited in Gordon, 1970) decreased the pressure to ambient within 1-35 minutes with few mortalities.

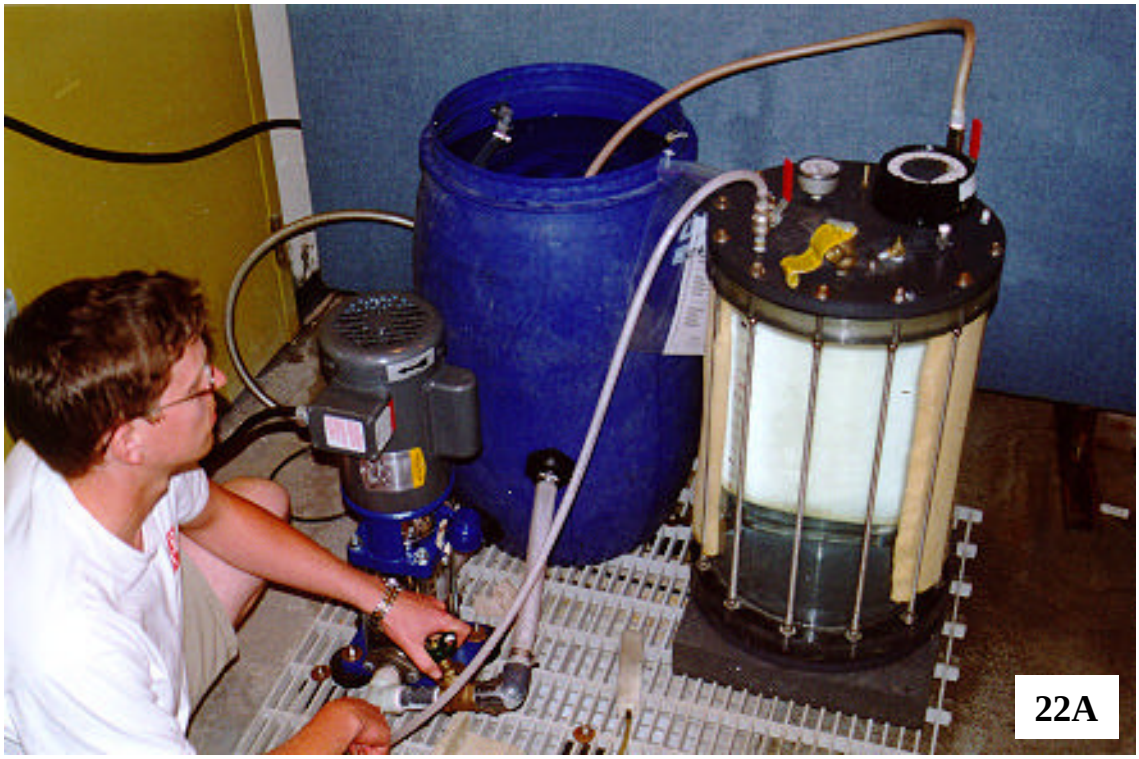
Longer periods (days to weeks) at lower pressure amplitudes are needed in order to estimate gas secretion abilities of bloater (see also section 2). On the other hand, longer periods (days to weeks) in a pressure chamber would mean designing a system in which the experimenter could add food and remove feces from the pressure chamber; both are logistically difficult. The large variability in the number and duration of swim-ups by individual bloater is probably the product of many interacting factors that may become more pronounced with the pressure increases of an imposed vertical migration protocol, especially for captive fish subjected to pressure (I am unaware of any studies that have examined differences in adaptability to pressure

between captive-raised and wild fish).

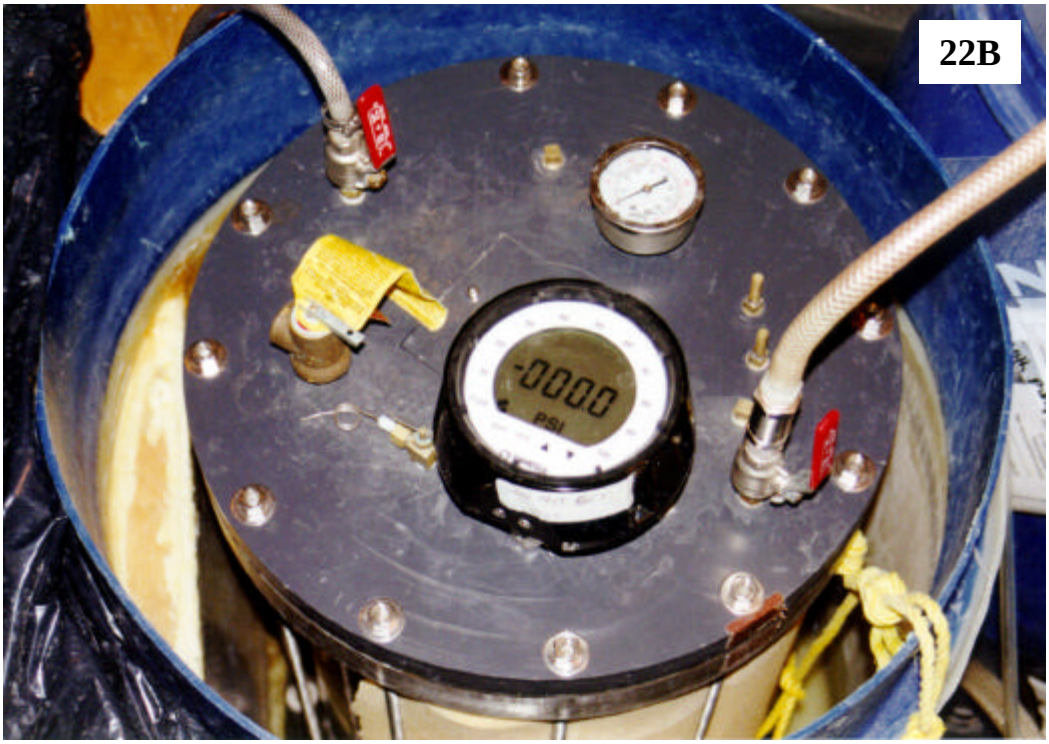
Pressure increases past a certain optimal range of amplitudes or rates may vary for individual fish, and thus effect their behaviour and/or survivability (Harden Jones & Scholes, 1985). The excitability and abnormal behaviours of fishes subjected to pressure, combined with a time lag in the behavioural and physiological response (i.e., gas secretion) can mask detectable patterns to stress at specific pressure intervals (Gibson, 1982; Sebert, 1997).

More work is needed on the effects of pressure on the behaviour of bloater before conclusions can be drawn. Three tentative conclusions can be gleaned from my study. One, it would appear that pressure effects the behaviour and survivability of individual bloater differently. Two, the first few initial increases in pressure may cause the most stress on the fish. Three, it does not appear that bloater would undergo a pressure change of 5.5 atm increase and decrease over 2 consecutive days. However, more research is needed in order to refute or confirm this latter claim.

Figure 22. A. Pressure system. Clockwise, from bottom left: pump with valve to change pressure, aerated reservoir (blue), and the pressure chamber. Not shown: cooling reservoir (into which the chamber is positioned) with a viewing window. **B.** Top-view of the lid of the pressure chamber (chamber is seated inside cooling reservoir). Note the analog (top) and digital pressure scales (middle). The inflow from the pump is at the top left, and the outflow from the chamber is at the right.



22A



22B

Figure 23. Schematic of the pressure system. **A.** Aerated reservoir of water. **B.** Pump with valve for pressure change. Arrows indicate the direction of flow of water. **C.** Pressure chamber seated inside cooling reservoir.

Figure 5.2

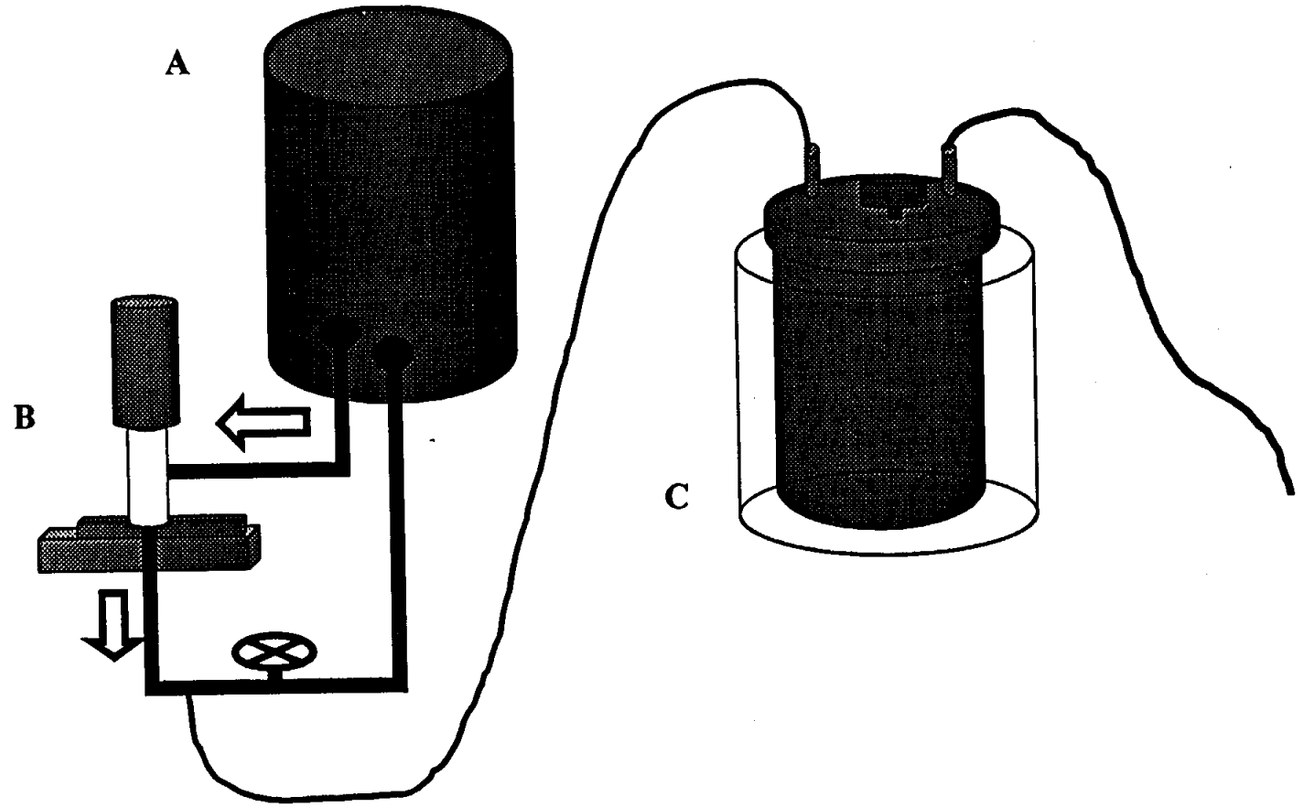
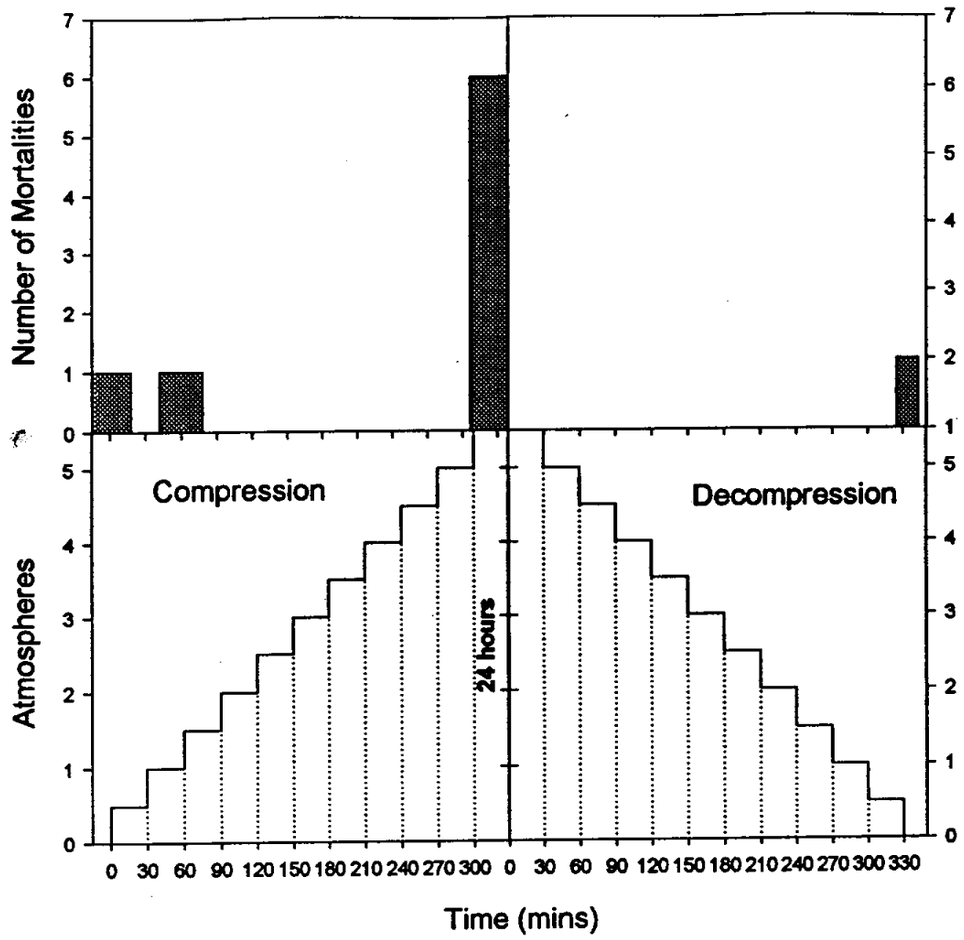


Figure 24. Mortalities in bloater subjected to a series of pressure changes (30 minute intervals). The top graph shows the number of mortalities in relation to the pressure change (bottom graph).

Fish no.	**Mass (g)**	**TL (mm)**	**FL (mm)**	**when Died**
1	97.4	225	202	Acclimation
8	177.6	246	223	Compression to 2.0 atm
7	127.9	260	236	24 hrs max pressure
3	138	259	232	24 hrs max pressure
4	113.8	231	207	24 hrs max pressure
5	178.5	267	241	24 hrs max pressure
10	168.6	255	230	24 hrs max pressure
12	148.5	236	240	24 hrs max pressure
2	103.3	230	205	Within 1 day of decompression
6	88.0	221	198	Within 1 day of decompression
9	**110.1**	**226**	**202**	**Alive**
11	**159.6**	**260**	**236**	**Alive**


Number of Mortalities
Atmospheres
Compression
Decompression
24 hours
0 30 60 90 120 150 180 210 240 270 300 0 30 60 90 120 150 180 210 240 270 300 330
Time (mins)

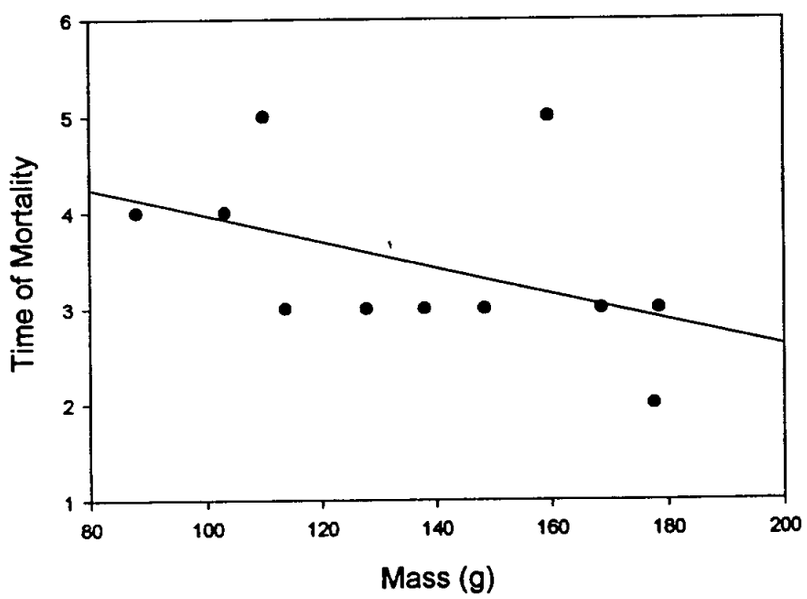

Time of Mortality
80 100 120 140 160 180 200
Mass (g)

Table 10. Comparison of ANOVA (n = 10) on pressure effects on each of the five measured variables; "trial" nested within pressure. There is a size effect for two of the variables. However, an increase in pressure did not have an affect on any of the five variables, regardless of whether size was used as a covariate or not.

	Pressure effect within each trial (no cov.)	**Pressure effect within each trial (size cov.)**	**Effect of Mass**	**Effect of Fork Length**
time start	F=0.88 p=0.663	F=0.97 p=0.538	**F=7.48 p=0.008**	**F=7.13 p=0.010**
duration of first	F=0.70 p=0.891	F=0.69 p=0.896	F=0.40 p=0.527	F=1.07 p=0.304
average duration	F=0.60 p=0.957	F=0.60 p=0.955	F=1.34 p=0.251	**F=16.47p=0.000**
number of swim-ups	F=1.22 p=0.235	F=1.21 p=0.247	F=0.36 p=0.552	F=0.59 p=0.446
total movements	F=1.15 p=0.300	F=1.14 p=0.316	F=0.23 p=0.633	F=0.51 p=0.477

Table 11. Comparison of ANOVA (n = 10) on trial effects on each of the five measured variables; "trial" nested within pressure. A size effect exists in two of the variables, and when used as a covariate, accounted for all of the variation in "time start". In the remaining four variables, the trial accounted for all of the variation, regardless of whether size was used as a covariate or not.

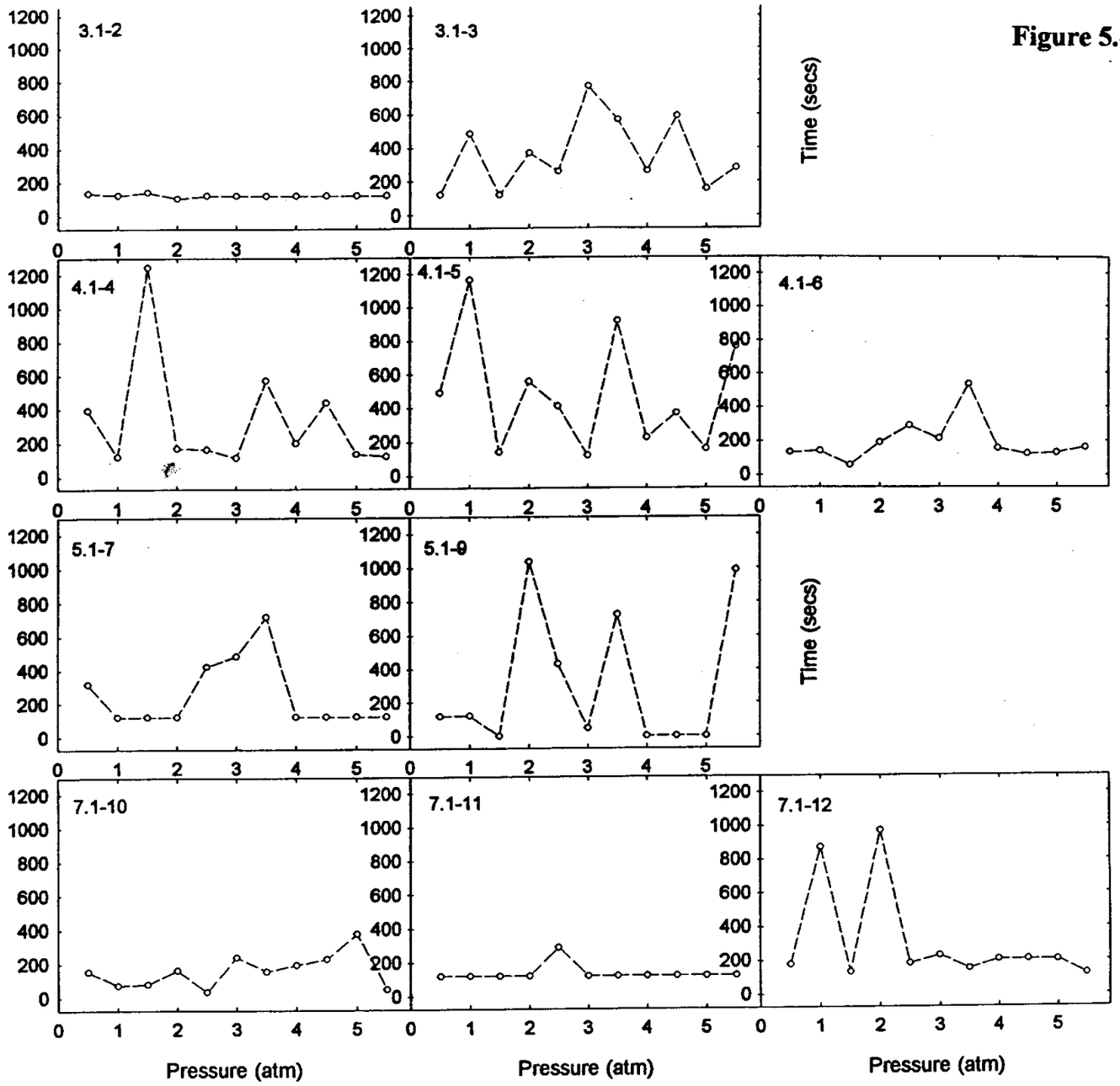
	Trial effect (no cov.)	**Trial effect (size cov.)**	**Effect of Mass**	**Effect of Fork Length**
time start	F=1.70 p=0.182	**F=3.67 p=0.018**	**F=7.48 p=0.008**	**F=7.13 p=0.010**
duration of first	**F=10.96 p=0.000**	**F=10.26 p=0.000**	F=0.40 p=0.527	F=1.07 p=0.304
average duration	**F=16.34 p=0.000**	**F=15.95 p=0.000**	F=1.34 p=0.251	**F=16.47 p=0.000**
number of swim-ups	**F=3.40 p=0.027**	**F=3.56 p=0.020**	F=0.36 p=0.552	F=0.59 p=0.446
total movements	**F=3.05 p=0.039**	**F=3.09 p=0.035**	F=0.23 p=0.633	F=0.51 p=0.477

Figure 25. Lapsed time (seconds) between the pressure increase and the first swim-up ("time start"). The trial and fish number are shown at the top left-hand corner. Each panel shows the responses of an individual fish. Each row of panels is for fish observed in the same trial. The bottom graph is the mean value for all fish for each pressure increase. Error bars are ±SEM.

Nested ANOVA (pressure effect nested within each trial) for lapsed time between the pressure change and the first swim-up ("time start") (Adjusted SS for Tests). Mass (g) used as a covariate.

Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**
mass	1	110119	479795	479795	**7.48**	**0.008**
Trial	3	686858	686858	228953	**3.67**	**0.018**
Pressure (Trial)	40	2481941	2481941	62049	0.97	0.538
Error	65	4171292	4171292	64174		
Total	109	7450209				

Figure 5.4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Time (secs)
Pressure (atm)

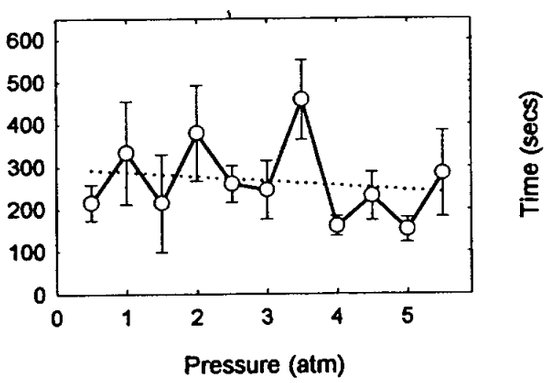
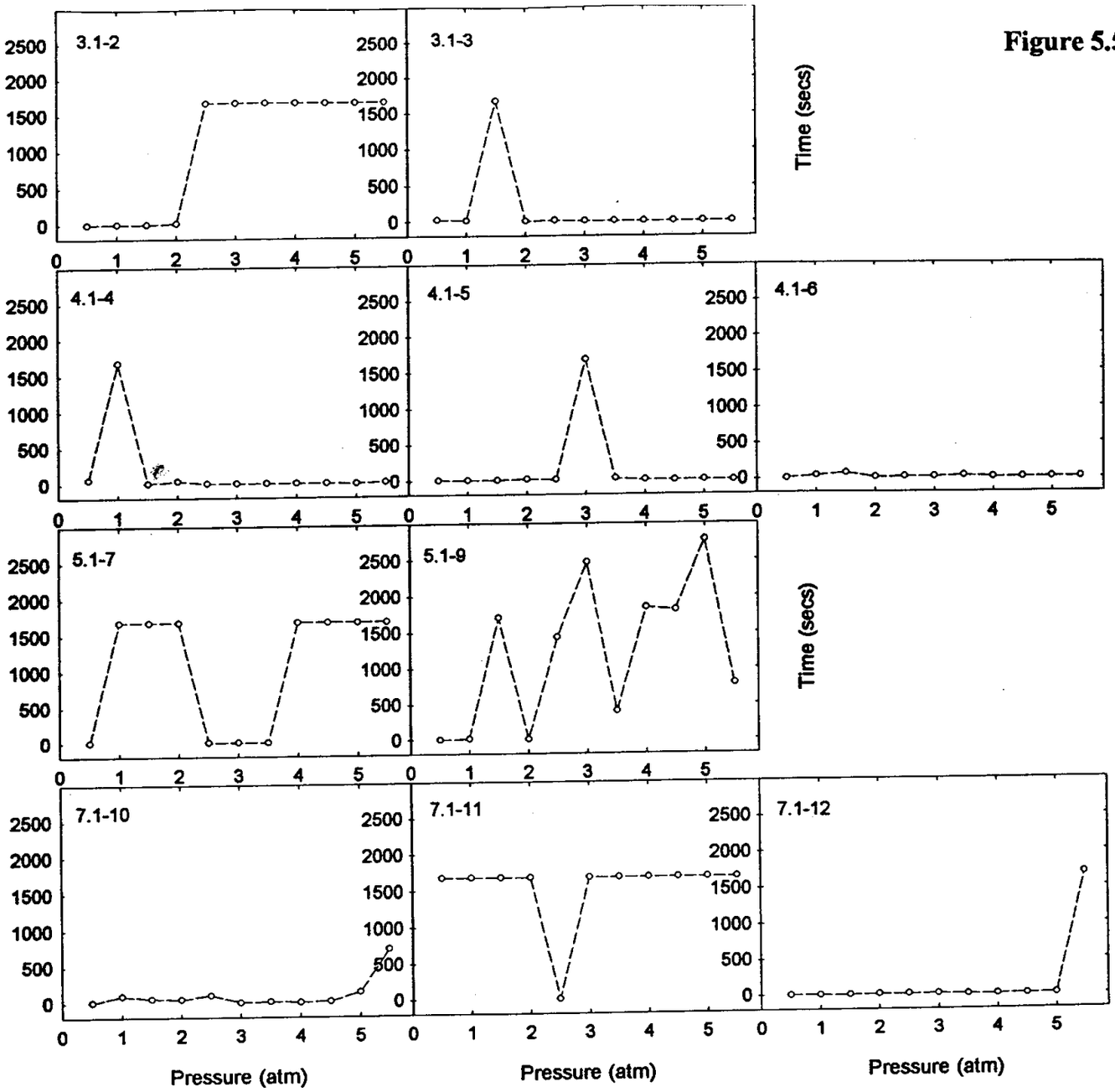

Time (secs)
Pressure (atm)

Figure 26. Duration of the initial swim-up following each pressure change ("duration of first"). The trial and fish number are shown at the top left-hand corner. Each panel shows the responses of an individual fish. Each row of panels is for fish observed in the same trial. The bottom graph is the mean value for all fish for each pressure increase. Error bars are ±SEM.

Nested ANOVA (pressure effect nested within each trial) for duration of the initial swim-up ("duration of first") following each pressure change (Adjusted SS for Tests).

Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**
Trial	3	13908730	13908730	4636243	**10.96**	**0.000**
Pressure (Trial)	40	16822461	16822461	420562	0.70	0.891
Error	66	39927280	39927280	604959		
Total	109	70658471				

Figure 5.5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Time (secs)
Pressure (atm)

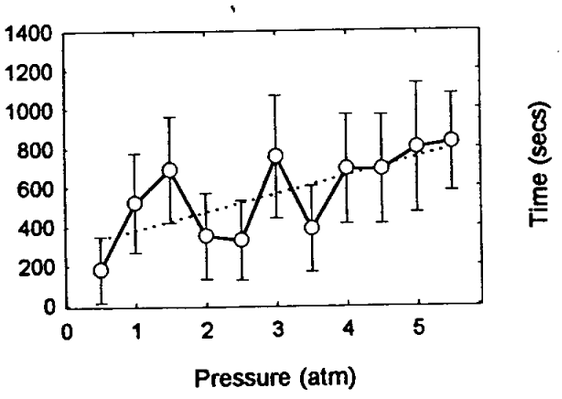
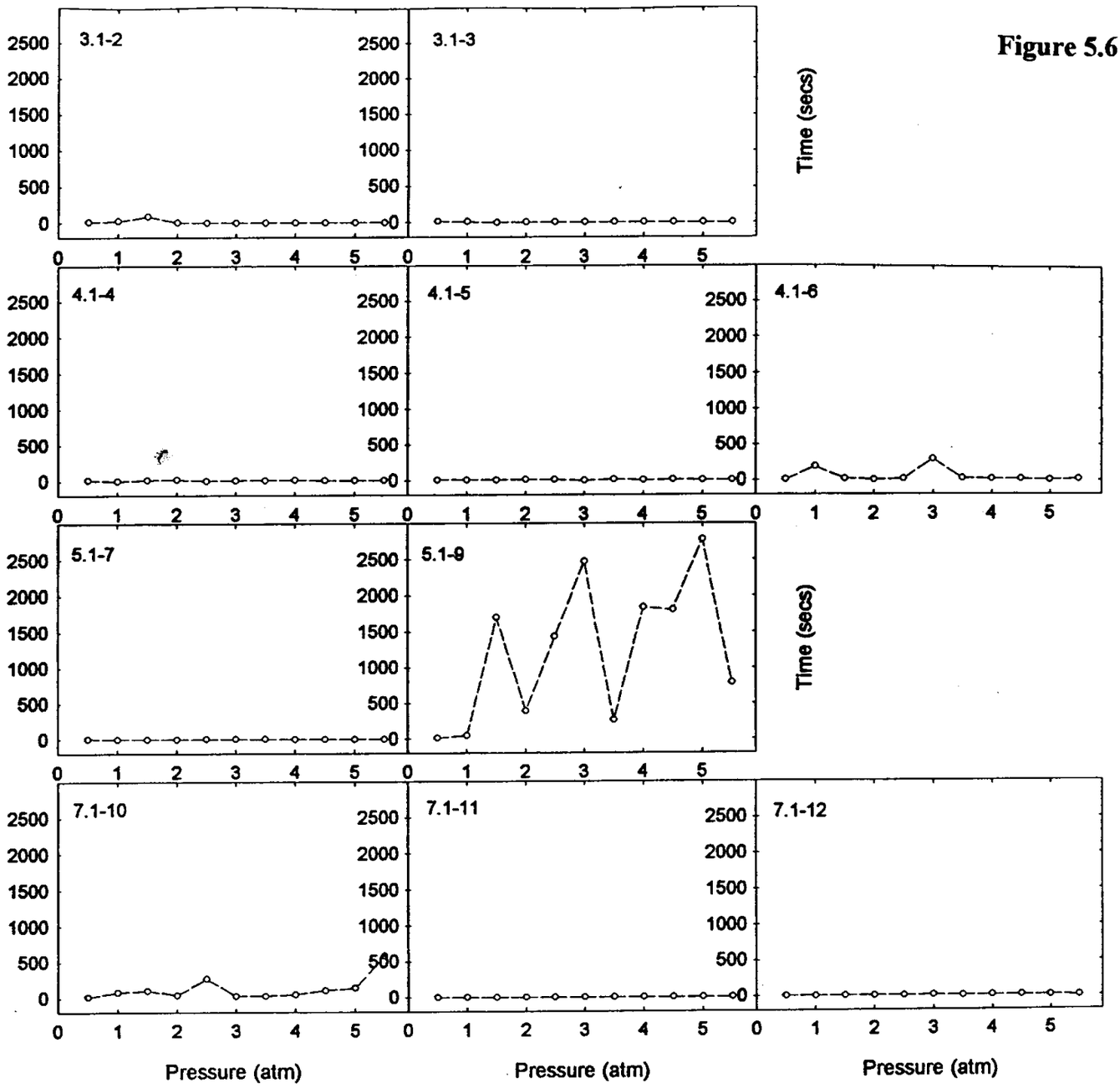

Time (secs)
Pressure (atm)

Figure 27. Average duration of time spent off the bottom. ("average duration"). The trial and fish number are shown at the top left-hand corner. Each panel shows the responses of an individual fish. Each row of panels is for fish observed in the same trial. The bottom graph is the mean value for all fish for each pressure increase. Error bars are ±SEM.

Nested ANOVA (pressure effect nested within each trial) for average duration of time spent off the bottom ("average duration") (using Adjusted SS for Tests).

Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**
Trial	3	6081138	6081138	2027046	**16.34**	**0.000**
Pressure (Trial)	40	4919477	4919477	122987	0.60	0.957
Error	66	13493737	13493737	204451		
Total	109	24494352				

Figure 5.6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7.1-12
Time (secs)
Pressure (atm)

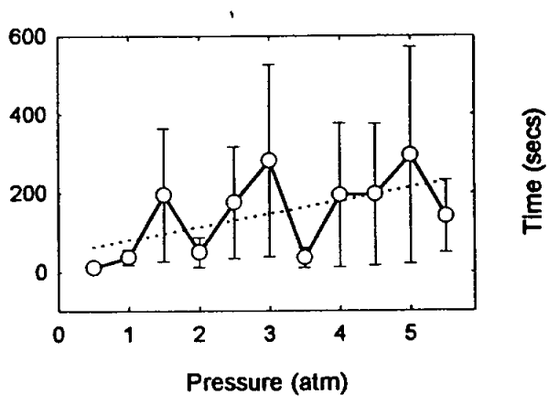
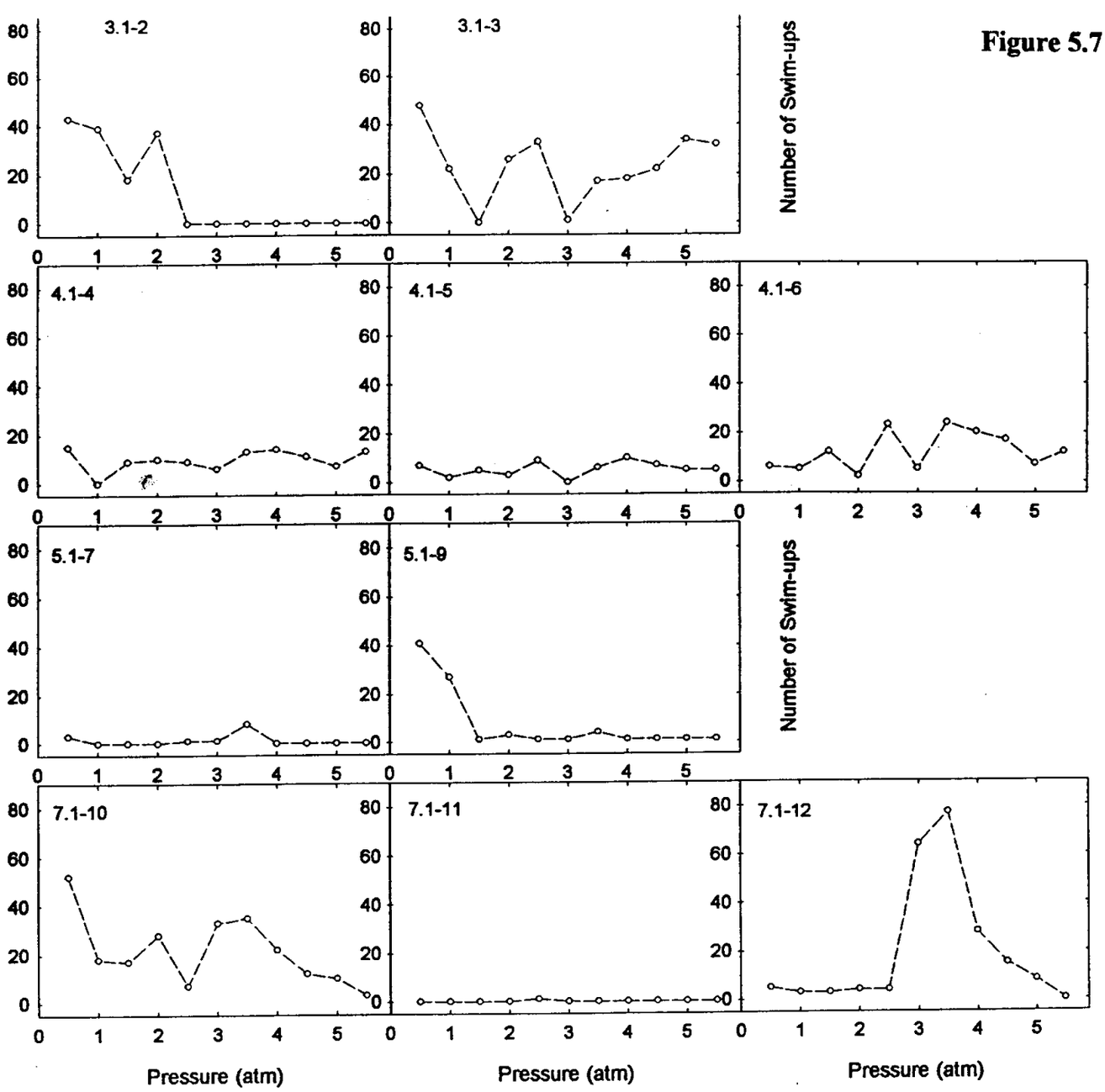

Time (secs)
Pressure (atm)

Figure 28. Number of swim-ups for each fish per each pressure increase. The trial and fish number are shown at the top left-hand corner. Each panel shows the responses of an individual fish. Each row of panels is for fish observed in the same trial. The bottom graph is the mean value for all fish for each pressure increase. Error bars are ±SEM.

Nested ANOVA (pressure effect nested within each trial) for the number of swim-ups (Adjusted SS for Tests).

Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**
Trial	3	2321.3	2321.3	773.8	**3.40**	**0.027**
Pressure (Trial)	40	9131.1	9131.1	228.3	1.22	0.235
Error	66	12363.8	12363.8	187.3		
Total	109	23816.2				

Figure 5.7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7.1-12
Number of Swim-ups
Pressure (atm)

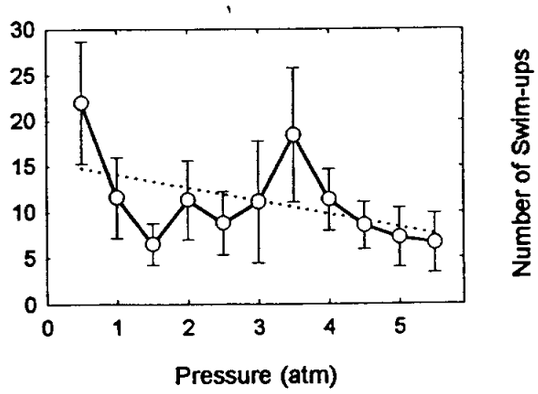
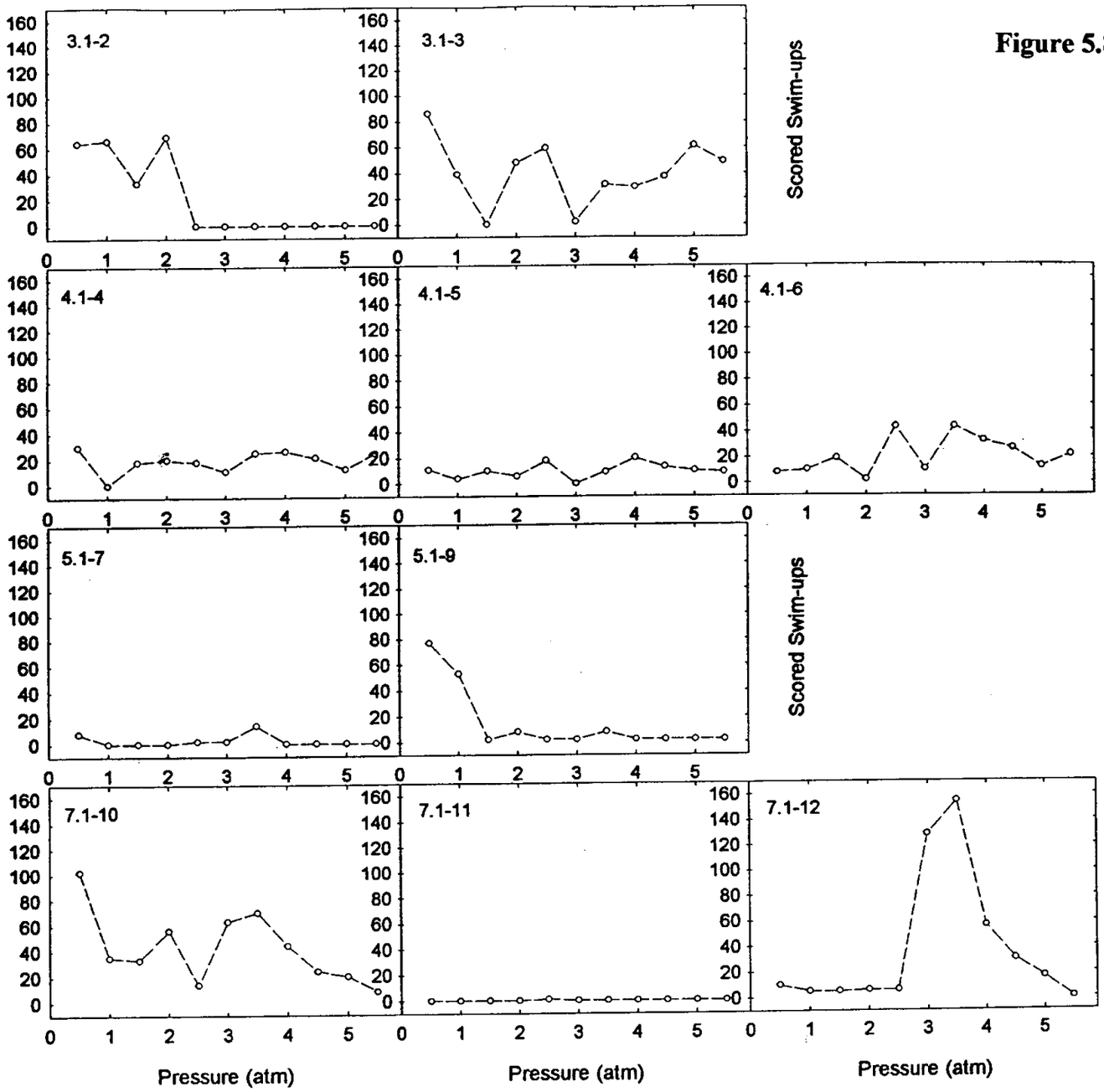

Number of Swim-ups
Pressure (atm)

Figure 29. Total number of vertical movements per each pressure increase ("total movements"). The trial and fish number are shown at the top left-hand corner. Each panel shows the responses of an individual fish. Each row of panels is for fish observed in the same trial. The bottom graph is the mean value for all fish for each pressure increase. Error bars are ±SEM.

Nested ANOVA (pressure effect nested within each trial) for scored vertical movements ("total movements") (Adjusted SS for Tests).

Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**
Trial	3	7309.9	7309.9	2436.6	**3.05**	**0.039**
Pressure (Trial)	40	31961.4	31961.4	799.0	1.15	0.300
Error	66	45765.5	45765.5	693.4		
Total	109	85036.8				

Figure 5.8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Scored Swim-ups
Scored Swim-ups
Pressure (atm)
Pressure (atm)
Pressure (atm)

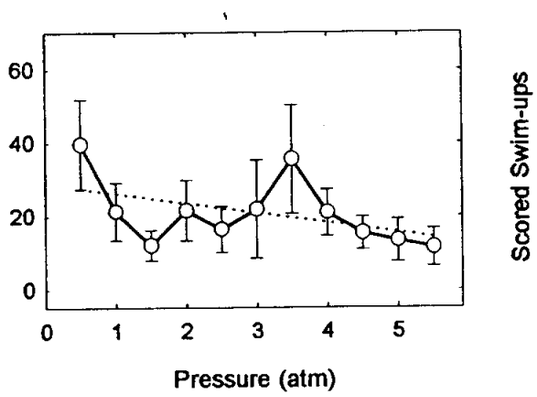

Scored Swim-ups
Pressure (atm)

Table 12. Comparison of ANOVA (n = 4; i.e., for the 4 fish that survived to the complete compression-decompression series of pressure changes) on pressure effects on each of five measured variables for bloater. Compression affected three of the five variables, while decompression only affected one of the variables. No variable was affected significantly in both compression and decompression.

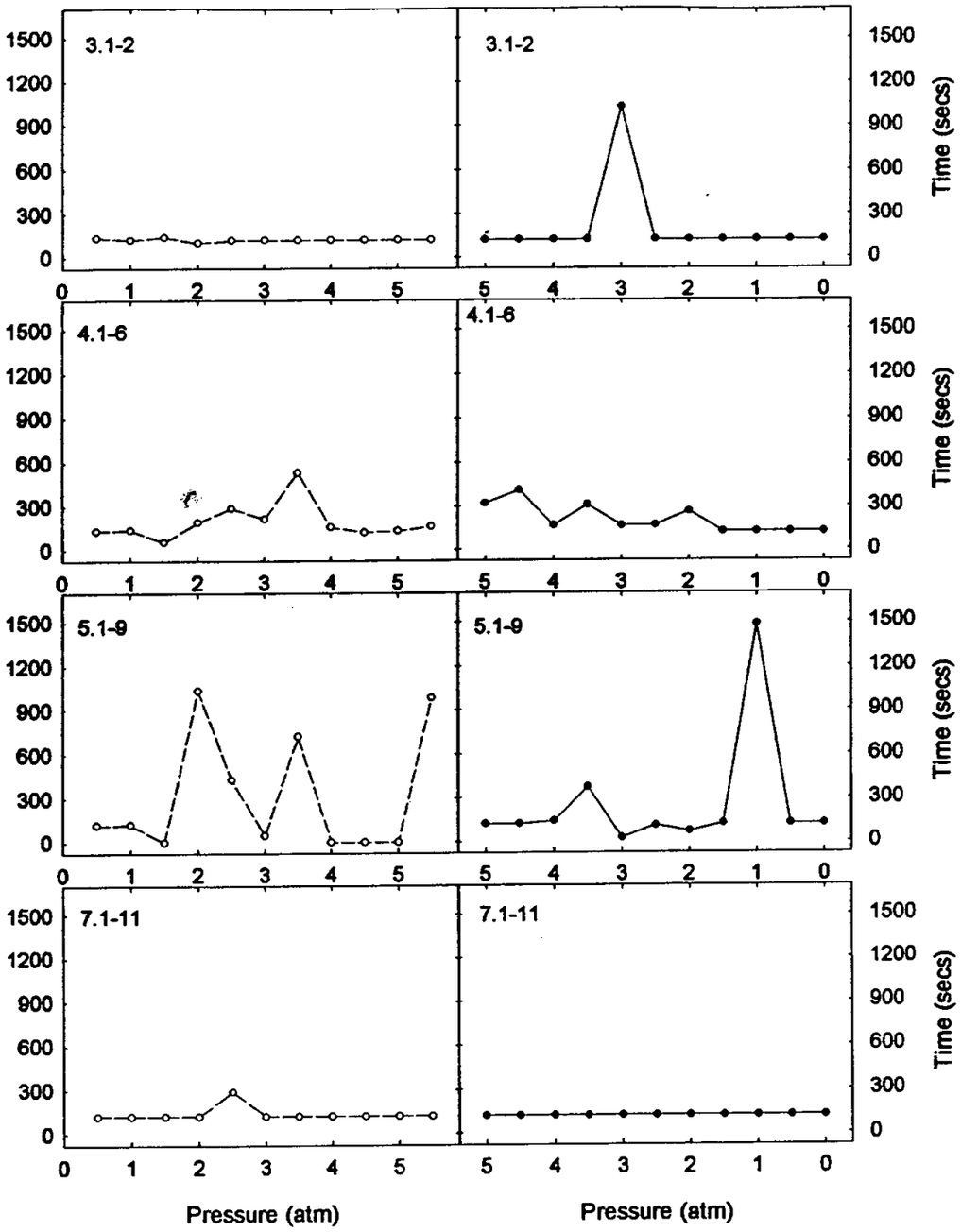
	Effect of Compression	**Effect of Decompression**
time start	F=0.23 p=0.635	F=0.00 p=0.984
duration of first	**F=5.83 p=0.020**	F=2.87 p=0.098
average duration	F=1.10 p=0.300	**F=5.33 p=0.026**
number of swim-ups	**F=8.51 p=0.006**	F=0.69 p=0.410
total movements	**F=8.55 p=0.006**	F=0.80 p=0.375

Figure 30. Lapsed time (seconds) between the pressure change and the first swim-up ("time start"). The trial and fish number are shown at the top left-hand corner. Each panel shows the responses of an individual fish. Each row of panels is for fish observed in the same trial. The bottom graph is the mean value for the 4 fish for each pressure change. Error bars are ±SEM.

ANOVA for elapsed time between the pressure change and the first swim-up ("time start"). Pressure changes did not affect this variable.

Compression	**DF**	**SS**	**MS**	**F**	**P**
Regression	1	11588	11588	0.23	0.635
Residual Error	42	2123063	50549		
Total	43	2134651			
Decompression	**DF**	**SS**	**MS**	**F**	**P**
Regression	1	27	27	0.00	0.984
Residual Error	42	2674712	63684		
Total	43	2674739			

Figure 5.9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5.1-9
5.1-9
7.1-11
7.1-11
Time (secs)
Pressure (atm)
Pressure (atm)

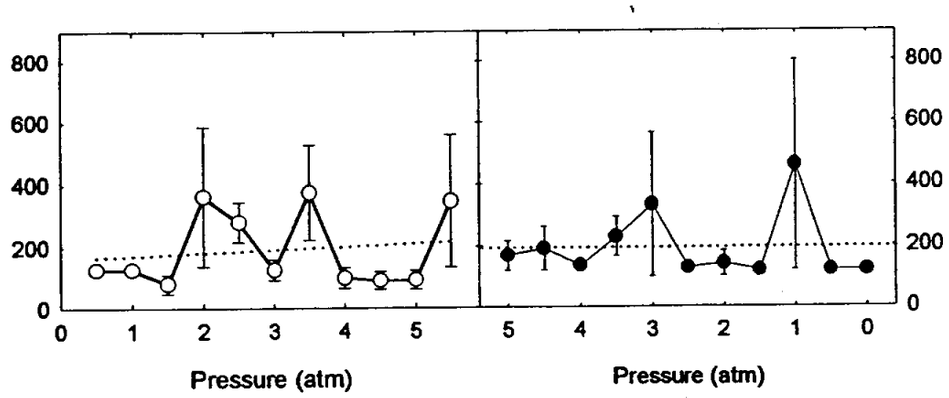
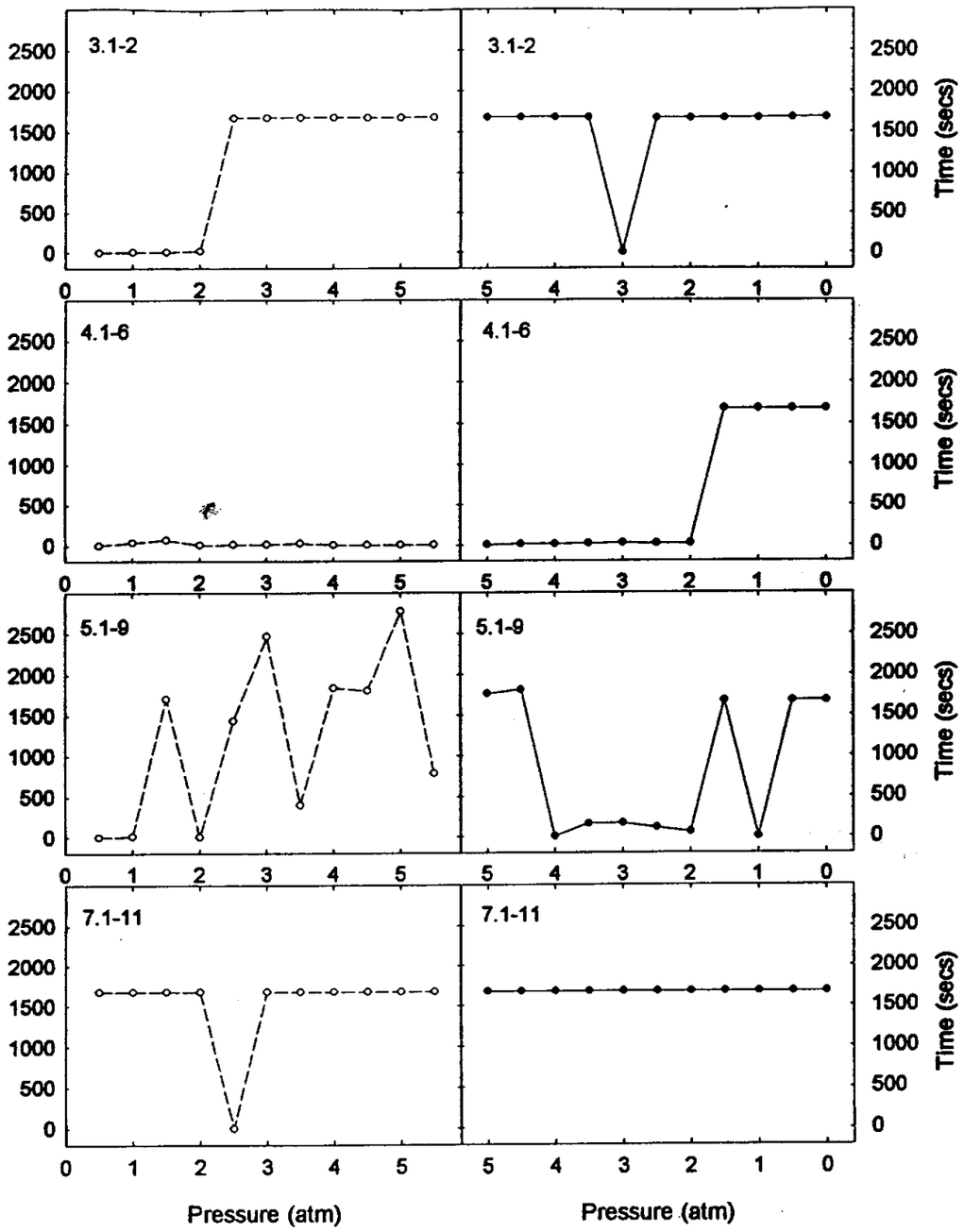

Pressure (atm)
Pressure (atm)

Figure 31. Duration of the initial swim-up following each pressure change ("duration first"). The trial and fish number are shown at the top left-hand corner. Each panel shows the responses of an individual fish. Each row of panels is for fish observed in the same trial. The bottom graph is the mean value for the 4 fish for each pressure change. Error bars are ±SEM.

ANOVA for duration of the initial swim-up ("duration first") following each pressure change. Compression did affect this variable.

Compression	**DF**	**SS**	**MS**	**F**	**P**
Regression	1	4145394	4145394	**5.83**	**0.020**
Residual Error	42	29873594	711276		
Total	43	34018988			
Decompression	**DF**	**SS**	**MS**	**F**	**P**
Regression	1	1649585	1649585	2.87	0.098
Residual Error	42	24123313	574365		
Total	43	25772898			

Figure 5.10


3.1-2
3.1-2
4.1-6
4.1-6
5.1-9
5.1-9
7.1-11
7.1-11
Time (secs)
Pressure (atm)
Pressure (atm)

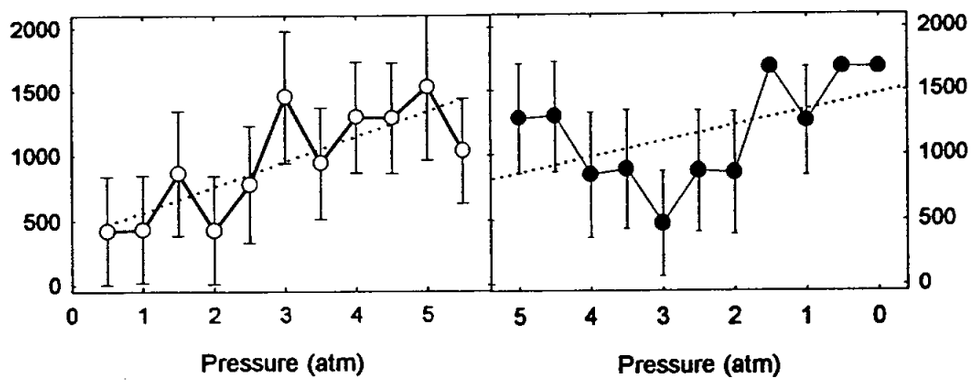
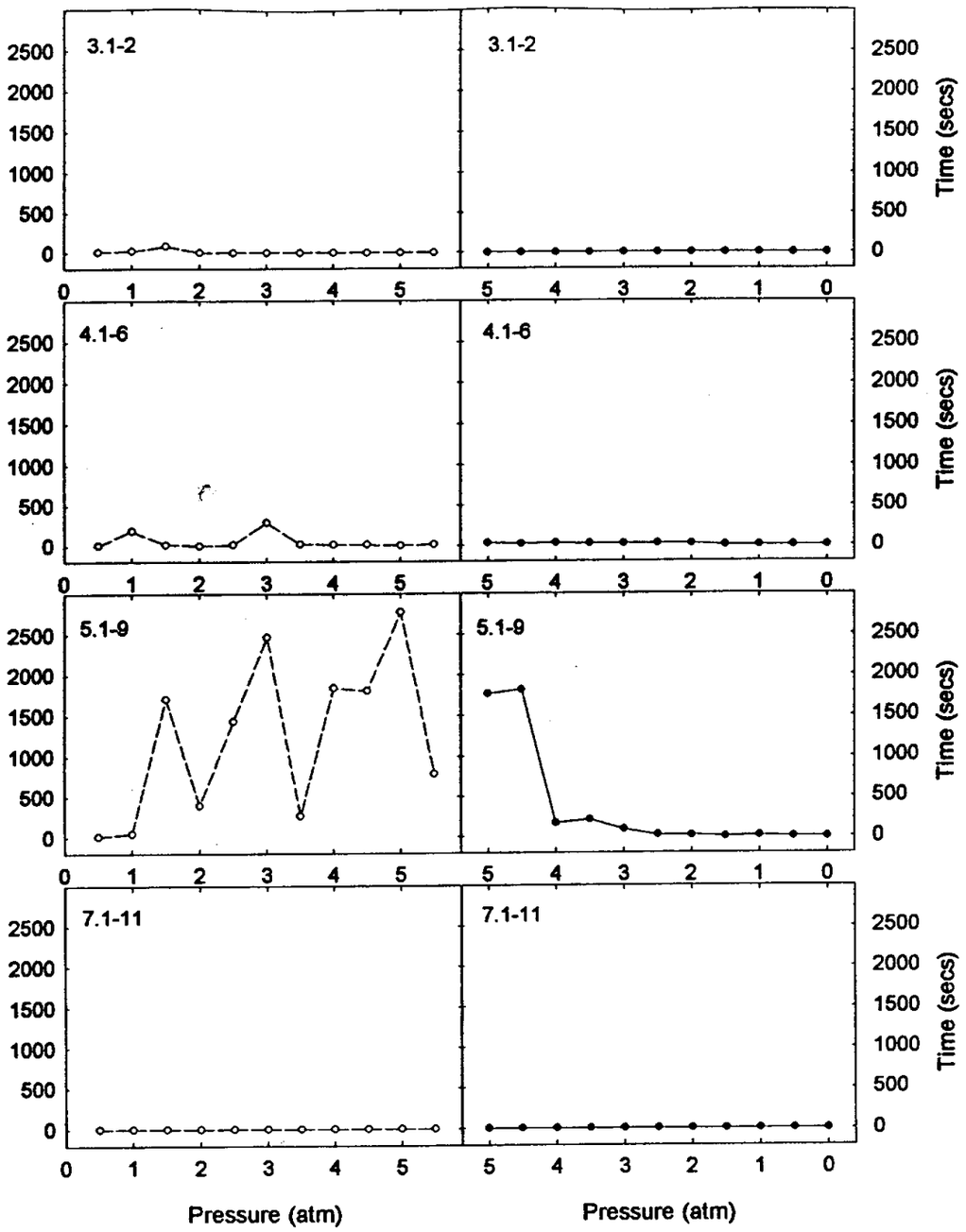

Pressure (atm)
Pressure (atm)

Figure 32. Average duration of time spent off the bottom ("average duration"). The trial and fish number are shown at the top left-hand corner. Each panel shows the responses of an individual fish. Each row of panels is for fish observed in the same trial. The bottom graph is the mean value for the 4 fish for each pressure change. Error bars are ±SEM.

ANOVA for average duration of time spent off the bottom ("average duration"). Decompression did affect this variable.

Compression	**DF**	**SS**	**MS**	**F**	**P**
Regression	1	554794	554794	1.10	0.300
Residual Error	42	21202738	504827		
Total	43	21757532			
Decompression	**DF**	**SS**	**MS**	**F**	**P**
Regression	1	685853	685853	**5.33**	**0.026**
Residual Error	42	5400677	128588		
Total	43	6086530			

Figure 5.11


3.1-2
3.1-2
4.1-6
4.1-6
5.1-9
5.1-9
7.1-11
7.1-11
Time (secs)
Pressure (atm)
Pressure (atm)

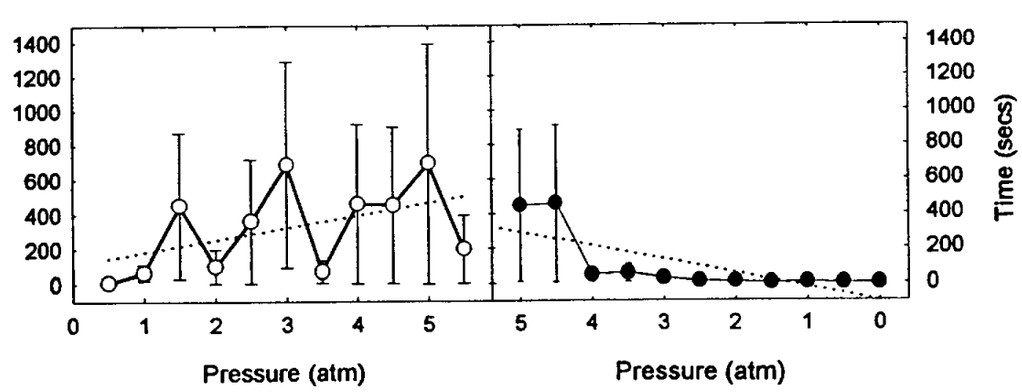
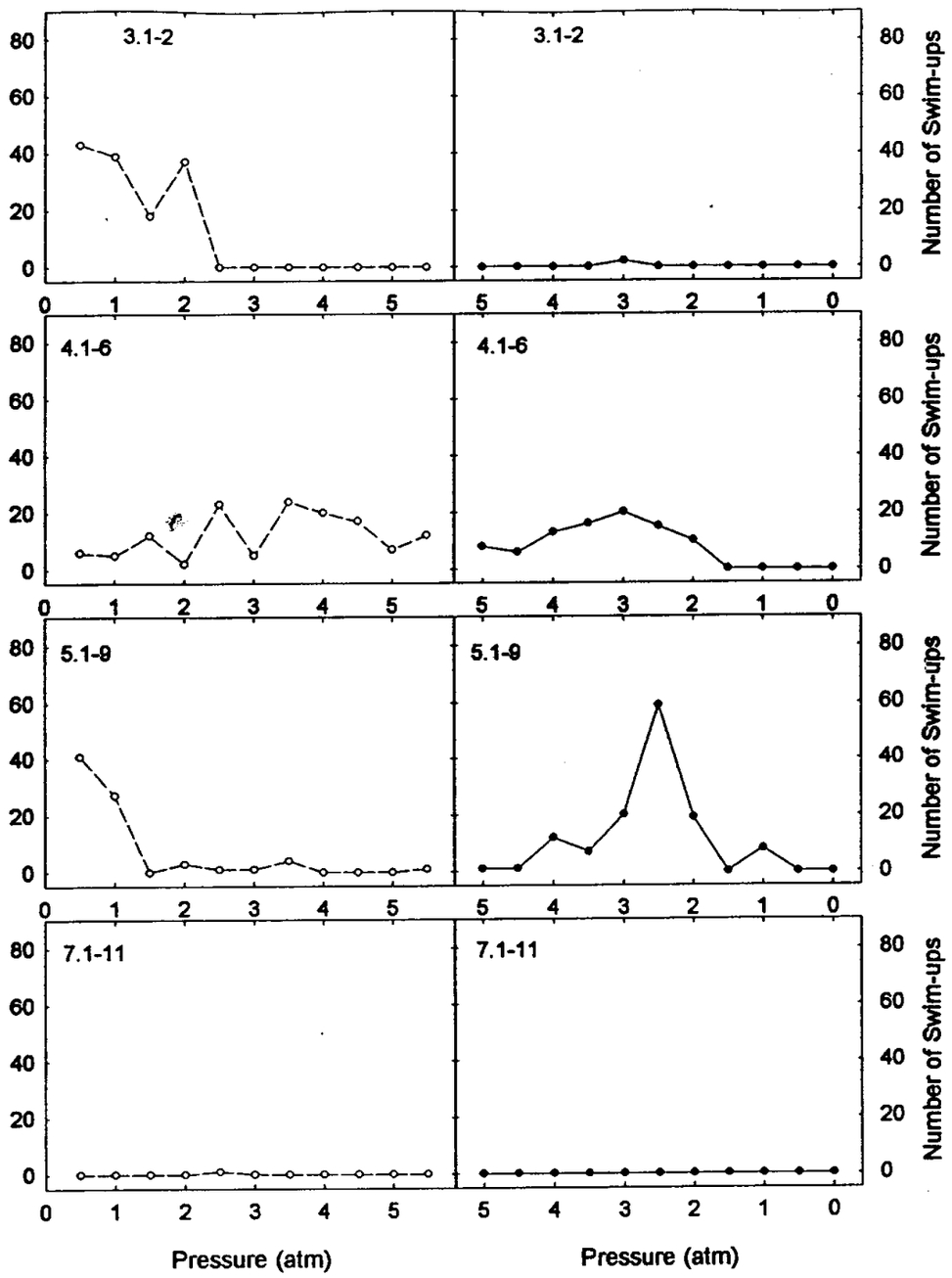

Time (secs)
Pressure (atm)
Pressure (atm)

Figure 33. Number of swim-ups. The trial and fish number are shown at the top left-hand corner. Each panel shows the responses of an individual fish. Each row of panels is for fish observed in the same trial. The bottom graph is the mean value for the 4 fish for each pressure change. Error bars are ±SEM.

ANOVA for the number of swim-ups. Compression did affect this variable.

Compression	**DF**	**SS**	**MS**	**F**	**P**
Regression	1	1161.9	1161.9	**8.51**	**0.006**
Residual Error	42	5733.1	136.5		
Total	43	6895.0			
Decompression	**DF**	**SS**	**MS**	**F**	**P**
Regression	1	76.1	76.1	0.69	0.410
Residual Error	42	4608.7	109.7		
Total	43	4684.8			

Figure 5.12


3.1-2
3.1-2
4.1-6
4.1-6
5.1-9
5.1-9
7.1-11
7.1-11
Number of Swim-ups
Pressure (atm)
Pressure (atm)

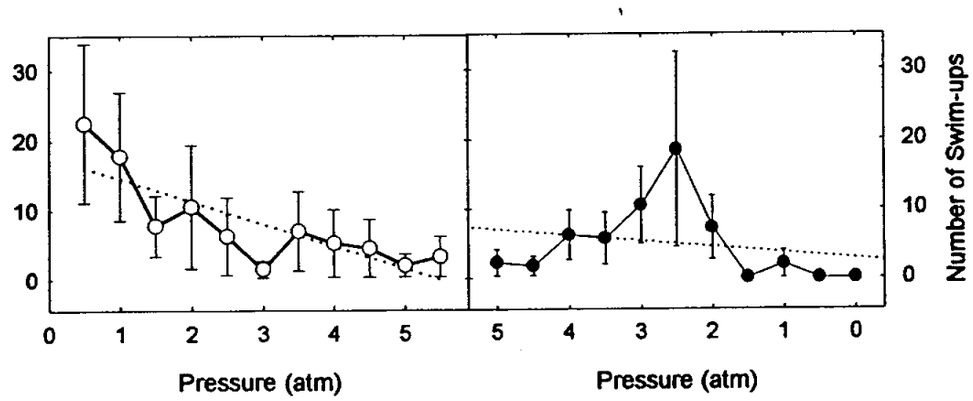
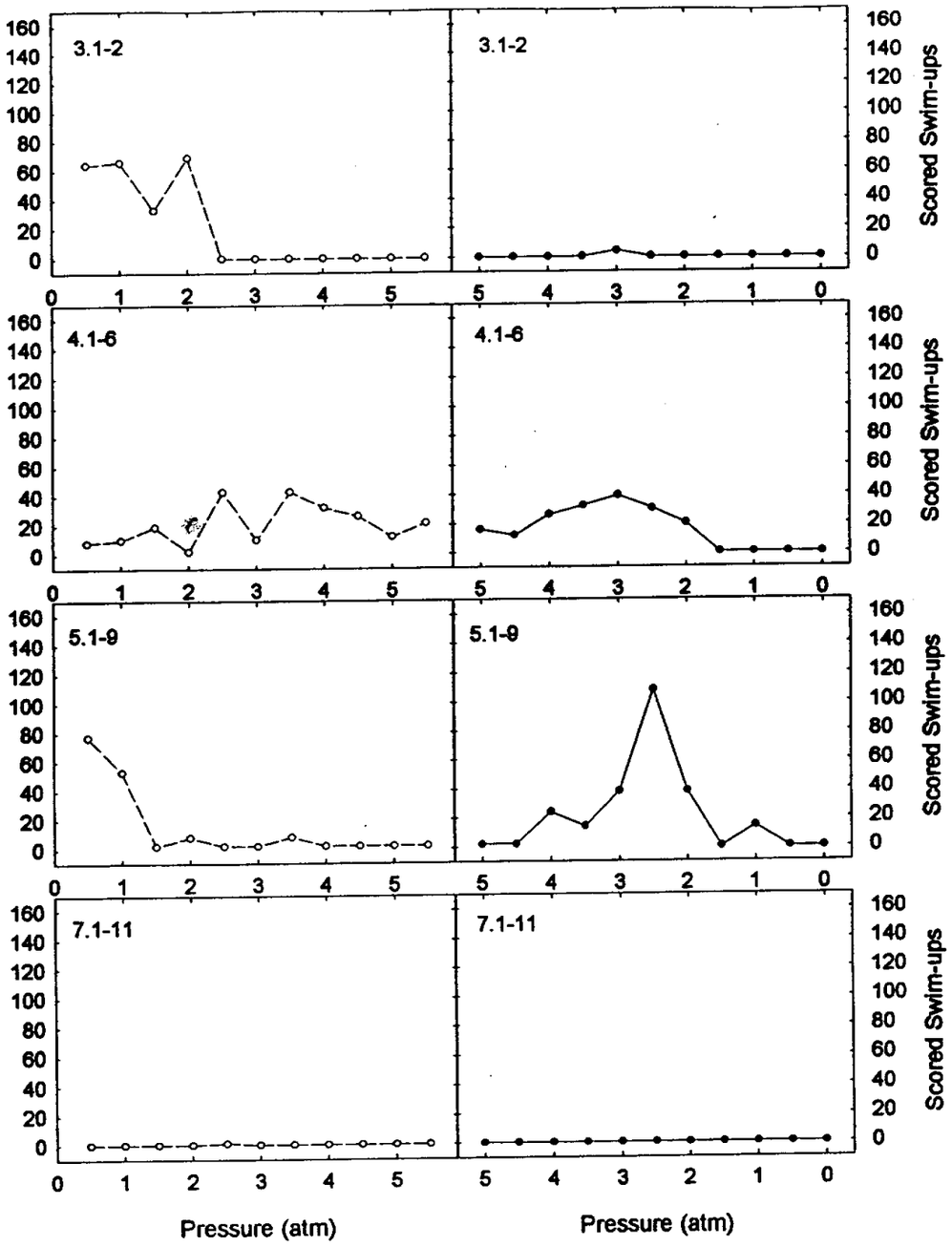

Number of Swim-ups
Pressure (atm)
Pressure (atm)

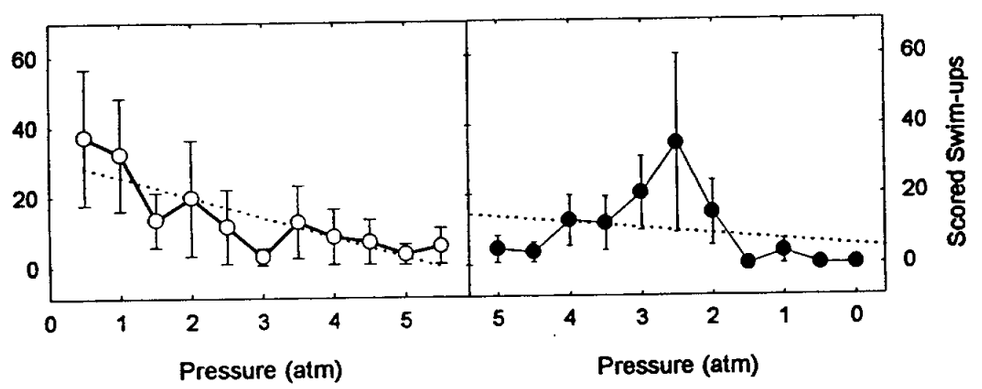
Figure 34. Total number of scored vertical movements per each pressure change ("total movements"). The trial and fish number are shown at the top left-hand corner. Each panel shows the responses of an individual fish. Each row of panels is for fish observed in the same trial. The bottom graph is the mean value for the 4 fish for each pressure change. Error bars are ±SEM.

ANOVA for scored vertical movements ("total movements"). Compression did affect this variable.

Compression	**DF**	**SS**	**MS**	**F**	**P**
Regression	1	3568.2	3568.2	**8.55**	**0.006**
Residual Error	42	17532.6	417.4		
Total	43	21100.8			
Decompression	**DF**	**SS**	**MS**	**F**	**P**
Regression	1	309.5	309.5	0.80	0.375
Residual Error	42	16165.5	384.9		
Total	43	16475.0			

Figure 5.13


3.1-2
3.1-2
4.1-6
4.1-6
5.1-9
5.1-9
7.1-11
7.1-11
Scored Swim-ups
Pressure (atm)
Pressure (atm)


Scored Swim-ups
Pressure (atm)
Pressure (atm)

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Appendices

Appendix I Flotation Pressure Data

Study	depth	FISH F.P.	ENV atm	%Diff. Buoy	Buoy. Status	depth of buoy	atm of buoy.
1953	18.4	2.1	2.8	-24	<(-)	11	2.1
1953	22.5	3.7	3.2	16	>(+)	28	3.7
1953	22.8	3.1	3.2	-3	<(-)	22	3.1
1953	23.0	4.2	3.2	29	>(+)	33	4.2
1953	23.3	3.5	3.3	7	>(+)	26	3.5
1953	23.4	2.7	3.3	-18	<(-)	17	2.7
1953	23.8	4.3	3.3	29	>(+)	34	4.3
1953	23.9	3.7	3.3	12	>(+)	28	3.7
1953	24.1	4.5	3.3	36	>(+)	36	4.5
1953	24.1	3.9	3.3	18	>(+)	30	3.9
1953	24.4	3.0	3.4	-10	<(-)	21	3.0
1953	24.4	4.1	3.4	21	>(+)	32	4.1
1953	35.7	5.1	4.5	15	>(+)	43	5.1
1953	38.3	6.1	4.7	30	>(+)	53	6.1
1953	38.3	4.7	4.7	0	>(+)	38	4.7
1953	38.3	4.5	4.7	-4	>(+)	37	4.5
1953	38.3	5.9	4.7	25	>(+)	50	5.9
1953	38.4	4.4	4.7	-7	<(-)	35	4.4
1953	38.4	4.2	4.7	-11	<(-)	33	4.2
1953	38.4	3.6	4.7	-23	<(-)	27	3.6
1953	38.5	5.7	4.7	20	>(+)	48	5.7
1953	38.6	5.0	4.7	6	>(+)	41	5.0
1953	39.6	5.9	4.8	21	>(+)	50	5.9
1953	41.4	6.1	5.0	21	>(+)	52	6.1
1953	41.5	5.7	5.0	13	>(+)	48	5.7
1953	41.8	4.6	5.0	-9	<(-)	37	4.6
1953	45.3	8.3	5.4	54	>(+)	76	8.3
1953	45.6	8.0	5.4	48	>(+)	72	8.0
1953	45.8	7.7	5.4	41	>(+)	69	7.7
1953	45.4	5.2	5.4	-4	<(-)	43	5.2
1953	45.6	7.0	5.4	29	>(+)	62	7.0
1953	45.9	6.7	5.4	23	>(+)	59	6.7
1953	46.1	8.2	5.5	50	>(+)	74	8.2
1953	46.0	4.7	5.5	-14	<(-)	38	4.7
1953	46.1	7.2	5.5	32	>(+)	64	7.2
1953	46.2	6.1	5.5	11	>(+)	52	6.1
1953	46.2	5.4	5.5	-2	<(-)	45	5.4
1953	46.4	6.3	5.5	15	>(+)	55	6.3
1953	46.7	5.9	5.5	6	>(+)	50	5.9
1953	46.7	7.1	5.5	28	>(+)	63	7.1
1953	46.7	7.1	5.5	28	>(+)	63	7.1
1953	46.9	7.6	5.5	37	>(+)	68	7.6
1953	47.0	5.2	5.6	-6	<(-)	43	5.2
1953	47.0	5.6	5.6	1	>(+)	47	5.6
1953	47.2	6.9	5.6	23	>(+)	60	6.9
1953	47.5	5.4	5.6	-3	<(-)	46	5.4
2002	55.4	6.3	6.4	-1	<(-)	55	6.3
2002	67	8.3	7.5	10	> (+)	75	8.3
2002	67.5	11.2	7.5	49	> (+)	106	11.2
2002	67.5	6.7	7.5	-12	< (-)	59	6.7

Appendix I (continued)

Study	depth	FISH F.P.	ENV atm	%Diff. Buoy	Buoy. Status	depth of buoy	atm of buoy.
2002	68.2	8.2	7.6	8	> (+)	74	8.2
2002	69.1	10.4	7.7	35	> (+)	97	10.4
2002	69.1	10.0	7.7	30	> (+)	93	10.0
2002	69.1	8.8	7.7	15	> (+)	81	8.8
2002	69.1	8.8	7.7	14	> (+)	80	8.8
2002	69.1	8.4	7.7	9	> (+)	76	8.4
2002	70.1	11.8	7.8	51	> (+)	111	11.8
2002	70.1	10.0	7.8	28	> (+)	93	10.0
2002	70.1	10.0	7.8	28	> (+)	93	10.0
2002	70.1	9.1	7.8	17	> (+)	84	9.1
2002	70.1	9.1	7.8	17	> (+)	84	9.1
2002	70.1	8.1	7.8	4	> (+)	74	8.1
2002	70.1	8.1	7.8	4	> (+)	73	8.1
2002	70.1	7.9	7.8	1	> (+)	71	7.9
2002	70.1	6.8	7.8	-12	< (-)	60	6.8
2002	70.1	5.6	7.8	-28	< (-)	48	5.6
2002	70.1	5.6	7.8	-29	< (-)	47	5.6
2002	70.4	9.7	7.8	24	> (+)	90	9.7
1953	74.6	9.2	8.2	12	>(+)	85	9.2
1953	74.7	11.7	8.2	42	>(+)	111	11.7
1953	74.8	10.0	8.2	22	>(+)	93	10.0
1953	74.8	9.5	8.2	15	>(+)	87	9.5
1953	74.8	8.9	8.2	8	>(+)	82	8.9
1953	74.8	10.7	8.2	30	>(+)	100	10.7
1953	74.9	8.3	8.2	1	>(+)	75	8.3
1953	75.0	10.3	8.3	25	>(+)	96	10.3
1953	75.0	11.0	8.3	33	>(+)	103	11.0
1953	75.0	10.5	8.3	27	>(+)	98	10.5
1953	75.3	10.1	8.3	22	>(+)	94	10.1
1953	75.3	10.8	8.3	30	>(+)	101	10.8
1953	83.9	11.8	9.1	30	>(+)	112	11.8
1953	84.0	11.6	9.1	27	>(+)	109	11.6
1953	84.0	11.3	9.1	24	>(+)	106	11.3
1953	84.2	11.4	9.2	25	>(+)	108	11.4
1953	84.6	9.2	9.2	0	neutral	85	9.2
2002	97	10	10	-7	< (-)	93	10
2002	97	8	10	-27	< (-)	72	8
2002	97.3	12	10	17	> (+)	114	12

Appendix II

Preliminary Pressure Chamber Studies

During Spring 2000, I observed behavioural changes of bloater (age-2+) to changes in ambient pressure using a pressure chamber constructed specifically for this purpose. The objective was to change the pressure and measure the time taken for the fish to adjust to the new pressure (i.e., to regain neutral buoyancy --- time to secrete gas into the gas bladder) as evidenced by changes in behaviour. Experimentally it turned out to be more interesting to look at the changes after a decrease in pressure.

The plexiglass chamber is cylindrical with in inside diameter of 33 cm and a length of 60.5 cm. The ends are constructed of very thick PVC and the chamber is reinforced with steel rods. Pressure can be changed by introducing gas from a compressed cylinder into a compliant section within the enclosed chamber. However, the chamber is now set-up exclusively as a flow-through system (see Figures 22 & 23). The chamber was designed to tolerate and has been tested to a pressure 150 psi (about 10 atm).

Captive-raised bloater (n = 12) were subjected to instantaneous pressure changes of 1 atm (above ambient pressure) in the pressure chamber (50 % compression). Fish behaviour was recorded with a video camera a few minutes prior to pressure increase and decrease, and up to 30 minutes following pressure change.

Conditions were not kept constant in these trials. For most of the trials the pressure chamber was behind a partition to avoid disturbance from others in the laboratory, and for the last two trials the chamber was partially covered with insulating material to reduce heat exchange with surrounding air. The protocol was to place one bloater in the chamber and close and seal the chamber but permit a flow-through of water to maintain oxygen and temperature levels. Pressure was instantaneously increased, the fish was left to adjust to the new pressure for 2 to 22 hours, and then the pressure was instantaneously decreased. I was especially interested in observing the response to the sudden return to ambient pressure at the end of the trial. In three trials the pressure was increased using compressed air. In all of the remaining trials the pressure was adjusted using water pressure and maintaining flow throughout the trial.

Some of the bloater compensated for increased ambient pressure through a positive tilt angle (head up with respect to the horizontal) not unlike that of Atlantic mackerel (*Scomber scombrus*) reported by He and Wardle (1986). At least 7 of 12 captive bloaters compensated for decreased pressure by expelling bubbles from their mouth (see below). All captive bloaters compensated for decreased ambient pressure by active swimming at negative tilt angles (head down) similar to what Harden Jones (1952) found in *Perca fluviatilis*. In some cases in which the fish released a bubble, the release was preceded by a rapid shaking of the head. This sort of behaviour was also seen when the pressure was increased. In some cases, attempts to remove all of the bubbles from the chamber were not successful. When the pressure was increased, the fish would get a bubble from the chamber wall into its mouth and then it would rapidly shake its head. Presumably the shaking of the head is associated with moving the bubble into or out of the

pneumatic duct.

I have shown that bloaters can add and release gas to and from the bladder via the pneumatic duct (see Ch. 3). In light of my observations on captive bloater subjected to pressure in a chamber, expansion (i.e. bloating) in wild-caught bloater may be considered. One possibility may be that the threshold for "burping" gas out of the pneumatic duct may be higher when a fish experiences fast ascension rates (Alexander, 1959a). It follows that the anatomy of the gas bladder of bloaters may restrict flow of gas from the gas bladder to the environment (Shrimpton *et al.*, 1990) during rapid ascent. One hypothesis is that the geometry of the pneumatic duct in relation to the esophagus and gas bladder may change when uncontrolled pressure differences are experienced.

Table 1 **Appendix II** **Time to Expel Gas Bubbles**

pressure chamber

dates	fish no.	** fed?	total time	amt. Incr	initial buoyancy	pressure decr.	gas secreted or gulped
Jun-20	42	n	2hrs	15psi	Neg.	no reaction	n
Jun-21	43	?	4hrs	15psi	Neg.	Pos.	y (bubble release)
6/22-6/23	44	n	5hrs 13min	10psi	Neut.	Pos.	likely (bubble release)
6/26-6/27	48	y	18hrs 54min	15psi	Neg.	Pos.	y (bubble release)
6/27-6/28	49	y	16hrs 13min	15psi	Neut.	Pos.	likely (bubble release)
7/3-7/4	50	n	19hrs 6min	15psi	Neg.	Neg.	n
7/4-7/5	52	y	18hrs 55min	15psi	Neut.	Pos.	likely (?)
7/5-7/6	53	y	17hrs 24min	15psi	Neg.	dead	xxx
7/10-7/11	54	n	17hrs 56min	15psi	Neg.	dead	xxx
7/11-7/12	55	n	18hrs 9min	15psi	Neg.	dead	xxx
7/12-7/13	56	?	16hrs 59min	14psi	Neg.	Pos.	y (bubble release)
7/13-7/14	57	y	18hrs 13min	14psi	Neg.	Neg.	n
7/16-7/17	58	n	19hrs 5min	14psi	Neg.	Neg.	n (bubble release)
7/28-7/29	59	n	21hrs 27min	14psi	Neg.	Neg.	n (bubble release)
8/2-8/3	61	y	21hrs 35min	14psi	Neg.	Pos.	y (bubble release)

** y = fed within last 24hrs
n = not fed in last 24hrs.

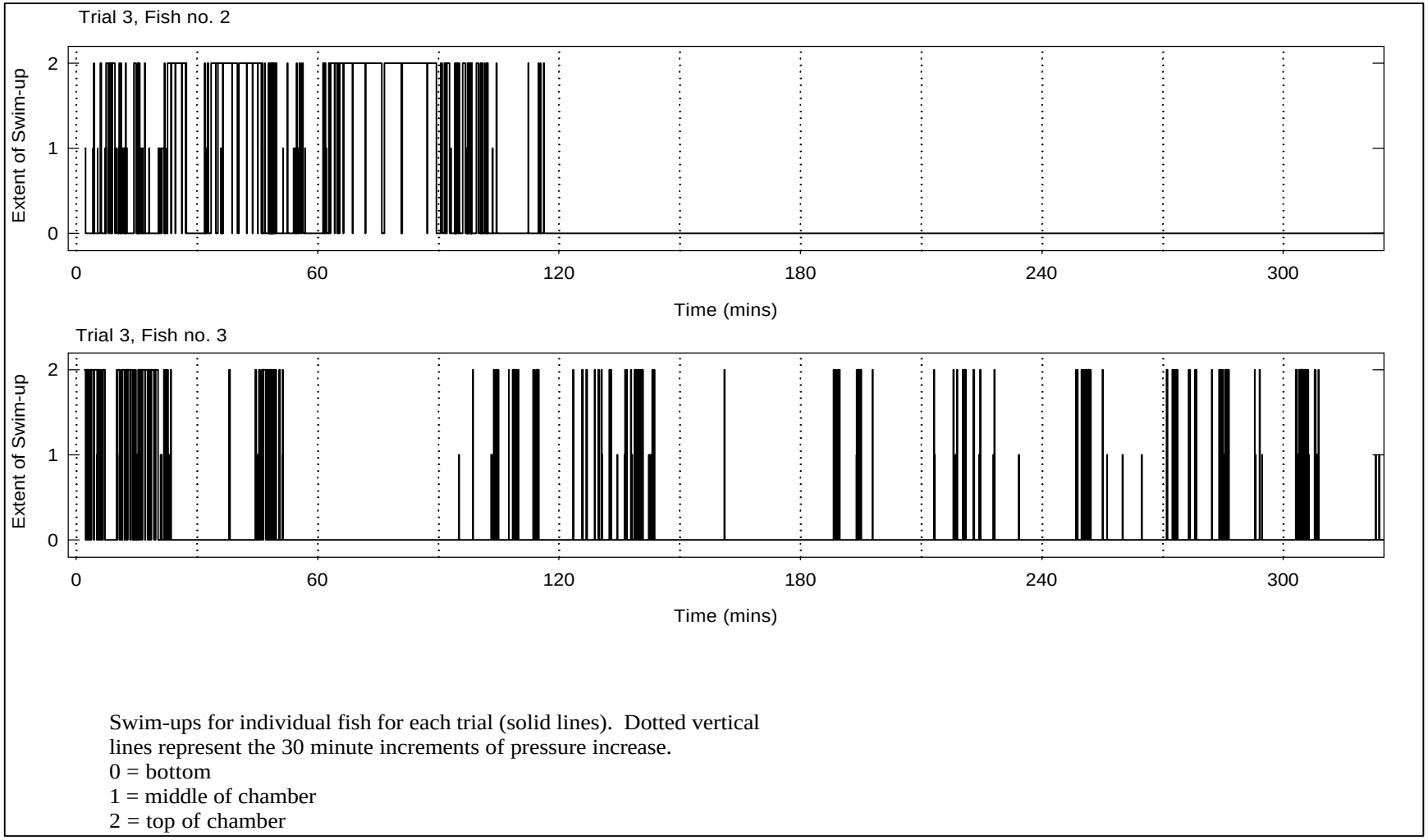
dates	fish no.	
Jun-20	42	
Jun-21	43	2 bubbles 1min and 3mins after pressure decrease
6/22-6/23	44	1 bubble 10mins after pressure decrease
6/26-6/27	48	10 bubbles: 1, 2, 4, 5, 6, 25, 26, 27, 29, and 36mins after pressure decrease
6/27-6/28	49	5 bubbles: 22, 23, 24, 25, 26mins after pressure decrease
7/3-7/4	50	(no swim bladder)
7/4-7/5	52	No, but camera view is too far away
7/5-7/6	53	
7/10-7/11	54	
7/11-7/12	55	
7/12-7/13	56	7 bubbles: 1, 2, 6, 9, 11, 12, 13mins after pressure decrease
7/13-7/14	57	
7/16-7/17	58	1 bubble 11mins after pressure decrease (condensation hide other bubbles?)
7/28-7/29	59	1 bubble 1min after pressure decrease (condensation hide other bubbles?)
8/2-8/3	61	2 bubbles 1min and 2mins after pressure decrease (condensation hide others?)

Pos. = positive buoyancy (tend to float)
Neut. = neutral buoyancy
Neg. = negative buoyancy (tend to sink)

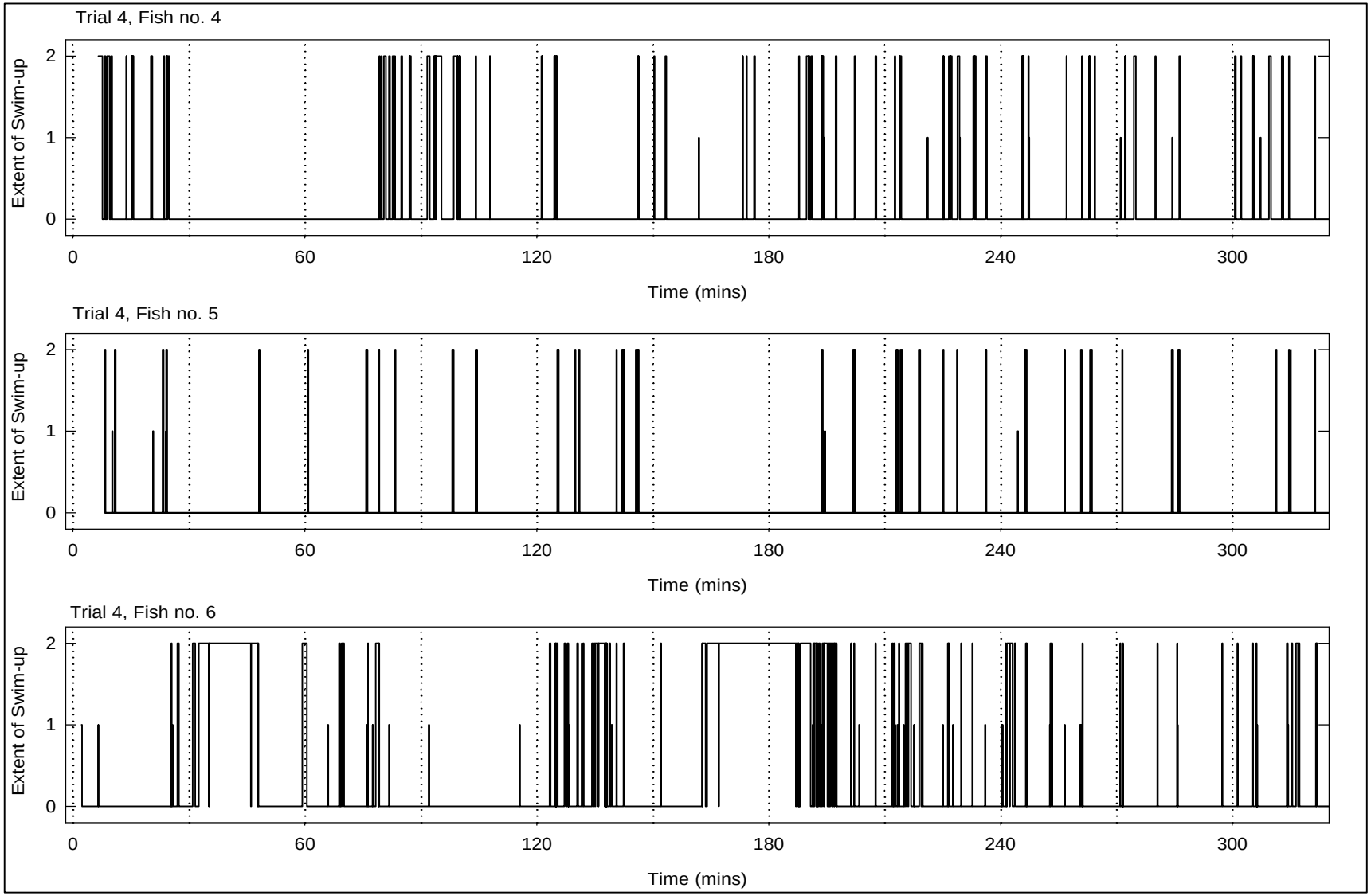
Appendix III:

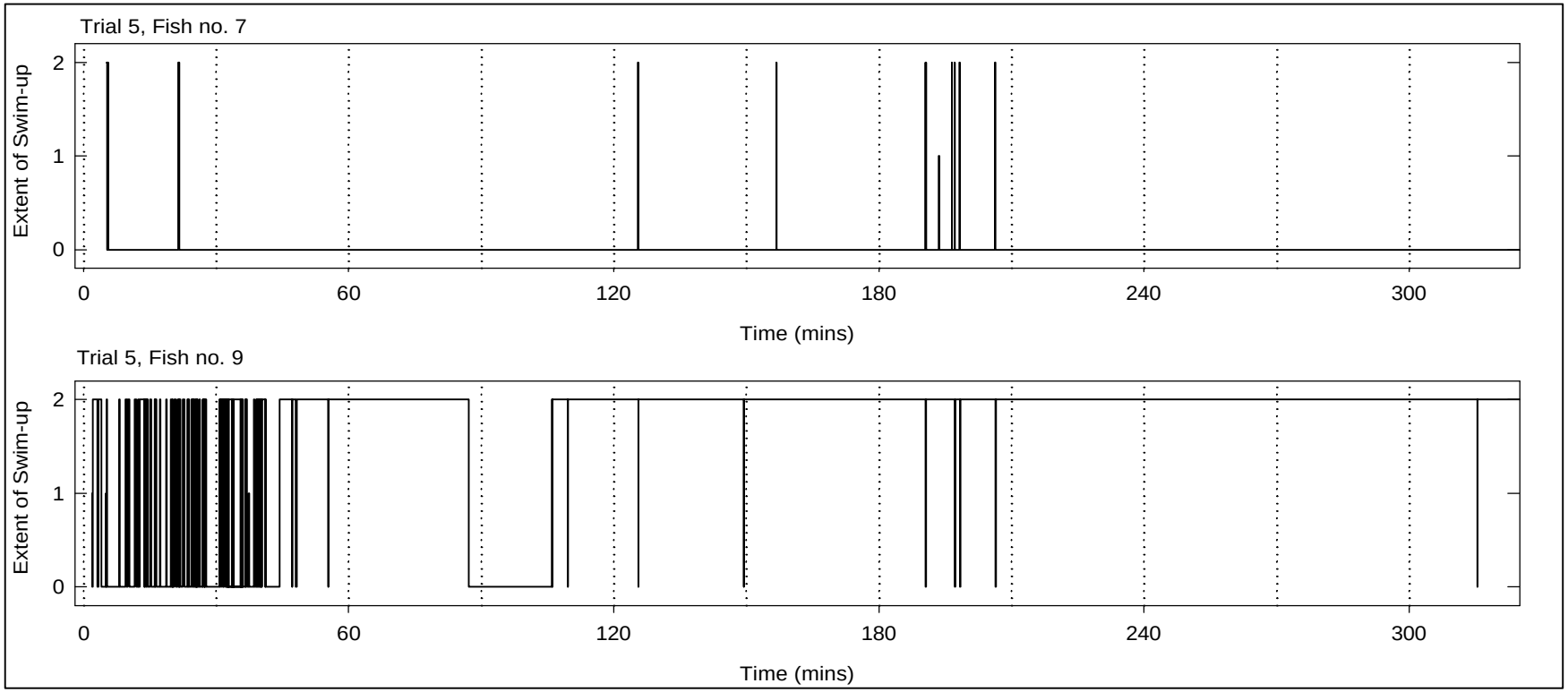
Number and Extent of Swim-ups

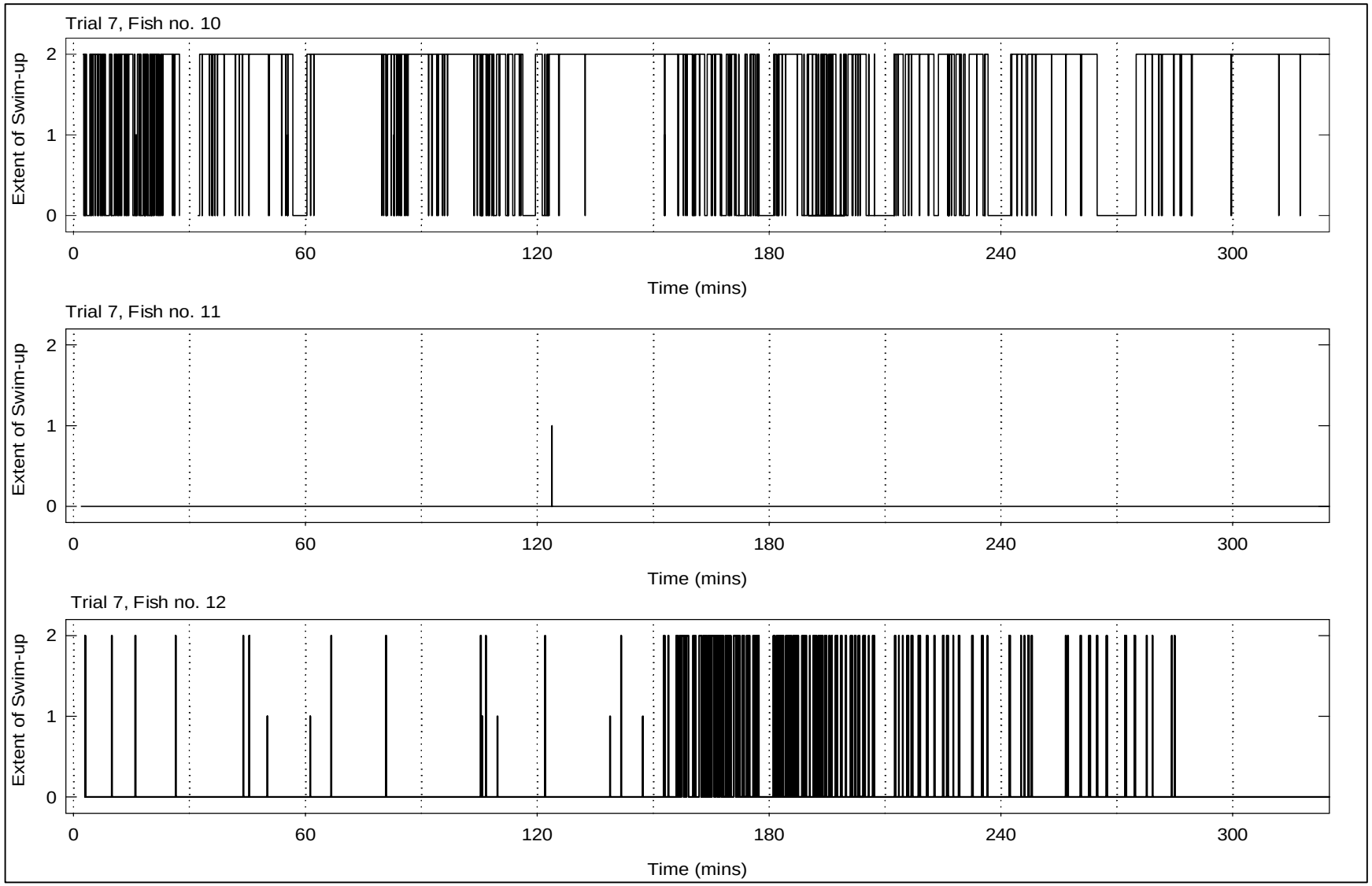
During Pressure Increase



Swim-ups for individual fish for each trial (solid lines). Dotted vertical lines represent the 30 minute increments of pressure increase.
0 = bottom
1 = middle of chamber
2 = top of chamber


Trial 4, Fish no. 4
Extent of Swim-up
0
1
2
0
60
120
180
240
300
Time (mins)
Trial 4, Fish no. 5
Extent of Swim-up
0
1
2
0
60
120
180
240
300
Time (mins)
Trial 4, Fish no. 6
Extent of Swim-up
0
1
2
0
60
120
180
240
300
Time (mins)


Trial 5, Fish no. 7
Extent of Swim-up
2
1
0
0
60
120
180
240
300
Time (mins)
Trial 5, Fish no. 9
Extent of Swim-up
2
1
0
0
60
120
180
240
300
Time (mins)


Trial 7, Fish no. 10
Extent of Swim-up
2
1
0
0
60
120
180
240
300
Time (mins)
Trial 7, Fish no. 11
Extent of Swim-up
2
1
0
0
60
120
180
240
300
Time (mins)
Trial 7, Fish no. 12
Extent of Swim-up
2
1
0
0
60
120
180
240
300
Time (mins)