THE SWIMBLADDER AS A HYDROSTATIC ORGAN IN THE NORTHERN PIKE, *Esox lucius* L.

by

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Pike, three inches long, perfect
Pike in all parts, green tigering the gold.
Killers from the egg: the malevolent aged grin.
They dance on the surface among the flies.

Or move, stunned by their own grandeur,
Over a bed of emerald, silhouette
Of submarine delicacy and horror.
A hundred feet long in their world.

In ponds, under the heat-struck lily pads -
Gloom of their stillness:
Logged on last year's black leaves, watching upwards.
Or hung in an amber cavern of weeds

The jaws' hooked clamp and fangs
Not to be changed at this date;
A life subdued to its instrument;
The gills kneading quietly, and the pectorals.

Three we kept behind glass,
Jungled in weed: three inches, four,
and Four and a half: fed fry to them -
Suddenly there were two. Finally one

With a sag belly and the grin it was born with.
And indeed they spare nobody.
Two, six pounds each, over two feet long,
High and dry and dead in the willow-herb -

One jammed past its gills down the other's gullet:
The outside eye stared: as a vice locks -
The same iron in this eye
Though its film shrank in death.

A pond I fished, fifty yards across,
Whose lilies and muscular tench
Had outlasted every visible stone
Of the monastery that planted them -

Stilled legendary depth:
It was as deep as England. It held
Pike too immense to stir, so immense and old
That past nightfall I dared not cast

But silently cast and fished
With the hair frozen on my head
For what might move, for what eye might move.
The still splashes on the dark pond,

Owls hushing the floating woods
Frail on my ear against the dream
Darkness beneath night's darkness had freed,
That rose slowly towards me, watching.

Ted Hughes

This thesis is the result of my own work.
It has not been, nor will be, submitted
for any other degree.

J. L. Webb (Candidate)

(Supervisor)

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ABSTRACT

The aim of this study was to investigate the ways in which the northern pike, Esox lucius L., adjusts its buoyancy by manipulation of the gas content of the swimbladder.

The pike, a physostome, was shown to employ two methods of increasing the volume of gas in the swimbladder. Firstly, by air-gulping in shallow water, and with free access to the surface. Secondly, by gas secretion, made possible because of the existence of a well-developed secretory apparatus at the anterior end of the swimbladder. Pike demonstrated the ability to secrete a gas, rich in oxygen, at rates similar to those demonstrated by some physoclists. Secretion occurred even when air-gulping was possible.

Gas release was shown to occur when deep-adapted pike were subjected to a reduction in ambient pressure. Swimbladder gas was also forcibly ejected via the pneumatic duct when pike were exposed to a water current, and under conditions of stress. In addition, swimbladder gas was lost as a result of simple diffusion across the swimbladder wall.

In still water, pike adapted to neutral or slight positive buoyancy, while those held in a current were negatively buoyant. Still-water adapted pike demonstrated substantial excess internal gas pressures, while in current-adapted fish, only slight excess internal pressures were developed.

Small transient changes in buoyancy, probably arising as a result of the action of the body wall muscles on the swimbladder contents were observed.

The physiological and ecological significance of these experimental results and observations are discussed.

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INTRODUCTION

The swimbladder of teleost fishes has been the subject of much attention for many years, and the numerous studies on the morphology and physiology of the organ are well documented.

The swimbladder, when present in adult fish, exists in two basic forms. The closed or physoclistous swimbladder has no persistent communication with the exterior, and has developed specialized areas for the secretion and reabsorption of gases (Corning, 1888; Saupe, 1940; Fänge, 1953). The more primitive open or physostomous swimbladder retains the pneumatic duct, present in early stages in all Teleostomi (Tracy, 1911) as a communication with the alimentary tract. This latter type has been described in the Anguillidae by Rauther (1923), Fänge (1953) and Steen (1963), in the Clupeidae by Hasse (1873), Ridewood (1891), Brawn (1962), and Fahlen (1967), in the Coregonidae by Hufner (1892), Saunders (1953), Scholander, van Dam and Enns (1956), and Sundnes (1959, 1963), in the Salmonidae by Cuvier and Valenciennes (1848), de Beaufort (1909), Jasinski (1963), and Fahlen (1971), in the Esocidae by Müller (1840), Corning (1888) and Rauther (1923), and in the Cyprinidae by Evans (1925), Evans and Damant (1929), Fänge and Mattisson (1956) and Alexander (1959).

Major review articles on the structure and function of the swimbladder include those of Harden Jones and Marshall (1953), Marshall (1960), Alexander (1966), Fänge (1966) and Blaxter and Tytler (1978).

Fish which lack swimbladders, and those from which the swimbladder has been removed, generally have specific gravities of between 1.06 and 1.09 (Delaroche, 1809). Possession of a gas-filled sac necessarily reduces the specific gravity of a fish, since the gas contained within it contributes to its volume without contributing materially to its

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weight. The theory that the swimbladder functions to reduce the density of a fish to around that of its environment and thus reduces the amount of energy required to maintain a given position in the water column was first proposed by Rondelet (1554). Since this proposal was made, much evidence has been collected in its favour. However, the role of the swimbladder in maintaining hydrostatic equilibrium became the subject of an extraordinary controversy in the 1930's when Rabaud and Verrier published the results of their experiments, including those conducted on a number of physostomes. In these experiments Rabaud and Verrier determined that the density of fish after removal of the swimbladder was similar to that of intact fish, and from these determinations, and the results of various decompression experiments, they concluded that the swimbladder does not function as a hydrostatic organ. Evidence for this conclusion and contrasting evidence from the work of Meierhans (1935 a, b, c), Guyenot (1909), Plattner (1937, 1938a, b, 1941), and Guyenot and Plattner (1938, 1939) has been reviewed in great detail by Harden Jones and Marshall (1953), who conclude that the results of Rabaud and Verrier cannot be considered valid because of considerable inaccuracies in their methods and experimental observations.

Fish with swimbladders are neutrally buoyant at one depth only, since a reduction in hydrostatic pressure, brought about by upward movement, will cause the swimbladder gas to expand, thus decreasing the density of the fish, and an increase in hydrostatic pressure brought about by downward movement will cause the gas to be compressed, thus increasing the density of the fish. Alexander (1959) found that in the 22 species he studied, the gas in the swimbladder obeyed Boyle's Law remarkably well, and only in the Cyprinidae did the swimbladder walls passively resist the expansion of gas when the ambient pressure was reduced.

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The problem of vertical instability imposed by possession of a swimbladder is reduced by the ability to remove and deposit gas when required. In physoclists, the Rete mirabile/Gas Gland system is responsible for concentrating blood gases into the lumen of the swimbladder, and the Oval is responsible for the removal of swimbladder gases into the bloodstream. In physostomes, the presence of the pneumatic duct allows them to swallow air at the surface, and to pass the air into the swimbladder for shallow adaptation, and to release gas rapidly from the swimbladder following a sharp decrease in hydrostatic pressure, and during the sounding response. In addition, a number of physostomes have been shown to possess a relatively weakly developed Rete mirabile/Gas gland system, capable of slow gas deposition (Rauther, 1923; Fänge and Mattisson, 1956; Fahlen, 1967, etc.).

Physostomes rendered negatively buoyant by the removal of gas from the swimbladder, by the attachment of a weight, or by an increase in ambient pressure attempt to compensate by introducing gas into the swimbladder. If access to the air-water interface is easily available, fish will swim to the surface to swallow air and pass it into the swimbladder *via* the pneumatic duct. Shallow adaptation may be attained in a relatively short time by air-gulping. Evans and Damant (1929) studied the behaviour of the goldfish, *Carassius auratus* following gas removal. Fish permitted free access to the surface gulped air and attained a state of neutral buoyancy within 90 minutes, while fish denied access to the surface by a glass bell jar remained negatively buoyant after 48 hours. Small roach (*Rutilus rutilus*) subjected to this treatment produced very similar results. Following these experiments, Evans examined the pneumatic duct of roach, tench (*Tenca tinca*), bream (*Abramis brama*), minnow,(*Phoxinus phoxinus*) and carp (*Cyprinus carpio*) and showed that the 'pneumatic bulb', present at the oesophageal end of the duct functioned as a pumping mechanism to facilitate the transfer of air from the oesophagus

into the swimbladder. Plattner (1941) conducted similar experiments on cyprinids, and showed that after section of the branches of the vagus nerve which run to the pneumatic duct sphincter, swallowed air did not pass into the swimbladder, but accumulated in the gut.

One of the major problems associated with the deposition of gas into the swimbladder is the first filling of the swimbladder in young fish. Although the swimbladder of newly-hatched fish is usually well-developed, it contains no gas, and its initial filling is usually coincident with the disappearance of the yolk-sac and the beginning of a food-seeking existence (Tait, 1960). In the physoclists Gasterosteus aculeatus, Lebistes reticulatus and Hippocampus hippocampus, the swimbladder never fills if they are prevented from reaching the surface during the first few days before their pneumatic ducts close (von Ledebur, 1928; Jacobs, 1938). However, in physostomes the duct remains patent even if the initial filling of the swimbladder is delayed. Tait (1960) showed that the salmonoids Salvelinus namaycush, Salmo gairdneri, Coregonus clupeaformis and Salmo trutta failed to fill their swimbladders while being reared without access to the atmosphere for 22, 50, 56 and 84 weeks respectively, but when subsequently permitted access, they filled them. Tait also showed that the fry of S. namaycush could swim to the surface from depths of up to 900ft to fill their swimbladders without marked fatigue, indicating that fish hatched in deep water can swim to the surface without the benefit of a gas-filled swimbladder.

If physostomes are rendered positively buoyant by the injection of gas into the body cavity, by the attachment of a float, or by a decrease in ambient pressure, they can release gas from the swimbladder via the pneumatic duct into the alimentary tract, where it escapes through the mouth and gills. In many clupeids, release of gas also occurs through an aperture close to the anus. In cyprinids, the pneumatic duct sphincter which controls the release of gas from the swimbladder is innervated

by the rami intestinalis vagi, and by sympathetic fibres (Evans, 1925; Franz, 1937). Kuiper (1915) showed that bilateral section of the vagus nerve behind the operculum caused an increase in the pressure reduction required for the release of gas, suggesting that one of the functions of the sphincter branch of the nerve is to inhibit the tonus of the sphincter muscles. He also found that section of the intestinal ramus close to the sphincter caused a decrease in the tonus, and concluded that the sympathetic fibres joined the vagus in this region, and that the stimuli from these fibres maintained the tonus of the sphincter. This conclusion is supported by the experiments of Franz (1937). Franz's results also indicate that swimbladder gas is not released passively during the "gasspuckreflex", but is forcibly ejected by contraction of smooth muscles in the swimbladder wall as the pneumatic duct sphincter relaxes.

This would certainly explain the release of gas following the attachment of a float, which, of course, will not affect the pressure of the swimbladder gas. In cyprinids, which develop an excess internal gas pressure (Evans and Damant, 1929; Alexander, 1959a), and other physostomes which do likewise (Gee, Machniak and Chalanchuk, 1974) relaxation of the pneumatic duct sphincter alone may bring about the expulsion of gas.

Fänge (1953) has examined the effects of acetylcholine on the pneumatic duct sphincter in S. gairdneri, and has shown that after topical application, the internal gas pressure required to cause the sphincter to open increased from 5 to 15mmHg. Harvey, Hoar and Bothern (1968) in studies on the smolts of Kokanee and Sockeye salmon (Oncorhynchus nerka) found that fright-induced sounding was accompanied by the active expulsion of gas, brought about by contraction of the circular muscles of the swimbladder walls. The amount of gas released was reduced after treatment with various adrenergic blocking agents, and enhanced following

treatment with sympathomimetics, suggesting that swimbladder contraction and concomitant gas release is under adrenergic control. Duct release pressure dropped after treatment with the anticholinergic drug atropine, supporting Fänge's proposal that the tonus of the pneumatic duct sphincter is under cholinergic control.

Tait (1970) has developed a large scale method of selecting salmonids for ability to retain swimbladder gas when subjected to a decrease in ambient pressure, with a practical application of some commercial importance. Observation that deep-swimming salmonids, such as the lake char (S. namaycush) retain swimbladder gas well, while shallow-dwelling species such as the brook char (S. fontinalis) lose gas fairly easily, led to the production of a hybrid char (S. fontinalis x S. namaycush) which combines the early maturing characteristics of the former species with the deep-swimming ability of the latter. This hybrid was produced for the purpose of stocking the Great Lakes with a fish which matured by age II or III, with a good chance of reproducing before being killed by fishermen or sea lampreys, and that could take advantage of the abundant food species in both deep and shallow water. Tait's selection method involved the use of flotation tanks in which large numbers of fish could be contained, and in which the pressure could be reduced by means of a vacuum pump. By slowly reducing the pressure, anaesthetized fish could be selected according to their ability to retain gas for use in breeding and stocking programmes.

Although physostomes are able to increase the gas content of the swimbladder by air-gulping, this method is not always ideally suited to the natural habits of the fish. For example, if a fish is to be neutrally buoyant at a depth greater than that to which it is initially adapted, it must swim to the surface to gulp air, then swim downwards against the considerable upwards force produced by the gulped

air. This is clearly inefficient in terms of energy expenditure, and may even be prevented since the temperature of the epilimnion in summer may exceed the upper lethal limit for some species, and in winter the presence of ice cover may prevent access to the surface. In addition, fish may only adapt to neutral buoyancy in relatively shallow water, since there are clearly limits to the quantity of air that can be accommodated in the swimbladder at the surface. It would, therefore, be to their advantage if physostomes which live at depth possessed some other mechanism by which gas could be accumulated into the swimbladder.

The structures and tissues associated with gas secretion in physoclists have also been found in several physostomous groups, although they are usually developed to a lesser degree. There is increasing evidence to suggest that the mechanism of gas secretion is fundamentally similar in physoclists and physostomes. Rauther (1923) found rete-like associations of blood vessels and glandular tissue in the walls of the posterior chamber of the swimbladder in the carp (C. carpio) and crucian carp (Carassius carassius). Fange and Mattisson (1956) also describe an area of gas gland tissue, separated from the lumen of the swimbladder by smooth muscle, and an internal layer of cylindrical epithelium surrounding the area where the pneumatic duct enters the swimbladder in the crucian carp. In the whitefish Coregonus lavaretus, Fahlen (1967) described ramification of arterial and venous vessels giving rise to an extensive plexus consisting of almost flat bundles of three or more parallel vessels averaging some 10μm in diameter. In the region of the pneumatic duct these bundles often consist of ten or more vessels, although more posteriorly they usually comprise only three vessels. The central vessel is always of arterial origin. In the larger vascular bundles, afferent and efferent vessels alternate regularly, with the lumina of adjacent vessels being separated only by the thin vascular walls. The bundles run inside, and parallel with

the walls of the swimbladder in what Fahlen describes as the "inner vascular plexus layer", before crossing to the base of the epithelium. The total length of these "micro-rete" vessels may exceed 50 metres in a swimbladder only 90mm long. Fahlen also reports that the epithelial cells lining the swimbladder have characteristics consistent with those of secretory tissue.

Of all of the physostomous groups, the most remarkable degree of development of the structures and tissues associated with gas secretion is to be found in the Anguillidae. In the common eel Anguilla anguilla, the swimbladder is divided functionally into two parts. The whole of the secretory part (the real swimbladder) is lined by a single-layered glandular epithelium, composed of cuboid cells, whose inclusions are typical of metabolically active secretory tissue (Fänge, 1953). In the resorbent part (the pneumatic duct) the lining epithelium is composed of flat cells of a type similar to that found in primitive lungs, and well suited to the passive process of diffusion (Dorn, 1961). Both the secretory and resorbent parts are highly vascularized. Krogh (1929) estimated the number of venous capillaries in the two bipolar retia of Anguilla anguilla at 88,000 and the number of arterial capillaries at 116,000, with the total length of these vessels exceeding 800 metres and their surface area exceeding 210 metres^2.

The composition of the gas present in the swimbladders of physoclists and physostomes often differs markedly with a high proportion of oxygen and correspondingly low proportion of nitrogen being commonly found in physoclists, and the converse in physostomes. As a result of these differences, several authors have argued that the secretory mechanism in the swimbladder of those physostomes capable of gas secretion must differ from that of physoclists. The high nitrogen content of the coregonid swimbladder has been investigated by Hufner (1892), Saunders

(1953), Scholander, van Dam and Enns (1956), Sundnes, Enns and Scholander (1958) and Sundnes (1959, 1963). Sundnes et al. (1958) working with Coregonus lavaretus and C. acronius were unable to find any specialization of blood vessels into a rete-type plexus, although they determined that the former species could apparently accumulate nitrogen and oxygen against considerable partial pressure gradients, while the latter only accumulated nitrogen. They concluded from this evidence that some unknown mechanism involving the epithelial cells of the swimbladder was involved. However, their anatomical investigation was shown to have been totally inadequate when Fahlen (1959) first demonstrated the presence of the countercurrent vascular system described above. The findings that C. acronius accumulated only nitrogen were also shown to be erroneous when Sundnes (1959) found that fish which had recently moved into deep water after spawning had significant proportions of oxygen in their swimbladders. These findings support the theory that the gas deposition mixture in physostomes consists mainly of oxygen, and that the high nitrogen content often found results from the loss of oxygen by diffusion and through preferential reabsorption of oxygen by the swimbladder tissues (Sundnes, 1963).

Although the majority of lentic physostomes strive to maintain a state of neutral buoyancy by means such as those described, neutral buoyancy is uncommon among lotic physostomes. More commonly, these fish are found to be negatively buoyant, and maintain position on or close to the bottom of streams in order to resist being swept away by the current. Even within a single species, the state of buoyancy may vary with stage in life history, season, and environment. Saunders (1965) demonstrated that the parr of Atlantic salmon (Salmo salar) held in a current were less buoyant than those held in still water, and Neave et al (1966) found that loss of gas and concomitant lowering of buoyancy occurred within twelve hours of an increase in current velocity. Pinder

and Eales (1969) showed that the buoyancy of parr held in a current was lowest in early winter, but increased throughout the spring until the onset of the parr-smolt transformation, when the pressure of neutral buoyancy in smolts in current was similar to that of smolts in still water. It is interesting to note that at no point during the course of these studies did parr or smolts ever attain a state of neutral buoyancy, either in current or in still water. The increase in the buoyancy of fish up to and during the parr-smolt transformation is probably associated with the migration of smolts downstream towards the sea. Smolts are less vigorous than parr, and this lack of vigour, coupled with their tendency to swim off the bottom of streams .... "results in their not reacting sufficiently to current by heading and swimming upstream for them to continue as river fish" (Huntsman, 1962).

Of the twelve species of physostomes investigated by Gee et al (1974), eleven were less buoyant in current than in still water, but Esox lucius became significantly more buoyant in current. According to their data, the flotation pressure (a measure of buoyancy) of pike held in current exceeded unity, indicating that they were positively buoyant. The fish which yielded these results were young-of-the-year, taken from the inlet streams of larger lakes, and Gee et al suggest that they may increase their buoyancy from just below neutral buoyancy in still water, to well above it on encountering a current, and that this shift assists in displacing the fish downstream into lakes.

The existence of an excess internal pressure (E.i.p.) in the swimbladder of several cyprinids has been determined by Alexander (1959a). The seventeen species of non-cyprinid fishes examined by Alexander (1959d) apparently showed no evidence of E.i.p. However, the methods used to study E.i.p. differed for cyprinids and non-cyprinids, and in the latter group, few individuals of each species were used. Gee et al have also shown the existence of E.i.p. in ten cyprinid and

and two non-cyprinid species. The ability to develop an excess internal pressure is of importance to physostomes for two main reasons. Firstly, its existence, coupled with the influence of swimbladder walls of limited extensibility, reduces the rate of swimbladder volume change with ambient pressure change, thus minimising the required alteration in buoyancy. In the extreme case of the bream Abramis brama, for a small depth change the swimbladder only changes its volume one-quarter as much as would a free air bubble of the same size (Alexander, 1959c). Secondly, it provides for precise control of buoyancy adjustment (McCutcheon, 1962) A considerable amount of the initial adjustment in swimbladder volume in response to variation in water velocity is brought about by changes in internal gas pressure (Gee, 1970; Machniak, 1973). In addition, in the Cypriniformes excess internal pressure produces tension in the swimbladder walls, necessary for the proper functioning of the Weberian ossicles (Alexander, 1959a).

Of the many studies conducted on the hydrostatic function of the swimbladder in physostomous fish, few have set out to evaluate the relative importance of the various means by which the swimbladder volume may be regulated. The present study was undertaken in an attempt to assess the capacity of the physostome Esox lucius to accumulate gas into the swimbladder by secretion, and to investigate the relative importance of air-gulping and gas secretion as means of increasing the gas content of the swimbladder. The pike was selected as the subject of this study on the basis of the findings of Jacobs (1934), who demonstrated that pike are capable of relatively rapid gas secretion.

In addition, the ability of pike to retain swimbladder gas and to regulate the buoyancy by manipulation of swimbladder volume under various conditions were investigated.

MATERIALS AND METHODS

THE ANIMALS

Specimens of the northern pike, Esox lucius L., were obtained from the Forth and Clyde canal, Stirlingshire and the Rosebery Reservoir, Midlothian, using gill nets (vertical knot to knot mesh size 55mm), and rod and line.

Animals caught using the gill nets were not generally suitable for use in physiological experiments extending over more than a few days, because damage caused by the nets commonly allowed infection by aquatic fungi of the genus Saprolegnia. Seriously damaged animals were killed on removal from the nets, and were used in morphological studies.

Fish caught using rod and line were available in excellent condition, and were transported live to the laboratory in 60 litre polythene bins, where they were transferred into 800 litre lidded holding tanks with a through-flow of fresh water. Fish were sorted according to size and held in separate tanks to prevent cannibalism.

Feeding of stock animals was carried out on alternate days, with small pike (approximately 0.05 to 1.00kg) being fed on live minnows Phoxinus phoxinus (L.), sticklebacks Gasterosteus aculeatus L., and rainbow trout fingerlings, Salmo gairdneri Richardson, and larger pike (approximately 1kg upwards) on small perch Perca fluviatilis L., and rainbow trout.

In order to reduce the problem of alarm-induced gas release caused by the handling of unhabituated pike during the course of the various experiments, stock animals were netted and handled on a daily basis for 10 to 14 days before the commencement of an experiment.

SURGICAL PROCEDURES

(i) Anaesthesia

Fish were anaesthetized by immersion in a 200 p.p.m. solution of ethyl-p-aminobenzoate (Benzocaine). The anaesthetic solution was prepared using a stock solution of 10% Benzocaine (w/v) in absolute ethanol, and fresh water at the holding temperature.

In circumstances where it was necessary to hold fish under anaesthesia for periods in excess of 3 to 4 minutes, they were maintained on a system with a recirculating supply of anaesthetic, which permitted the gills to be perfused with a 100 p.p.m. solution of Benzocaine.

On completion of procedures requiring extended periods of anaesthesia, the anaesthetic supply was turned off, and fresh water was substituted to facilitate rapid recovery.

(ii) Sampling of swimbladder gas

Swimbladder gas samples were collected using 1 cm^3 disposable polythene syringes containing 0.05 cm^3 of acid rinsing solution (Appendix 1) as a gas seal, and 25-gauge hypodermic needles.

Fish were lightly anaesthetized and placed flank down on a cork board covered with several layers of damp tissue. The hypodermic needle was inserted into the swimbladder through the epaxial musculature at a point just posterior to the margin of the operculum and slightly above the lateral line. In this position the needle could be passed through the line of attachment of the swimbladder with the body wall, thus minimising any leakage of swimbladder gas. A 0.5 cm^3 sample of swimbladder gas was aspirated into the syringe, the needle was withdrawn, and the syringe was sealed by pushing the tip of the needle into a rubber stopper. Following the collection of gas samples, fish were returned to their tanks to allow recovery from the effects of the anaesthetic.

(iii) Implantation of indwelling catheters

In short-term experiments where it was necessary to gain access to the swimbladder without having to handle the animals involved, catheters of the type illustrated in Figure 1 were used.

Fish were anaesthetized, and the gills were perfused with a maintenance concentration of anaesthetic on the recirculating system. The body of the catheter was attached to the dorsal musculature by passing the polypropylene thread through a hypodermic needle embedded in the musculature, then threading on an acetate button and sealing the end of the thread with heat. A pointed surgical seeker was used to produce a small fistula through which the tip of the catheter could be passed into the swimbladder. Following recovery from the effects of the anaesthetic, catheterized fish were placed into shallow tanks for 5 to 7 days to allow healing of the wounds. After satisfactory healing of the wounds the catheter was closed off by means of the closure lever on the three-way tap.

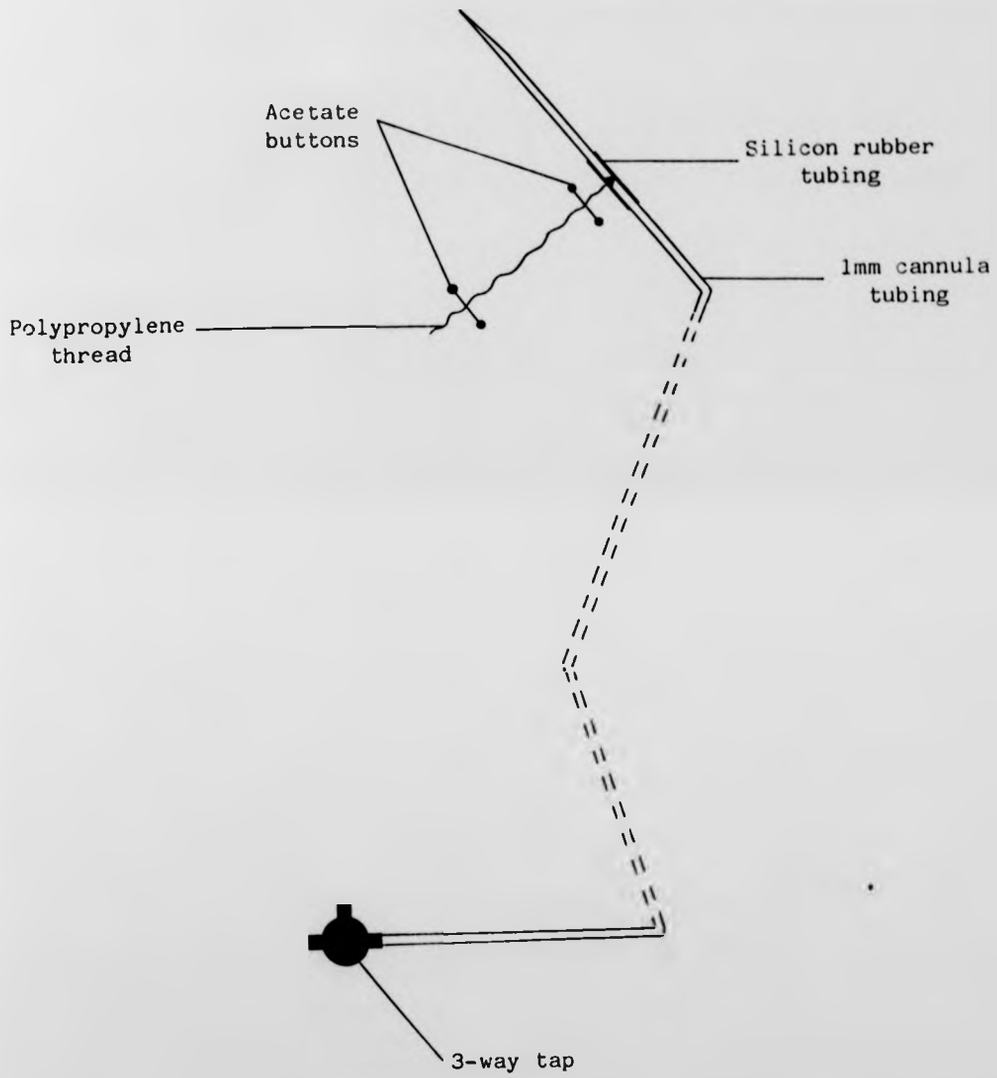
Approximately 55% of fish treated in this manner could be used in the various experiments. In the remaining 45%, problems such as catheter rejection and infection of wounds necessitated removal of the catheter and/or destruction of the fish.

THE SWIMBLADDER

(i) The volume of the swimbladder

The swimbladder volume required to confer neutral buoyancy was estimated using information on tissue density obtained by two methods. (a) Freshly killed fish were blotted dry and weighed to the nearest 0.05g on a top-pan balance. The swimbladders of these fish were exposed by cutting down the ventral midline, and a small incision was made in the swimbladder wall. The swimbladder was then emptied of gas by holding the cadaver under water and running a finger down the swimbladder towards

FIGURE 1 The indwelling catheter designed to allow access to the swimbladder of confined fish.


Acetate
buttons
Silicon rubber
tubing
1mm cannula
tubing
Polypropylene
thread
3-way tap

the incision. After checking the cadaver for the presence of gas bubbles, the gas-free fish were again weighed to the nearest 0.05g in distilled water, using a balance provided with a cradle suspended in a water-filled tank.

The densities of the gas-free fish were then calculated by relating the weight of the fish in water to the weight in air using the expression:-

$$p = \frac{p'}{p' - (w/W)} \qquad (a)$$

Where: p = Tissue density (g cm^{-3})

p' = Density of suspending medium (g cm^{-3})

w = Weight in suspending medium (g)

W = Weight in air (g)

(b) The densities of gas-free fish as determined using the method described above were checked using a method modified after Harvey (1963). A series of saline baths providing media with densities of 1.050 g cm^{-3} to 1.090 g cm^{-3} in increments of 0.002 g cm^{-3} was prepared. The gas-free fish were placed into each of the baths in ascending order of concentration, and their densities were taken to be equivalent to the density of the medium in which they just floated.

Swimbladder volumes required to confer neutral buoyancy were calculated from density data using the equation of Alexander (1966):

At neutral buoyancy : $p' = W/(W/p + X)$

therefore $X = (p - p') \cdot W/p.p' \qquad (b)$

where p = Tissue density (g cm^{-3})

p' = Density of suspending medium (g cm^{-3})

W = Weight in air (g)

X = Swimbladder volume (cm^3)

(ii) The structure of the swimbladder

The general morphology of the pike swimbladder was investigated in fresh and Formalin-fixed specimens, using various standard dissection techniques, often with the aid of a binocular dissecting microscope.

The position and proportions of the swimbladder in live, intact animals were determined using a Watson Mobilix X-ray Apparatus. Radiographs were produced on Kodak Industrex C X-ray plates by exposing the fish for 2½ to 3 seconds at 51 kv/56 ma. Exposure of the plates was followed by standard development in DX 80 (Kodak), and fixation in FX 40 (Kodak). The radiographs were used to produce black and white contact prints.

The general histology of the swimbladder wall was investigated in Formalin-fixed tissue using Paraffin wax/Gelatin and Nitrocellulose embedding to reduce the problems of layer separation during sectioning. Erlich's Haematoxylin and Eosin was used as a general stain, with Van Gieson's stain for collagen, and Orcein Elastica for elastic fibres (Mahoney, 1966).

(iii) The vascularization of the swimbladder

Two techniques were employed in order to confirm existing information on the origin of the blood vessels which supply and drain the swimbladder walls, and to further investigate the associations of blood vessels within the swimbladder.

The body cavity of anaesthetized fish was opened by cutting down the ventral mid-line, and the viscera were carefully displaced to expose the ventral surface of the swimbladder. The swimbladder was then opened and cut blood vessels were sealed by heat cautery to prevent excessive bleeding. After recording the configuration of the blood vessels on colour photographic film, the animals were killed by spinal section, without recovery from the effects of the anaesthetic.

Further investigations were carried out using pigmented neoprene rubber solutions, which were injected into the blood vessels of the swimbladder *via* two of the major vessels of the blood vascular system.

The blood supply to the posterior portion of the swimbladder was examined by opening the body cavity of freshly killed fish, and exposing the swimbladder. The caudal fin was removed by cutting across the caudal peduncle at the posterior margins of the dorsal and anal fins, and a catheter was introduced into the caudal artery. Vessels were cleared of residual blood by connecting a syringe to the catheter and injecting a quantity of 0.1 N ammonium hydroxide into the artery. After clearing the vessels, the syringe was replaced by another containing pigmented neoprene rubber solution, diluted with 0.1 N ammonium hydroxide, and the neoprene was injected into the artery using minimal pressure. The preparation was then placed into a bath containing acidified formalin in order to fix the tissue, and to accelerate the setting of the neoprene. Fixation was normally complete within 3 to 4 days, and the preparation was then removed from the bath and the swimbladder opened for examination.

The blood supply to the anterior part of the swimbladder was examined by injecting diluted neoprene rubber solution into the ventricles of freshly killed fish. The heart was exposed by making a small incision along the ventral midline at a point just posterior to the gill isthmus. A fine catheter was introduced into the ventricle and a quantity of 0.1N ammonium hydroxide was injected to clear residual blood. A syringe containing neoprene rubber solution diluted 1 : 1 with ammonium hydroxide was connected to the catheter, and the solution was slowly injected into the vascular system. After a few minutes, the body cavity was opened and the preparation was placed into acidified formalin to fix. After fixation and setting of the injected rubber was complete, the preparation was removed from the fixative, and the swimbladder was opened along

its ventral surface.

Both types of preparation were dissected and examined with the aid of a dissecting microscope to allow a general map of the blood system to be constructed.

(iv) The purine content of the swimbladder wall

The purine content of the pike swimbladder wall was determined using an assay technique modified after Ross (1977). This technique involved the use of two of the enzymes of the purinolytic pathway, which are responsible for the breakdown of guanine and xanthine to uric acid. The reaction pathway is shown in Figure 2.

Tissue samples were taken by punch biopsy from the anterior and posterior portions of freshly excised swimbladders, and each sample was digested in a tube containing 3 cm^3 of 0.1 N sodium hydroxide for 72 hours at 25°C. The tubes were then centrifuged to remove any suspended material, and a 0.25 cm^3 aliquot of the supernatant was taken from each tube. The supernatant samples were added to tubes containing 2.75 cm^3 of Tris buffer pH 8.1. These tubes were placed into a water bath at 25°C and after the solutions had reached this temperature, 10µl of xanthine oxidase (B.D.H.) and 15µl of guanase (Sigma) were added to each tube. After incubating the solutions for 35 minutes, the optical density of each solution was measured at 290 nM against a standard solution of 50 µg xanthine and 50 µg guanine/cm^3, using a Pye Unicam S.P.1800 Recording Spectrophotometer.

THE PHYSIOLOGY OF THE SWIMBLADDER

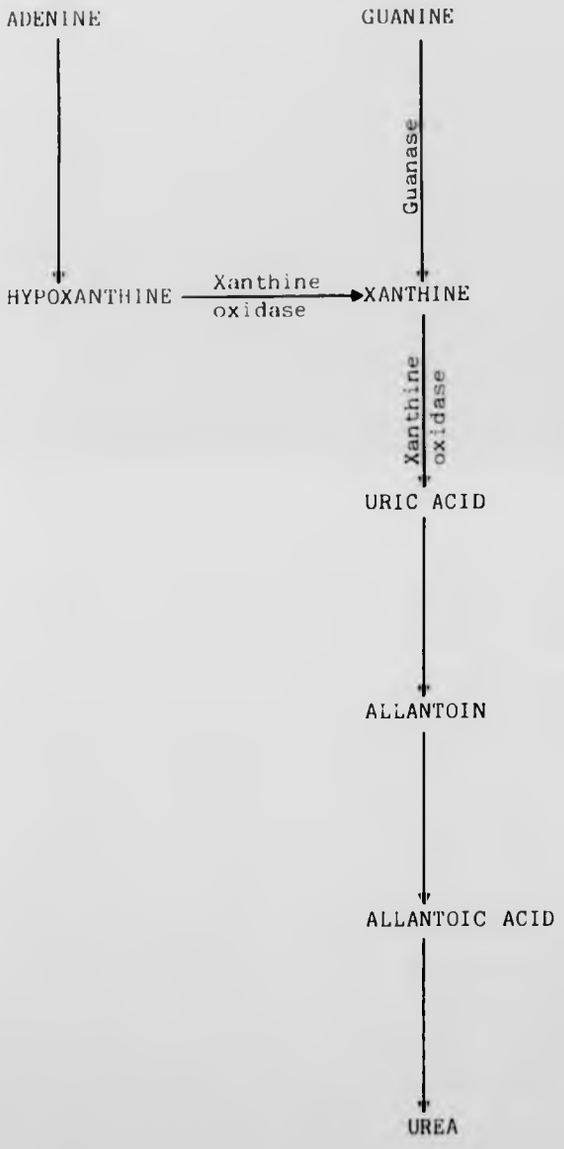
(i) Inflatory Mechanisms

(a) Air-gulping experiments

The behaviour of fish rendered negatively buoyant by emptying of the swimbladder was investigated by placing them into tanks where free access to the air-water interface was available. Categorisation

FIGURE 2 The Purinolytic pathway in fish.

(after Florkin, 1949)


ADENINE
GUANINE
Guanase
HYPOXANTHINE
Xanthine oxidase
XANTHINE
Xanthine oxidase
URIC ACID
ALLANTOIN
ALLANTOIC ACID
UREA

and classification of behavioural events and sequences was carried out using information collected during preliminary behavioural studies.

The swimbladders of lightly anaesthetized fish were emptied as fully as possible by aspirating the gas into a syringe, using the method described previously. Since it was impossible to completely empty the swimbladder by aspiration, the volume of gas remaining was determined by weighing the fish in air and in distilled water, and comparing the weight in water to that of a gas-free fish of the same size. The fish were then placed into an 800 litre perspex observation tank containing water at 13°C, and permitted to recover from the effects of the anaesthetic. After recovery, their swimming and air-gulping behaviour was recorded with the aid of a cassette tape recorder and stop-watch.

In further trials, a polypropylene mesh screen was fixed about 5 cm below the water surface in order to determine the response of fish when access to the air was prevented. The mesh was removed from the tank 6 hours after the beginning of each trial.

Changes in the composition of swimbladder gas in fish refilling their swimbladder when access to the surface was available were determined during experiments similar to those described above. After emptying the swimbladders as fully as possible by aspiration, and determining the volume of gas remaining, the fish were placed into lidded glass tanks containing water to a depth of 30 cm, at a temperature of 13°C. Gas samples were subsequently removed from the swimbladders of these fish for analysis at 3 to 4 day intervals.

The oxygen and carbon dioxide content of swimbladder gas samples were determined using Radiometer oxygen (E5046) and carbon dioxide (E5036) electrodes and a Radiometer P.H.M.72 Blood Gas Analyser. The gas electrodes were mounted inside D616 thermostatted cuvettes, which

were supplied with water at constant temperature by a Metofrig C.T. bath and pump (Figure 3).

Just prior to the analysis of swimbladder gas samples, the system was calibrated by passing humidified oxygen/carbon dioxide/nitrogen mixtures (B.O.C. Special Gases) through the sample chamber of the cuvettes.

The electrode membranes were wetted by injecting a small quantity of distilled water into the sample chambers and and 70 µl sub-samples of swimbladder gas were slowly injected into the sample chambers. After allowing 90 to 120 seconds for the readings to stabilise, the p O_2 and p CO_2 of the samples were read from the analyser display unit. After the initial readings had been obtained, the chambers were flushed with nitrogen and the procedure was repeated.

(b) Gas secretion experiments

The capacity for adjustment of buoyancy by gas secretion was investigated using the pressure chamber illustrated in Figure 4. The use of the pressure chamber permitted assessment of daily gas secretion rates, and allowed the pattern of buoyancy adjustment to be determined.

The glass chamber had a volume of 20 litres and a maximum internal diameter of 19 cm. Water was supplied to the chamber *via* polythene pipes from a header tank situated 13 metres above the datum line. The flow rate of water through the chamber and the internal pressure were controlled by coarse and fine inlet needle valves (V.1 and V.2 respectively), and a fine outlet valve (V.3). Two additional outlets (a and b) permitted the removal of gas bubbles from the system, and allowed access to fish *via* indwelling catheters.

The internal pressure was monitored by means of a mercury manometer, and the flow rate by a Gapmeter flow gauge. The temperature of the water passing through the chamber was monitored by a thermometer connected in series with the flow gauge. In order to minimise any possible

FIGURE 3 The Radiometer D616 thermostatted gas electrode cuvette.

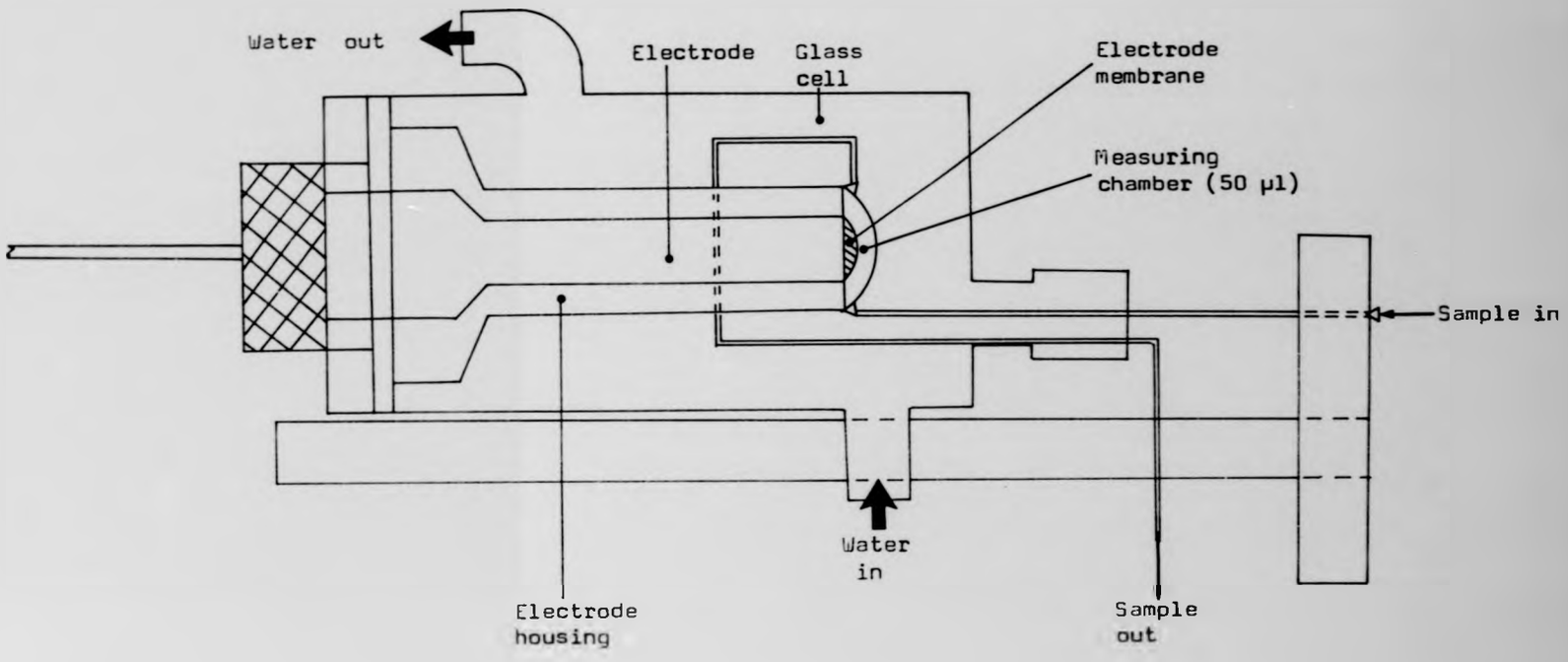
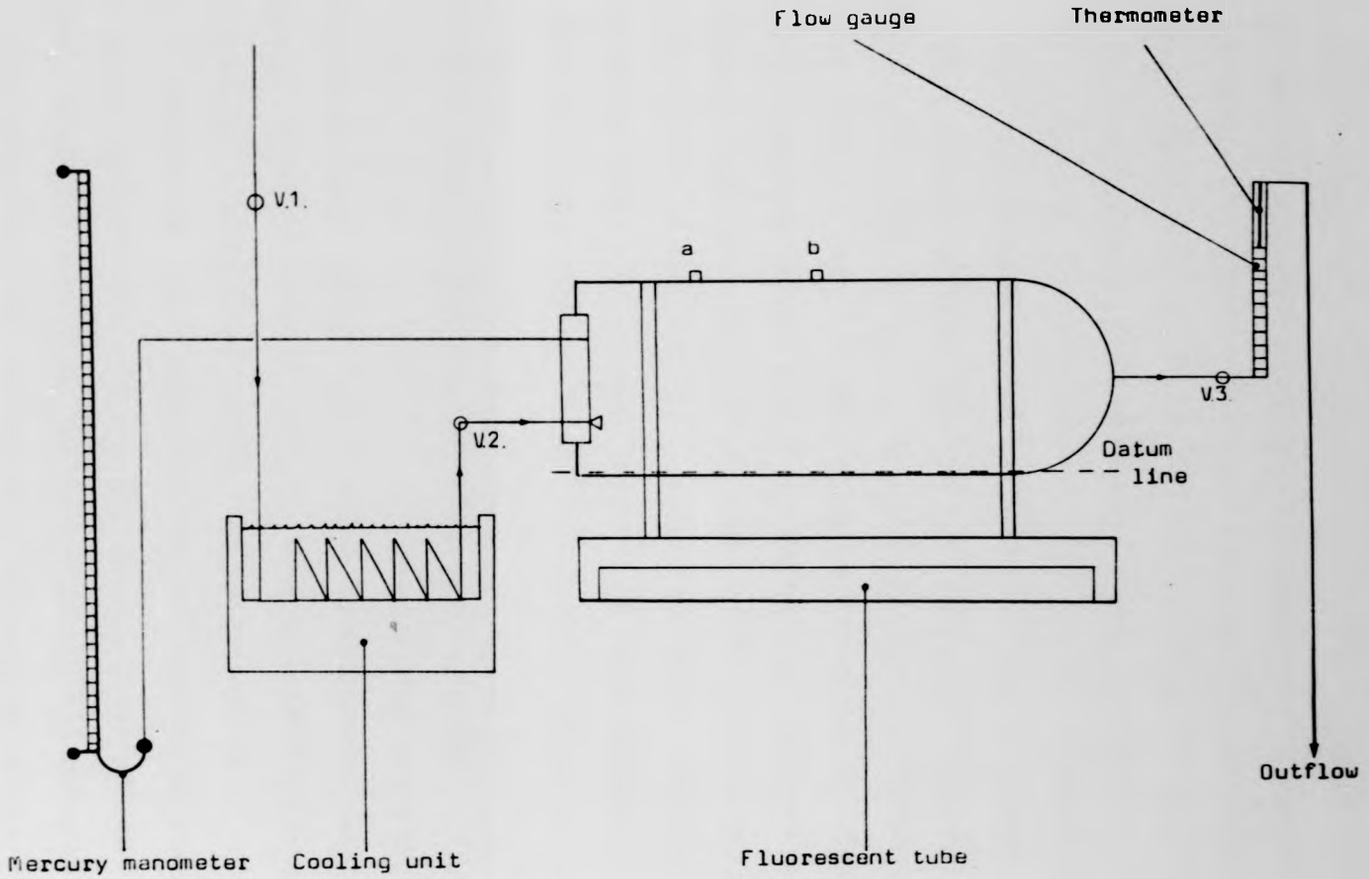

Water out
Electrode
Glass cell
Electrode membrane
Measuring chamber (50 µl)
Sample in
Water in
Electrode housing
Sample out

FIGURE 4 The pressure chamber.

a, b - access points

V_1, V_2 - inlet valves

V_3 - outlet valve


Flow gauge
Thermometer
V.1.
a
b
V.3.
V.2.
Datum
line
Outflow
Mercury manometer
Cooling unit
Fluorescent tube

fluctuation in the temperature of the water during the course of an experiment, the water supply line between the inlet valves was connected to a cooling coil immersed in the reservoir of a Hetofrig constant temperature unit. Illumination of the chamber was provided by a diffused 15 watt fluorescent tube set into the supporting framework, and the chamber was enclosed from above and behind by a protective box.

The rate of gas secretion was determined by confining individual fish in the pressure chamber and exposing them to increased hydrostatic pressure, then decompressing them to the pressure of neutral buoyancy (P.O.N.B.) at given intervals.

Before the commencement of each trial, the fish was permitted to preadapt at the proposed experimental temperature in a shallow tank for 7 to 10 days. The fish was lightly anaesthetized, blotted dry, weighed to the nearest 0.05g, and a 0.5 cm^3 sample of swimbladder gas was removed for analysis. It was then placed inside the pressure chamber, and the chamber was sealed and filled. The inlet and outlet valves were balanced to allow maximum flow through the chamber without allowing the internal pressure to fall below atmospheric pressure. After a suitable period for recovery from the effects of the anaesthetic, and to allow the fish to adjust to conditions inside the chamber (generally 48 to 72 hours), the initial P.O.N.B. was determined, and the inlet and outlet valves were adjusted to increase the internal pressure to the required level.

The P.O.N.B. was determined at 24 to 72 hour intervals during each trial by slowly decompressing the fish from the holding pressure by closing down the fine inlet valve. The P.O.N.B. was reached when the fish rose slowly from the bottom of the chamber without the aid of fin movements. After duplicate measurement of P.O.N.B., pressure was reapplied to the holding level. The trial was terminated when no further increase in P.O.N.B. was noted.

At the conclusion of each trial, the fish was decompressed in a controlled manner, then removed from the chamber and lightly anaesthetized. It was blotted dry, reweighed, and a further gas sample was removed from the swimbladder for analysis. The fish was then placed into a glass tank containing 30 cm of water at the experimental temperature, where it was held without access to the surface. Swimbladder gas samples were removed at given intervals to allow compositional changes to be followed.

Information on changes in the composition of swimbladder gas during secretion was obtained in experiments similar to those described in the previous section. After emptying the swimbladders of fish as fully as possible by aspiration, they were held in shallow tanks without access to the surface, and the swimbladder gas was sampled and analysed at given intervals.

(ii) Deflatory mechanisms

(a) Pressure thresholds for gas release during decompression

Pressure thresholds for the release of gas from the swimbladder *via* the pneumatic duct were determined in some fish at the end of buoyancy adjustment experiments conducted in the pressure chamber.

Fish were slowly decompressed from the holding pressure to their P.O.N.B. by closing down the fine inlet valve on the pressure chamber supply line. After noting the P.O.N.B., the pressure was further reduced until bubbles of gas were released *via* the mouth and opercula. The pressure at which gas release occurred was noted, and the fish was then recompressed or further decompressed to the new P.O.N.B. After noting this pressure, the fish was permitted to settle, and the procedure was repeated until the P.O.N.B. was reduced to below atmospheric pressure.

(b) Pressure thresholds for gas release in stunned fish

The degree to which fish could influence swimbladder gas

retention during decompression was assessed using stunned fish. The internal gas pressure was continuously monitored using the transducer system shown in Figure 5, as the pressure was slowly increased to the point of gas release.

A Bell and Howell physiological pressure transducer (0 to 750 mm Hg) was connected to a Devices DC.2D preamplifier and M2 pen recorder, and a three-way tap was attached to each of the Luer-lock fittings on the outlets of the transducer dome. In order to calibrate the system, a mercury manometer was connected to outlet C of tap 1 by a short section of 1 mm cannula tubing, and a 10 cm^3 polythene syringe provided with a device which allowed the plunger to be locked in position was similarly connected to the outlet C of tap 2.

The system was calibrated by opening the transducer to the atmosphere and setting the recorder pen deflection to zero. The closure levers of the three-way taps were turned to position B, and the plunger of the syringe was depressed to increase the pressure of gas in the system. The plunger was then locked in position, and after reading the gas pressure as indicated on the manometer, the equivalent pen deflection was set on the recorder using the preamplifier gain.

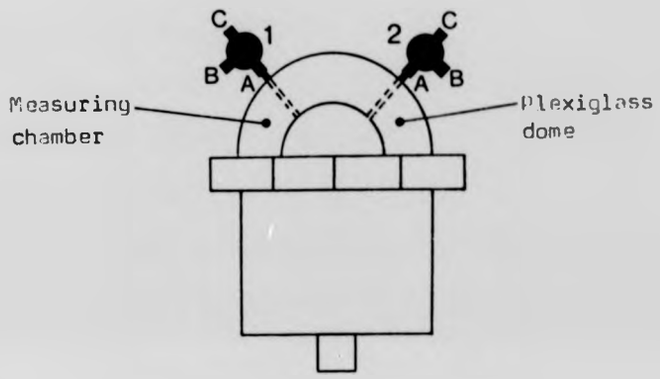
After calibration of the system, the manometer was disconnected from tap 1 and replaced by a 30 cm length of 1 mm cannula tubing with a 21-gauge hypodermic needle inserted into the distal end.

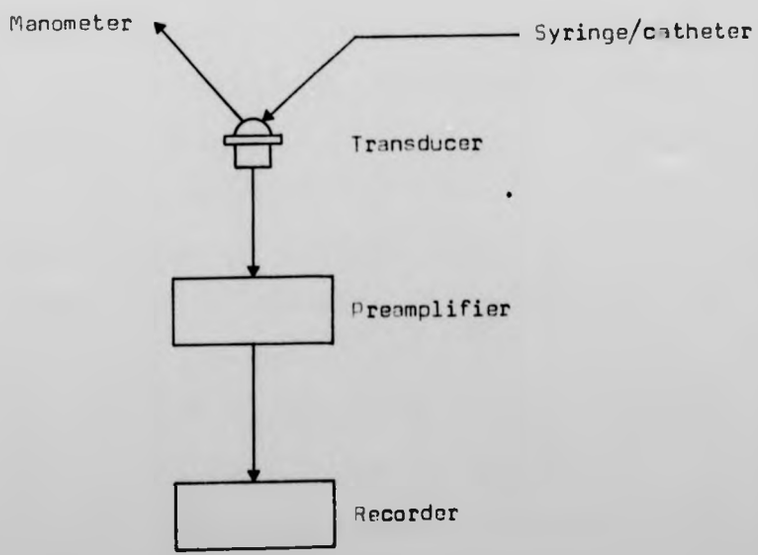
Fish were stunned by delivering a sharp blow to the top of the skull, placed flank down on a cork board, and the hypodermic needle attached to the cannula tubing was inserted into the swimbladder through the epaxial musculature. They were then transferred into a small glass tank containing sufficient water to cover them, in order to check for gas leakage at the point of entry of the needle. Pressure thresholds for gas release were then determined by slowly depressing the plunger of the syringe to increase the gas pressure inside the swimbladder,

FIGURE 5 The pressure transducer system.

1, 2 - 3-way taps

a, b, c - closure positions


C
1
2
C
B
A
A
B
Measuring chamber
Plexiglass dome


Manometer
Syringe/catheter
Transducer
Preamplifier
Recorder

until the pneumatic duct sphincter opened, allowing the escape of gas. This point was indicated by a sudden dip in the trace, and the release of gas bubbles *via* the mouth and opercula. After noting the internal pressure at gas release, the procedure was repeated several times to give a number of pressure readings for each animal.

(c) Oxygen diffusion experiments

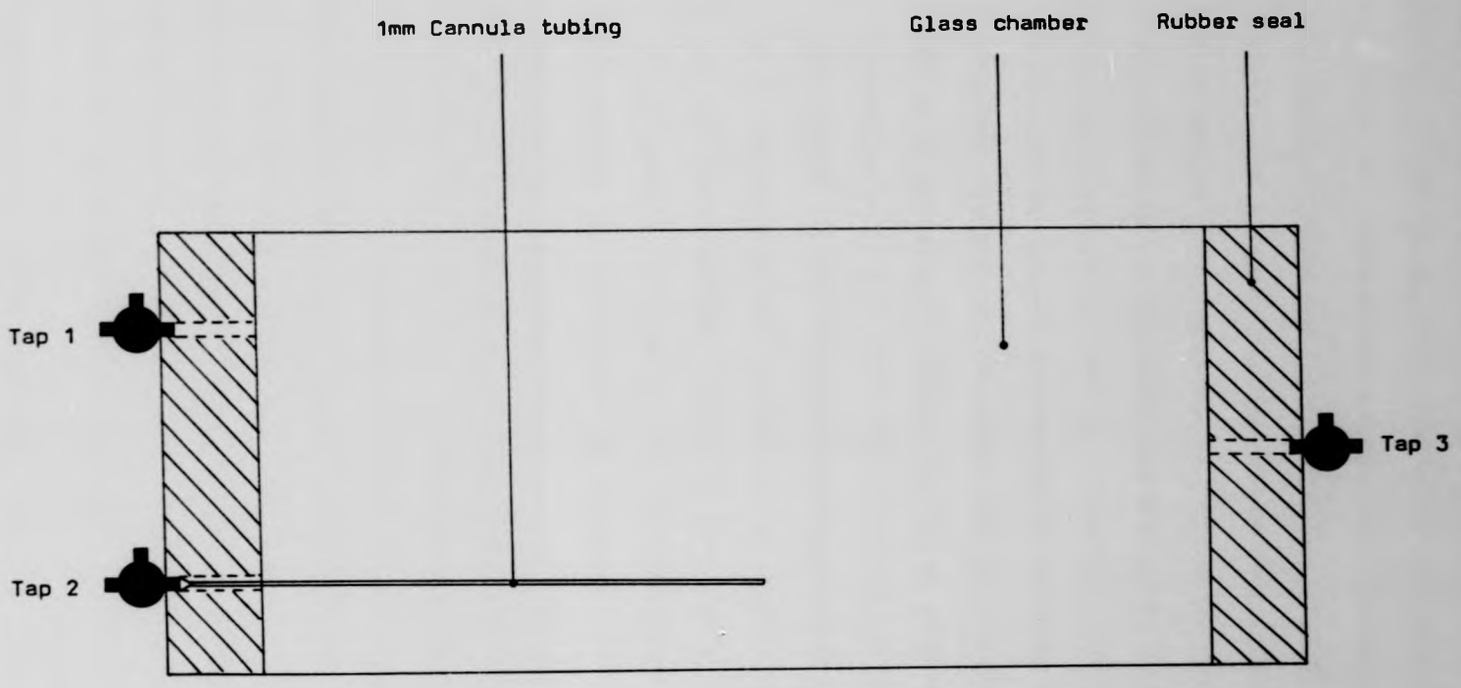
The rate of oxygen loss by diffusion through the swimbladder walls was measured at a constant temperature of 13°C, using the glass chamber illustrated in Figure 6.

The swimbladder was excised from a freshly killed fish, and the 1 mm diameter cannula tubing attached to tap 2 was passed into the pneumatic duct and secured in place with a length of surgical silk. After placing the cannulated swimbladder inside the diffusion chamber, a small quantity of physiological saline solution (Burnstock, 1958 - Appendix 2) was added to ensure that the tissue remained moist, and the swimbladder was emptied of gas by aspirating its contents into a syringe connected to tap 2. The chamber was then filled with nitrogen by passing the gas through the chamber for 2 to 3 minutes, then closing tap 3. The pressure transducer described in the previous section was then connected to tap 2 to monitor the swimbladder gas pressure during reinflation, and the swimbladder was inflated to atmospheric pressure by injecting oxygen through the dome of the transducer.

An initial 0.5 cm^3 sample of the gas in the chamber was removed for analysis *via* tap 1 after 30 minutes to check for leakage in the preparation. Further 0.5 cm^3 samples were removed at 4 to 6 hour intervals for up to 12 hours, with 0.5 cm^3 volumes of nitrogen being injected to replace the gas removed from the chamber for analysis.

The samples were analysed for oxygen content and at the conclusion of the experiment, the gas remaining inside the swimbladder was analysed for carbon dioxide.

FIGURE 6 The diffusion chamber used to measure swimbladder oxygen permeability.


1mm Cannula tubing
Glass chamber
Rubber seal
Tap 1
Tap 2
Tap 3

(iii) Buoyancy and Swimbladder Gas Pressure

(a) Excess internal pressure development under increased ambient pressure

Changes in swimbladder gas pressure during gas secretion under increased hydrostatic pressure were investigated by conducting the experiments described in Section (i)(b), using pike with catheterized swimbladders. The free end of the catheter was connected to the transducer system described previously, and the internal gas pressure was determined along with the pressure of neutral buoyancy.

(b) Stillwater experiments

All fish were held in 30 cm of water at 13°C for 10 to 14 days before buoyancy assessment and measurement of swimbladder gas pressure were carried out. Buoyancy was assessed by removing fish from the tanks and lightly anaesthetizing them. Fish which released gas at any point during the procedure were discarded. Anaesthetized fish were taken to be negatively buoyant if they came to rest horizontally on the bottom of the glass anaesthetic bath, neutrally buoyant if they floated clear of the bottom but did not rise to the surface, or if they came to rest with only the head or the tail touching the bottom of the bath, and positively buoyant if they floated at the surface. Swimbladder gas pressure was measured in anaesthetized fish using the pressure transducer system described previously. Following calibration of the system, the transducer dome was filled with physiological saline solution to reduce the compressible dead-space of the system. The syringe and cannula tubing were disconnected from the dome and replaced by a 30 cm length of 1 mm cannula tubing with a 21-gauge hypodermic needle inserted into its distal end. The swimbladder gas pressure was measured by turning the closure lever of tap 1 to position A, and inserting the tip of the needle into the swimbladder through the epaxial musculature. After determination of gas pressure, fish were returned to their tanks to permit recovery from the effects of the anaesthetic.

(c) Current experiments

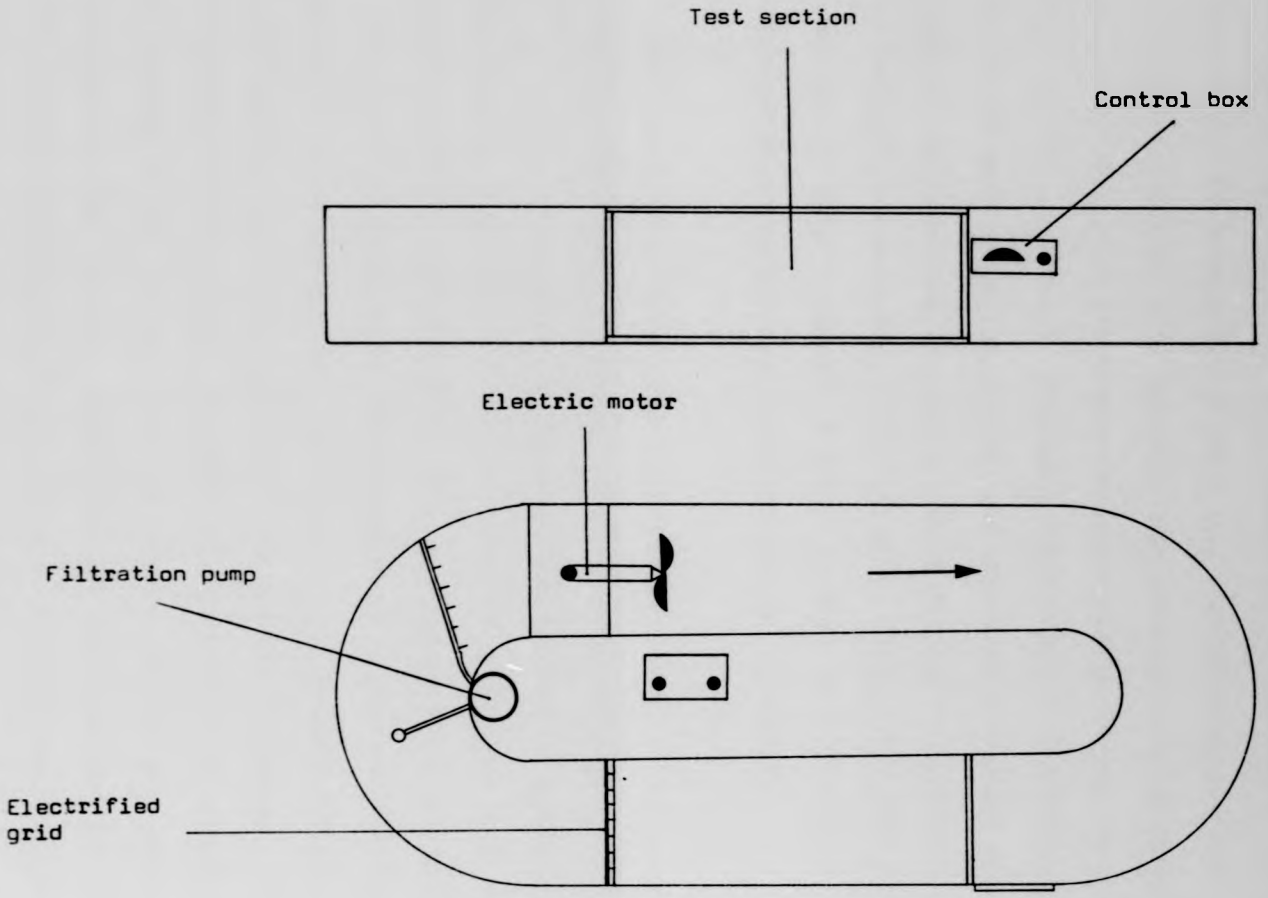
The buoyancy and swimbladder gas pressure of pike held in a current were investigated using the endless flume shown in Figure 7, modified from the basic design of Priede (1973) by Ross (in press). The water temperature was maintained at 13°C by situating the apparatus inside a controlled temperature room, and the flume water was filtered through charcoal and recirculated using an Eheim filtration pump. Current was produced by means of a 1.1 Kw electric outboard motor, powered by a 12 v motor car battery. The velocity profile of the water in the test section was partially smoothed by a system of vanes and screens, and the water velocity during experiments was set with the aid of a calibration curve constructed using a Braystoke current meter. In order to prevent fish from resting against the grid at the downstream end of the test section during the experiments, the grid was electrified using a 9 v a.c. source. Because of the internal dimensions of the test section, the maximum length of fish used in this type of experiment was 35 cm.

With the flume motor turned off, individual fish were transferred from still water tanks into the test section, where they were left undisturbed for 48 hours prior to the commencement of an experiment to allow them to settle. After this time the flume motor was switched on, and the flow rate was gradually increased to 30 cm sec^{-1}. The fish were exercised at this velocity for 11 to 13 days before assessment of buoyancy and measurement of swimbladder gas pressure was carried out. The buoyancy and swimbladder gas pressure of fish held in current were assessed using the method described above.

(d) Measurement of changes in gas pressure in the exposed swimbladder

In order to determine the degree to which swimbladder gas could be compressed by the smooth muscles of the swimbladder wall, the internal gas pressure was monitored as adrenalin and acetylcholine solutions were applied to the exposed swimbladders of freshly killed fish.

FIGURE 7 The swimming flume.

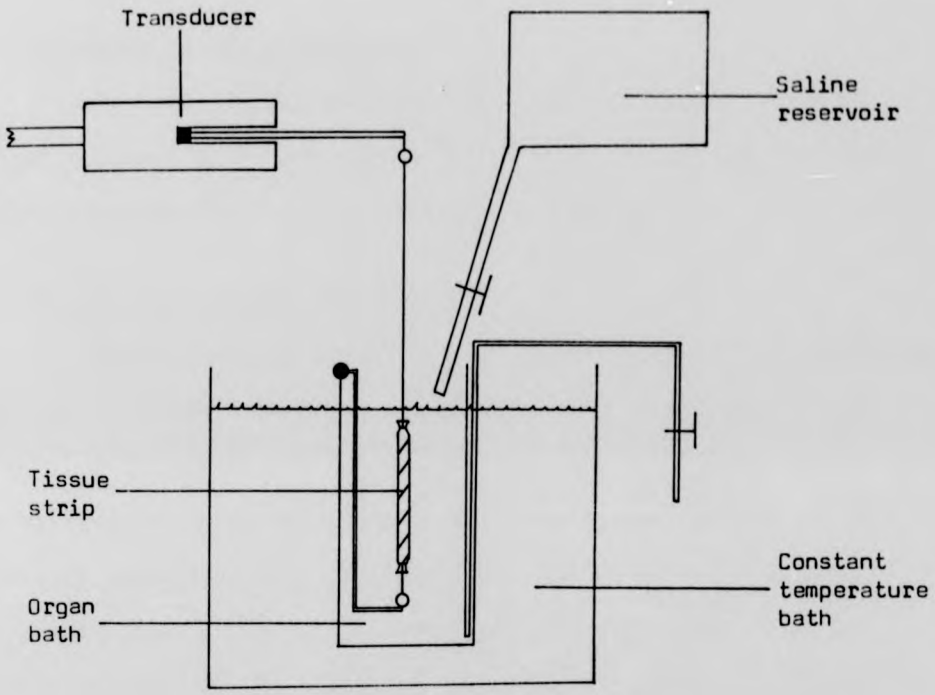

Test section
Control box
Electric motor
Filtration pump
Electrified grid

Fish were stunned, and the body cavity was opened by cutting down the ventral midline. The body wall was pinned back and the viscera were displaced to expose the swimbladder. If necessary, the pneumatic duct was tied off with a length of surgical silk. The swimbladder was then connected to the pressure transducer system using the method described above. Adrenalin, in solution in physiological saline (Appendix 2), at concentrations of 10^{-6} to 10^{-3} g cm^{-3} was applied to the swimbladder with a pasteur pipette in ascending order of concentration, with the preparation being washed with fresh saline between successive applications. After each application of adrenalin solution, any change in swimbladder gas pressure recorded by the apparatus was noted.

This procedure was then repeated after allowing a 10 minute recovery period, using acetylcholine solutions at concentrations of 10^{-7} to 10^{-3} g cm^{-3}.

Studies on isolated strips of swimbladder tissue were carried out in parallel with those on intact swimbladders, using a technique employed by Ross (1978). An R.C.A. deformable-anode isotonic transducer was connected to a Tektronix oscilloscope and Devices M2 pen recorder. Freshly isolated longitudinal and circular strips of swimbladder tissue were attached to the arm of the transducer as shown in Figure 8. Adrenalin and acetylcholine, at concentrations similar to those used above, were administered by micro-organ bath technique at a temperature of $15^{o}C$, with the bath being flushed with fresh saline between successive doses. Changes in the tonus of the tissue strips were monitored and recorded.

FIGURE 8 The micro-organ bath system.


Transducer
Saline reservoir
Tissue strip
Organ bath
Constant temperature bath

RESULTS, OBSERVATIONS AND ANALYSIS

THE SWIMBLADDER

(i) The volume of the swimbladder

The mean percentage swimbladder volume required to confer neutral buoyancy in the pike was found to be 6.9% (s.e $\pm$ 0.3%) volume/weight, and 7.5% (s.e $\pm$ 0.3%) volume/volume (Table 1).

(ii) The structure of the swimbladder

The results show that the pike swimbladder is of the physostomous type, and is a single chambered, elongate sac which lies dorsally in the body cavity between the alimentary canal and the kidney. It is outwith the peritoneum which covers only its ventral surface. It was found that the swimbladder is very firmly attached to the body wall musculature along lines which run ventrally to the lateral margins of the kidney. The presence and location of these lines of attachment permitted access to the swimbladder with reduced risk of gas leakage into the body cavity.

A single connection between the alimentary canal and the swimbladder exists at the anterior end of the swimbladder, where the pneumatic duct joins it with the oesophagus. A muscular sphincter at the posterior end of the duct closes the swimbladder. The pneumatic duct appears to be a relatively simple structure, showing no particular specialization for pumping air into the swimbladder such as the 'pneumatic bulb' found in the cyprinids (Evans and Damant, 1929).

The position and proportions of the swimbladder, and the location of the pneumatic duct and its sphincter in a live, intact animal are shown in Plate 1.

The terminology adopted by various authors for the layers

TABLE 1 Mean tissue density and percentage swimbladder volumes in *Esox lucius*.

	Mean	± S.e.	n
Mean tissue density ($g\ cm^{-3}$)	1.077	0.004	14
Percentage swimbladder volume (v/w)	6.9	0.3	14
Percentage swimbladder volume (v/v)	7.5	0.3	14

PLATE 1 The position and proportions of the swimbladder in a live, intact specimen of Esox lucius, as shown by X-ray.

P.d. = Pneumatic duct.

S = Sphincter.


P.d.
S


P.d.
S

of tissue in the swimbladder wall is very varied. Fänge (1953) reviewed the existing terminology, and proposed a new system of nomenclature based on the layers of the gut wall, from which the swimbladder is derived embryologically, and his system is adopted in this thesis.

The wall of the pike swimbladder consists of two clearly distinguishable layers; the outer tunica externa, covered ventrally by the peritoneal serosa, and the complex tunica interna.

The tunica externa in comprised mainly of a dense matrix of collagenous material with associated elastic fibres. The tunica interna contains fine vessels of the blood vascular system, and circular and longitudinal smooth muscle fibres in the lamina propria, which underlies the thin columnar epithelium lining the swimbladder. The general microstructure of the pike swimbladder is shown in Plate 2.

(iii) The vascularization of the swimbladder

Studies on the vascularization of the pike swimbladder indicate that two blood supply systems exist. The anterior part of the swimbladder is supplied with blood by the swimbladder artery, a branch of the coeliacomesenteric artery. After penetrating the wall of the swimbladder near the pneumatic duct, the artery divides into four branches, and each of these ramifies to produce a plexus of closely-packed capillaries. Afferent and efferent capillaries run in close proximity to each other beneath the lamina propria and epithelial layer, forming microretia mirabilia, and efferent capillaries coalesce to drain into the hepatic portal system.

Blood is supplied to the walls of the posterior portion of the swimbladder by short vessels which run from the segmental intercostal arteries in the body wall. On penetrating the swimbladder walls, these branch, giving rise to arboriform capillary patterning, and the efferent capillaries coalesce to drain into the posterior cardinal vein. These

PLATE 2 The general microstructure of the swimbladder wall in Esox lucius (Erlich's H and E).

T.Ext. = Tunica externa.
T.Int. = Tunica interna.
E. = Epithelium.
L.P. = Lamina propria.
L. = Lumen of swimbladder.


L
E


L
E
L

findings generally confirm the results of Corning (1888) and Coggi (1889).

The vascular system of the pike swimbladder is shown schematically in Figure 9, and the capillary branching patterns in the anterior and posterior parts of the swimbladder are shown in Plate 3.

(iv) The purine content of the swimbladder

The purine content of the anterior and posterior parts of the pike swimbladder was determined using tissue samples removed from five animals. The mean purine content of the anterior portion was 43.0μgcm^{-2} (s.e. $\pm$ 2.20μgcm^{-2}), and that of the posterior portion was 32.1μgcm^{-2} (s.e. $\pm$ 1.88μgcm^{-2}) (Table 2).

The mean purine level found in the anterior part of the pike swimbladder is significantly greater than the level found in the posterior part ($p < 0.01$).

FIGURE 9 Schematic representation of the vascular system of the pike swimbladder.

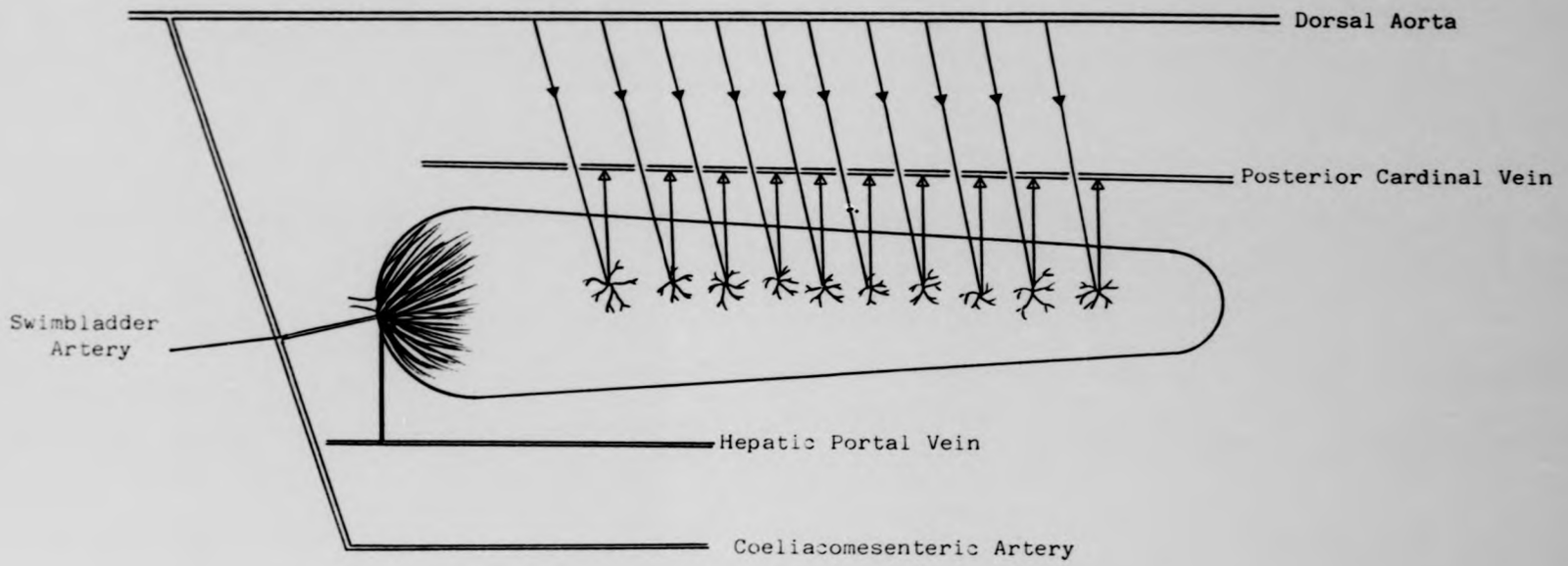

Dorsal Aorta
Posterior Cardinal Vein
Swimbladder Artery
Hepatic Portal Vein
Coeliacomesenteric Artery

PLATE 3 The capillary branching patterns in the anterior and posterior parts of the pike swimbladder.

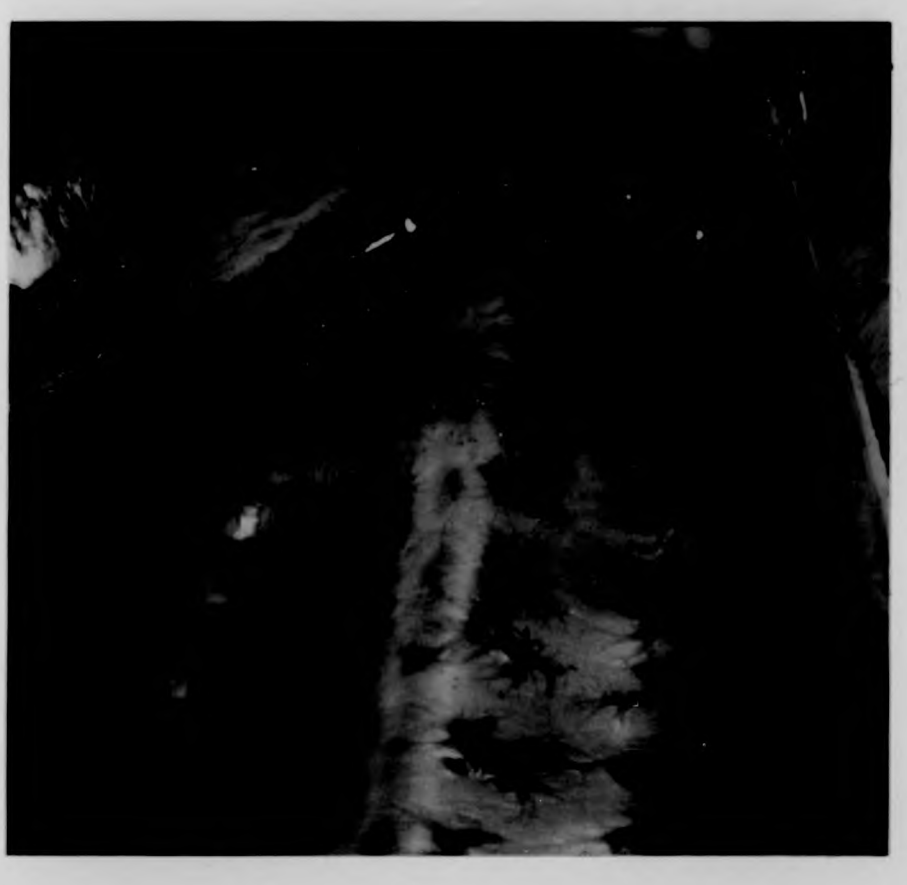


TABLE 2 The purine content of the swimbladder in *Esox lucius*.

	Purine content ($\mu g\ cm^{-2}$)	± S.e.	n
Anterior swimbladder	43.0	2.20	5
Posterior swimbladder	32.1	1.88	5
Mean	37.6	3.23	5

THE PHYSIOLOGY OF THE SWIMBLADDER

(i) Inflatory Mechanisms

(a) Air-gulping experiments

The swimming and air-gulping behaviour of pike rendered negatively buoyant by gas removal was determined using ten fish, which ranged in size from 54.0 to 263.3g.

In experiments where free access to the air-water interface was available, the characteristic pattern of behaviour exhibited involved fish swimming from the bottom of the tank to break the surface and take air into the mouth. Initially, upward excursions appeared to be undertaken only with great difficulty, with fish making several attempts to reach the surface. Successful excursions resulted in fish reaching the surface, where the snout was pushed out of the water, while station was held at approximately 45^{o} to the horizontal by vigorous movement of the caudal and ventral paired fins. The mouth was then opened and closed rapidly, thus enclosing a volume of air, as the fish altered its body attitude to descend. Descent was usually accompanied by the escape of bubbles *via* the opercula.

The position occupied in the water column by fish during the process of refilling the swimbladder by air-gulping changed with time. At the beginning of the trials, fish spent a high proportion of the time resting on the bottom, but as the swimbladder was gradually refilled, the fish swam actively in midwater, until such times as they closely approached or attained a state of neutral buoyancy. At around neutral buoyancy, the body was held almost motionless with only very slight movements of the paired ventral fins, and the posterior margins of the dorsal and anal fins, being discernible.

In trials where access to the air-water interface was temporarily

prevented by the presence of the surface screen, fish attempted to gain access to the air, but being prevented from doing so, settled on the bottom of the tank. When the screen was removed after 6 hours, an identical pattern of behaviour to that described above was observed after a mean latency period of 6 minutes (s.e. $\pm$ 1.4 minutes).

Tables 3(a) and 3(b) show air-gulping frequencies and times taken to attain neutral buoyancy by air-gulping after gas removal.

The relationship between the volume of gas removed and the time taken to attain neutral buoyancy by air-gulping, after the removal of a relatively constant proportion of the swimbladder contents by aspiration is shown in Figure 10(a). In both plots, a positive correlation of time with volume removed exists. The correlation coefficients of 0.64 and 0.69 respectively for "free access" and "delayed access" data are significant at the 5% level. No significant difference was found to exist between the mean times required to refill the swimbladder when free access to the surface was available, and when access was delayed for six hours.

The relationship between the gas volume removed and the number of occasions air was gulped to produce neutral buoyancy is shown in Figure 10(b). The positive correlation coefficients of 0.78 for "free access" data and 0.69 for "delayed access" data are significant at the 1% and 5% levels respectively. No significant difference was found to exist between the mean number of times air was gulped to produce neutral buoyancy with free and delayed access. Analysis also indicates that the frequency of air-gulping was relatively uniform throughout the refilling process (Tables 3(a) and 3(b)). No significant difference was found between the mean number of gulps of air taken in successive thirds of the total time taken to attain neutral buoyancy.

Changes in the composition of swimbladder gas in fish permitted

TABLE 3(a) Air-gulping frequency in pike following swimbladder gas removal by aspiration. (In these trials, free access to the surface was immediately available).

T_1, T_2, T_3 The number of occasions on which air was gulped in each third of the total time from the first gulp to neutral buoyancy.

Fish No.	Swimbladder volume (cm^3)	Gas volume removed (cm^3)	% Reduction in gas content	Time from 1st gulp to neutral buoyancy (mins)	Number of gulps to neutral buoyancy	T_1, T_2, T_3
1	3.7	2.8	76	32	5	3, 1, 1.
2	4.7	4.3	91	42	7	4, 2, 2.
3	5.7	5.1	89	40	12	3, 3, 6.
4	7.6	6.7	88	38	12	3, 8, 1.
5	9.5	8.9	94	134	11	8, 2, 1.
6	12.0	10.9	91	51	15	4, 0, 11.
7	12.1	10.2	84	128	42	17, 14, 11.
8	13.4	12.1	90	58	21	4, 7, 10.
9	14.2	10.8	76	161	34	14, 13, 7.
10	18.1	14.3	79	133	45	16, 17, 12.
	$\bar{x}$ = 10.1	$\bar{x}$ = 8.6	$\bar{x}$ = 85.8	$\bar{x}$ = 81.7	$\bar{x}$ = 20.4	$\bar{x}$ = 7.6, 6.7, 6.1.
	S.e. = 1.5	S.e. = 1.2	S.e. = 2.1	S.e. = 16.0	S.e. = 4.6	S.e. = 1.8, 1.9, 1.5.

Fish No.	Swimbladder volume (cm^3)	Gas volume removed (cm^3)	% Reduction in gas content	Time from 1st gulp to neutral buoyancy (mins)	Number of gulps to neutral buoyancy	T_1, T_2, T_3
1	3.7	2.8	76	32	5	3, 1, 1.
2	4.7	4.3	91	42	7	4, 2, 2.
3	5.7	5.1	89	40	12	3, 3, 6.
4	7.6	6.7	88	38	12	3, 8, 1.
5	9.5	8.9	94	134	11	8, 2, 1.
6	12.0	10.9	91	51	15	4, 0, 11.
7	12.1	10.2	84	128	42	17, 14, 11.
8	13.4	12.1	90	58	21	4, 7, 10.
9	14.2	10.8	76	161	34	14, 13, 7.
10	18.1	14.3	79	133	45	16, 17, 12.
	$\bar{x}$ = 10.1	$\bar{x}$ = 8.6	$\bar{x}$ = 85.8	$\bar{x}$ = 81.7	$\bar{x}$ = 20.4	$\bar{x}$ = 7.6, 6.7, 6.1.
	S.e. = 1.5	S.e. = 1.2	S.e. = 2.1	S.e. = 16.0	S.e. = 4.6	S.e. = 1.8, 1.9, 1.5.

TABLE 3(b) Air-gulping frequency in pike following swimbladder gas removal by aspiration. (In these trials, access to the surface was delayed for 6 hours).

T_1, T_2, T_3 The number of occasions on which air was gulped in each third of the total time from the first gulp to neutral buoyancy.

Fish No.	Swimbladder volume (cm^3)	Gas volume removed (cm^3)	% Reduction in gas content	Time from 1st gulp to neutral buoyancy (mins)	Number of gulps to neutral buoyancy	T_1, T_2, T_3
2	4.7	4.1	87	27	9	5, 3, 1
3	5.7	5.0	88	39	15	9, 2, 4
5	9.5	8.7	91	95	7	3, 3, 1
6	12.0	11.0	92	42	19	8, 7, 4
7	12.1	10.5	87	56	24	9, 6, 9
8	13.4	12.2	91	76	16	7, 5, 3
9	14.2	12.0	84	134	22	9, 6, 7
10	18.1	13.2	73	112	34	13, 11, 10
	$\bar{x}$ = 11.2	$\bar{x}$ = 9.6	$\bar{x}$ = 86.6	$\bar{x}$ = 72.6	$\bar{x}$ = 18.2	$\bar{x}$ = 7.8, 5.4, 4.9
	S.e. = 1.6	S.e. = 1.2	S.e. = 2.2	S.e. = 13.6	S.e. = 3.0	S.e. = 1.1, 1.0, 1.2

FIGURE 10(a) The relationship between gas volume removed from the swimbladder, and the total time taken to attain neutral buoyancy by air-gulping.

FREE ACCESS DATA (▲)

Regression line (---) with 95% confidence intervals: $y = 8.7x + 6.8$

$r = 0.642$

DELAYED ACCESS DATA (△)

Regression line (——) with 95% confidence intervals: $y = 7.6x - 0.4$

$r = 0.689$

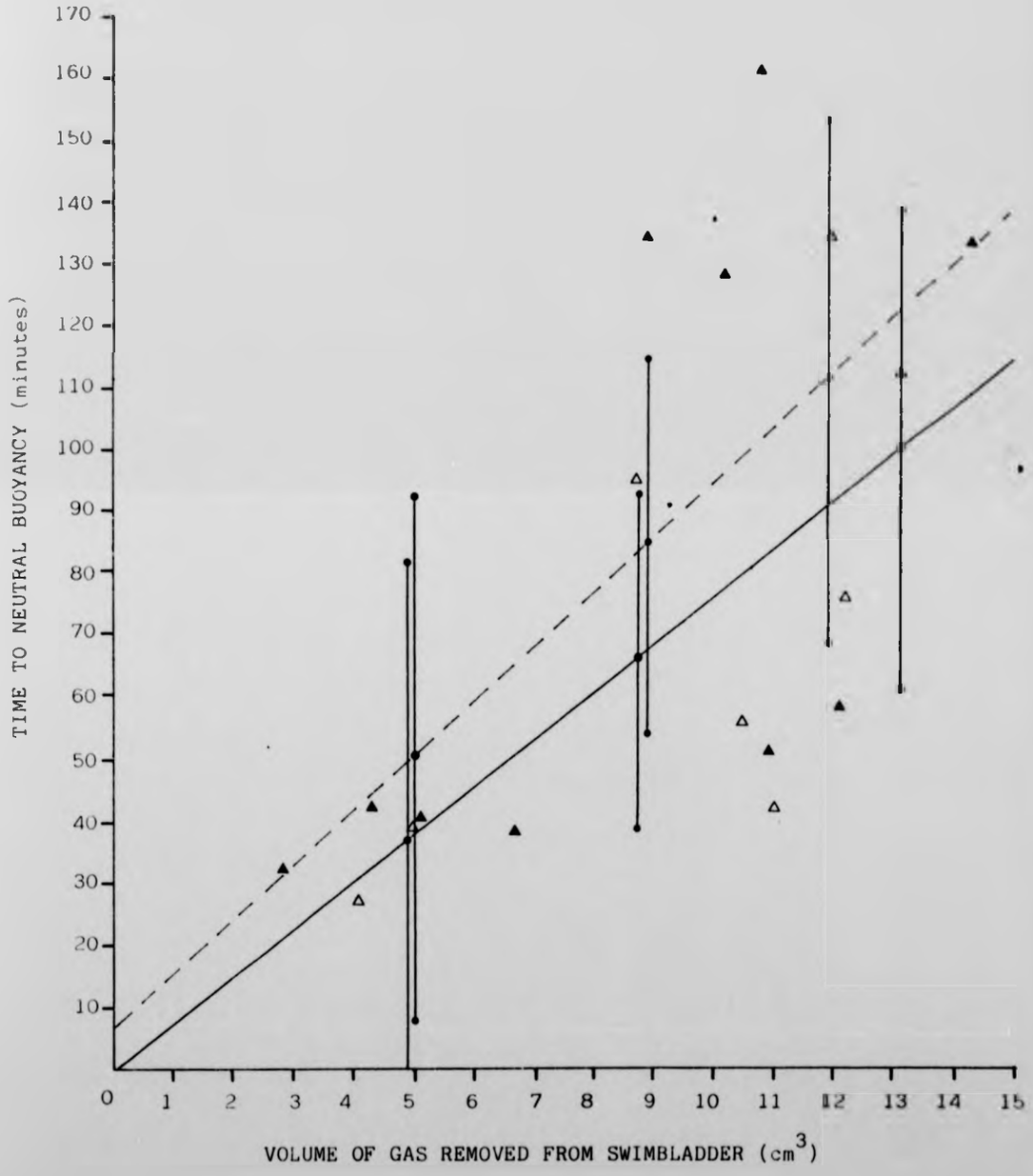

TIME TO NEUTRAL BUOYANCY (minutes)
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
VOLUME OF GAS REMOVED FROM SWIMBLADDER (cm3)

FIGURE 10(b) The relationship between gas volume removed from the swim-bladder, and the total number of occasions air was gulped to produce neutral buoyancy.

FREE ACCESS DATA (▲)

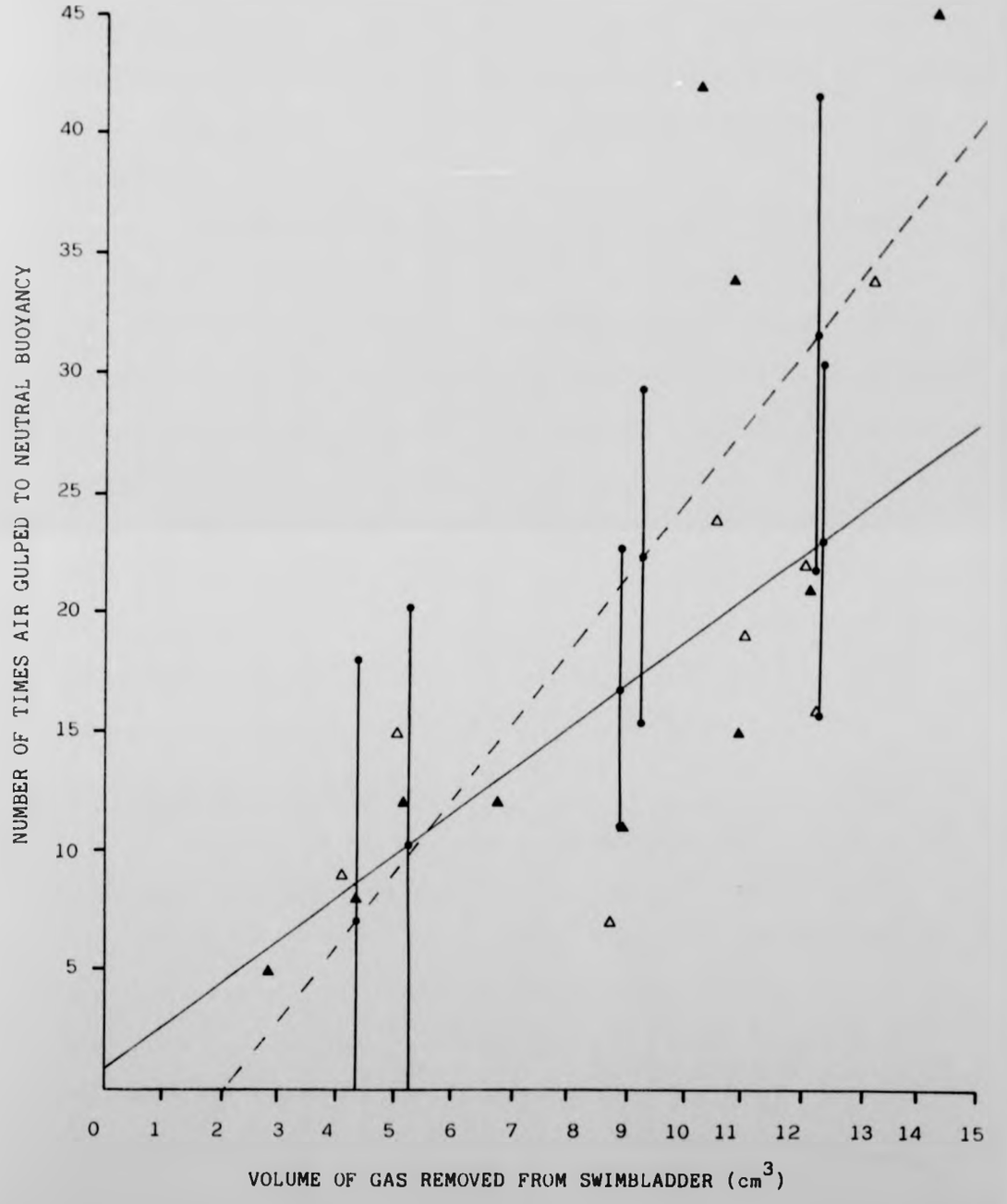
Regression line (---) with 95% confidence intervals: $y = 3.1x - 5.9$

$r = 0.779$

DELAYED ACCESS DATA (△)

Regression line (——) with 95% confidence intervals: $y = 1.8x + 0.9$

$r = 0.693$


NUMBER OF TIMES AIR GULPED TO NEUTRAL BUOYANCY
45
40
35
30
25
20
15
10
5
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
VOLUME OF GAS REMOVED FROM SWIMBLADDER (cm3)

to refill their swimbladders with free access to the surface were determined using eight pike over 20 day periods. The results are shown in Figure 11. The plotted oxygen and carbon dioxide values are corrected for the influence of gas remaining in the swimbladders after these were emptied as fully as possible.

The mean percentage of oxygen present in gas samples taken two days after the swimbladders were emptied was significantly greater than the level of oxygen present in atmospheric air ($p < 0.01$). After four days, the level of swimbladder oxygen had fallen to that of atmospheric air, and this downward trend continued to 20 days, when gas sampling was discontinued.

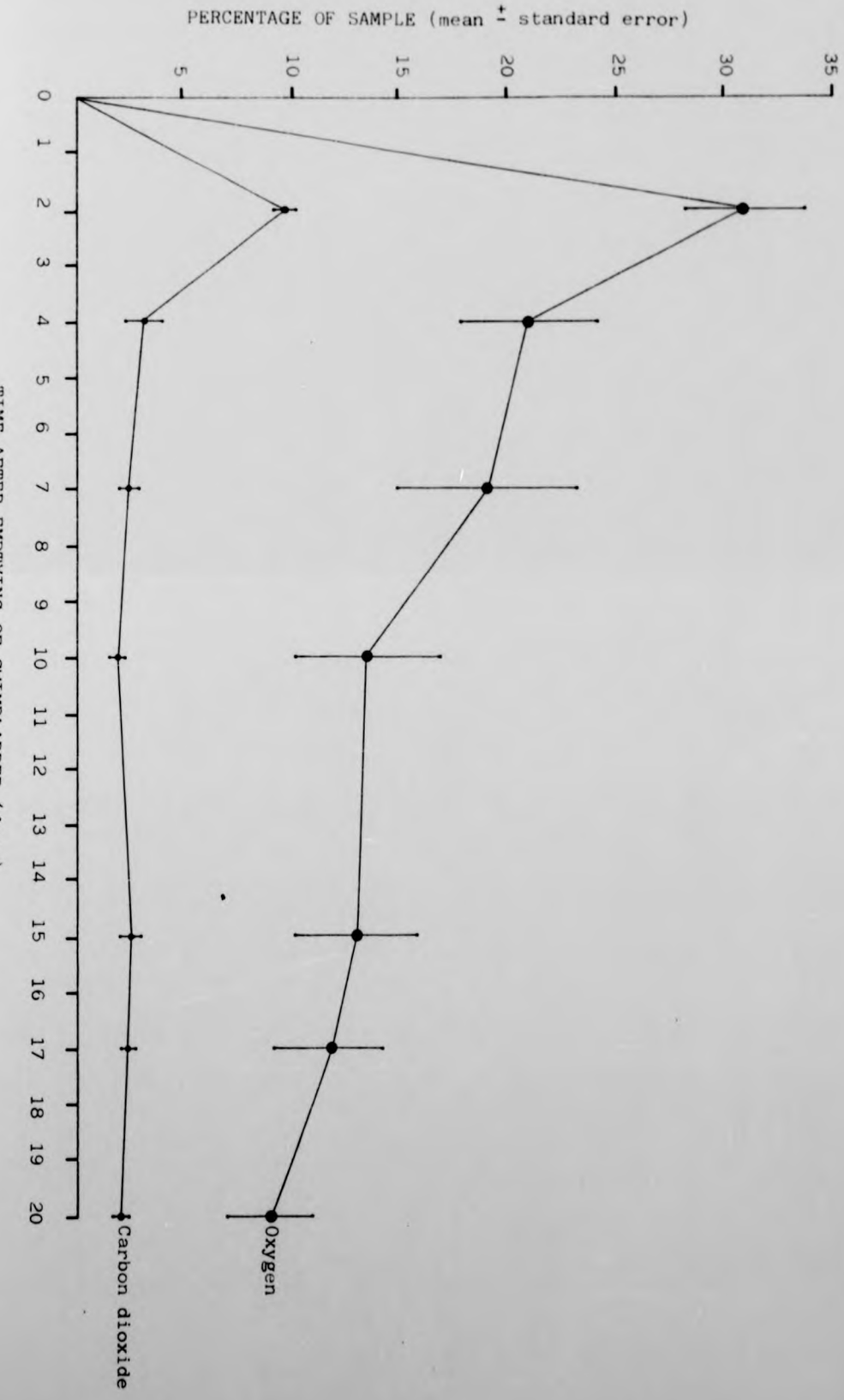
The mean percentage of swimbladder carbon dioxide was also significantly greater than atmospheric carbon dioxide at two days ($p < 0.001$), and although the initially high level of the gas in the samples was reduced after two days, it still remained significantly greater than the level of atmospheric carbon dioxide up to 20 days.

(b) Gas secretion experiments

The capacity for adjustment of buoyancy by gas secretion under conditions of increased hydrostatic pressure was determined using 16 pike, ranging in size from 63.9 to 360.0g. Plots of pressure of neutral buoyancy against time for each experimental temperature are shown in Figures 12(a) to 12(f). The net mean and maximum rates of gas secretion were calculated using Boyle's Law, making the assumption that any excess internal pressure developed was negligible. The results are given in Table 4.

The rates and extent of adjustment of buoyancy by gas secretion in response to an increase in hydrostatic pressure of 760mmHg (to 2 ATA) are very variable, and no relationship between buoyancy adjustment rates and environmental temperature or size is apparent. Mean rates of gas

FIGURE 11 Changes in the composition of the swimbladder contents in pike which refilled their swimbladders with free access to the surface. (n = 8).


PERCENTAGE OF SAMPLE (mean ± standard error)
5
10
15
20
25
30
35
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
TIME AFTER EMPTYING OF SWIMBLADDER (days)
Oxygen
Carbon dioxide

FIGURES 12(a) to 12(f) Buoyancy adjustment by gas secretion in pike exposed to increased hydrostatic pressure at ambient temperatures of 10 to 20°C.

(1 - 16 : fish numbers, Table 4).

Figure 12 (a) 10°C

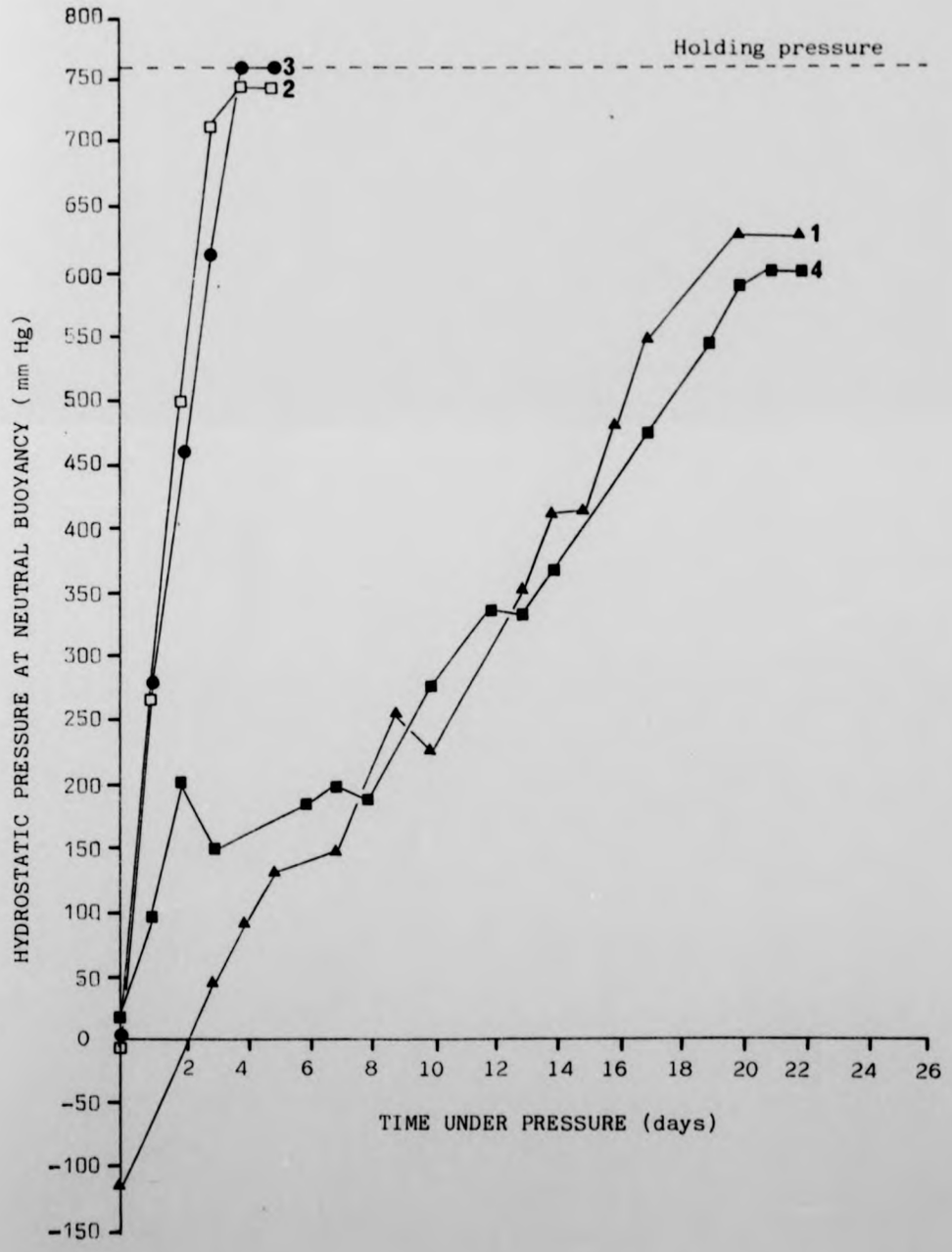


Figure 12 (b) 12°C

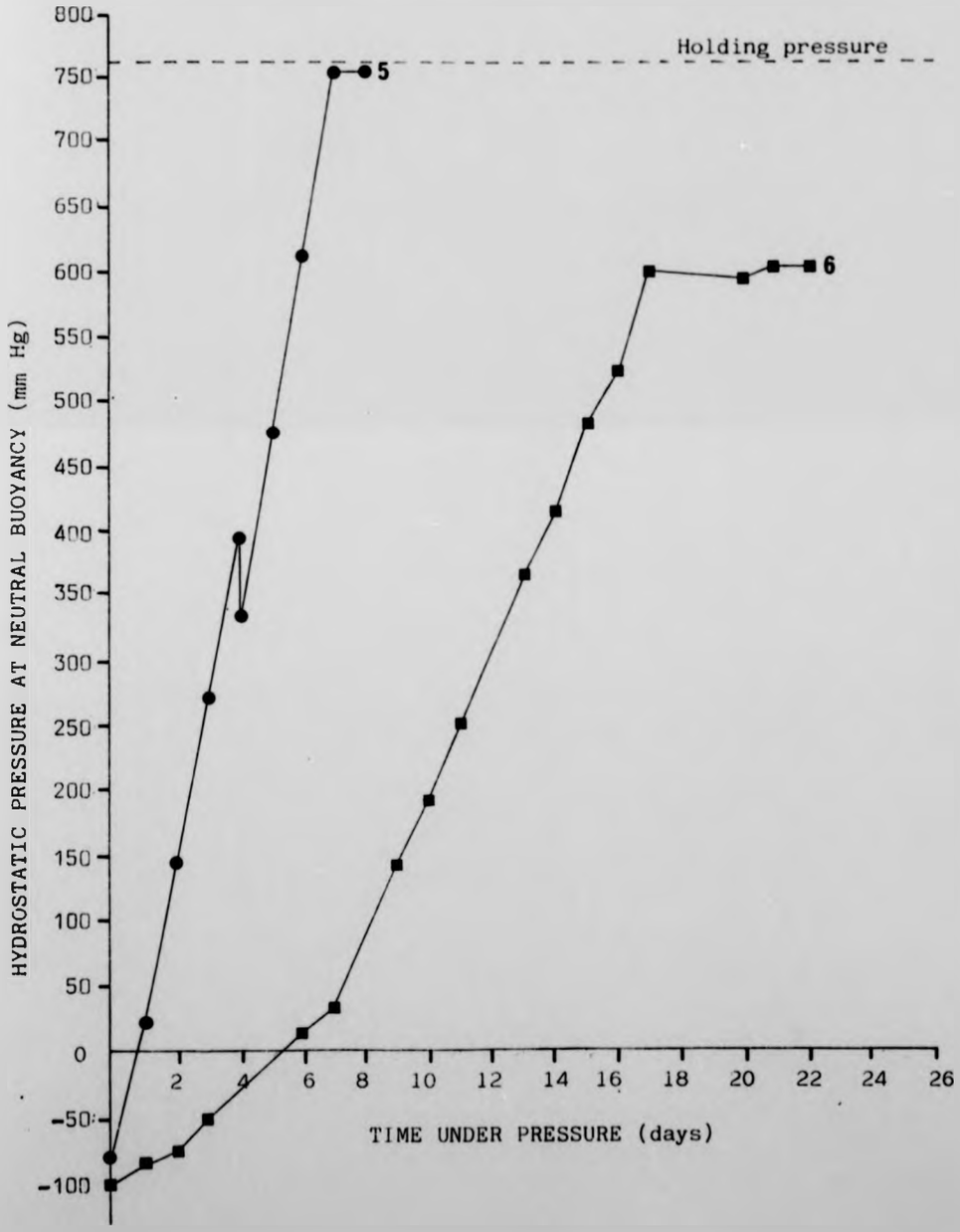


Figure 12 (c) $14^{o}C$

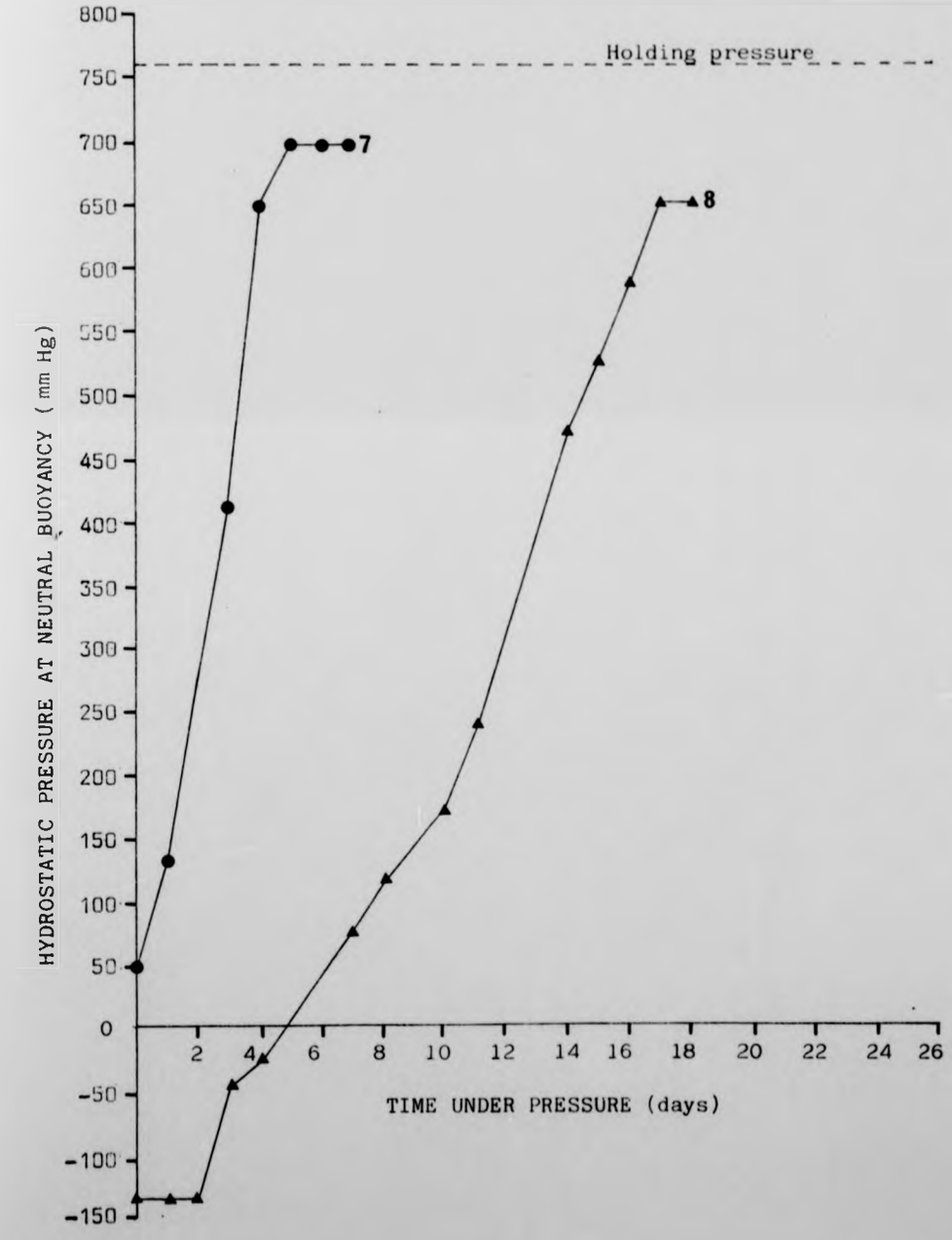


Figure 12 (d) $16^{\circ}C$

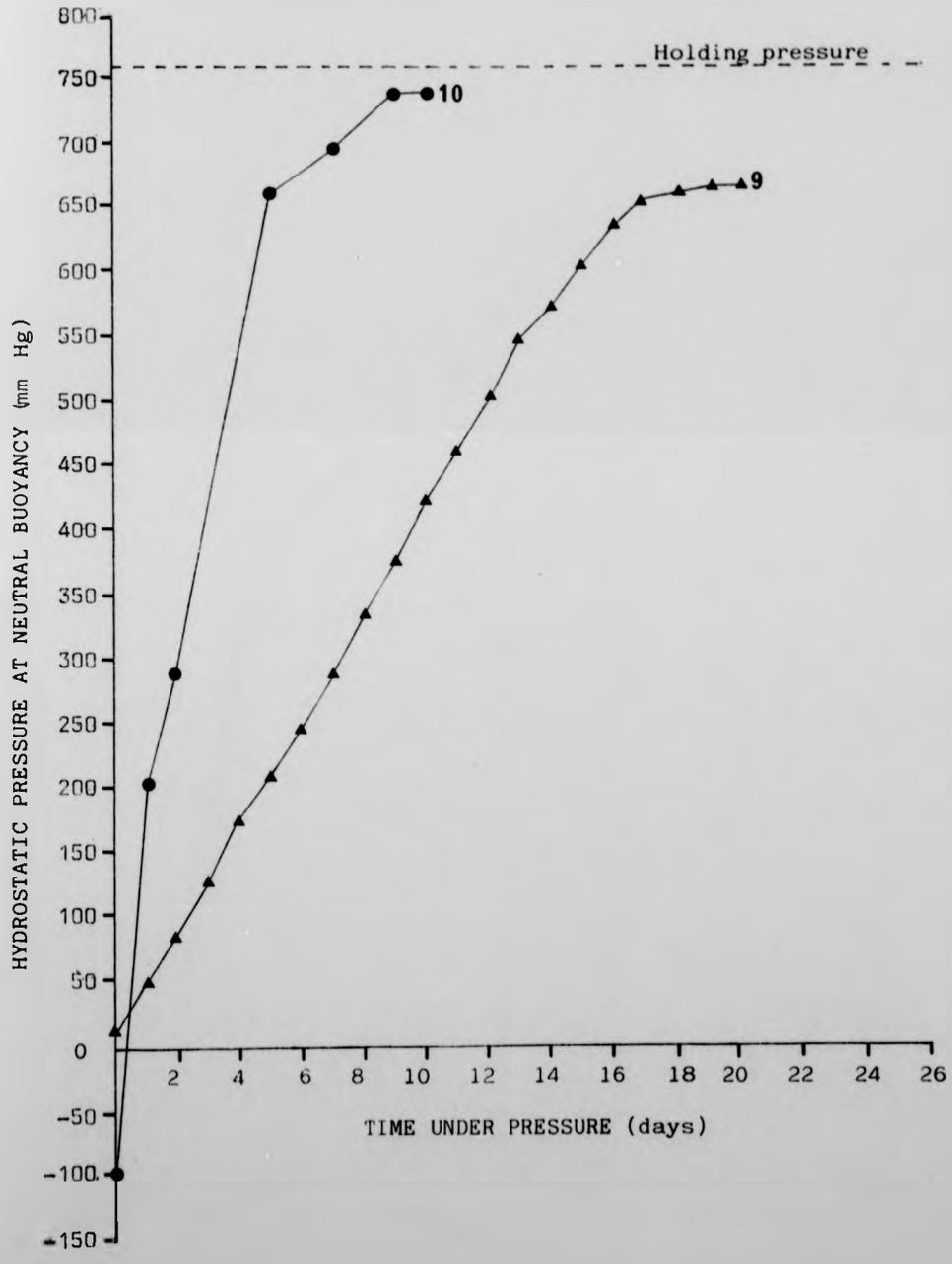


Figure 12 (a) $18^{o}C$

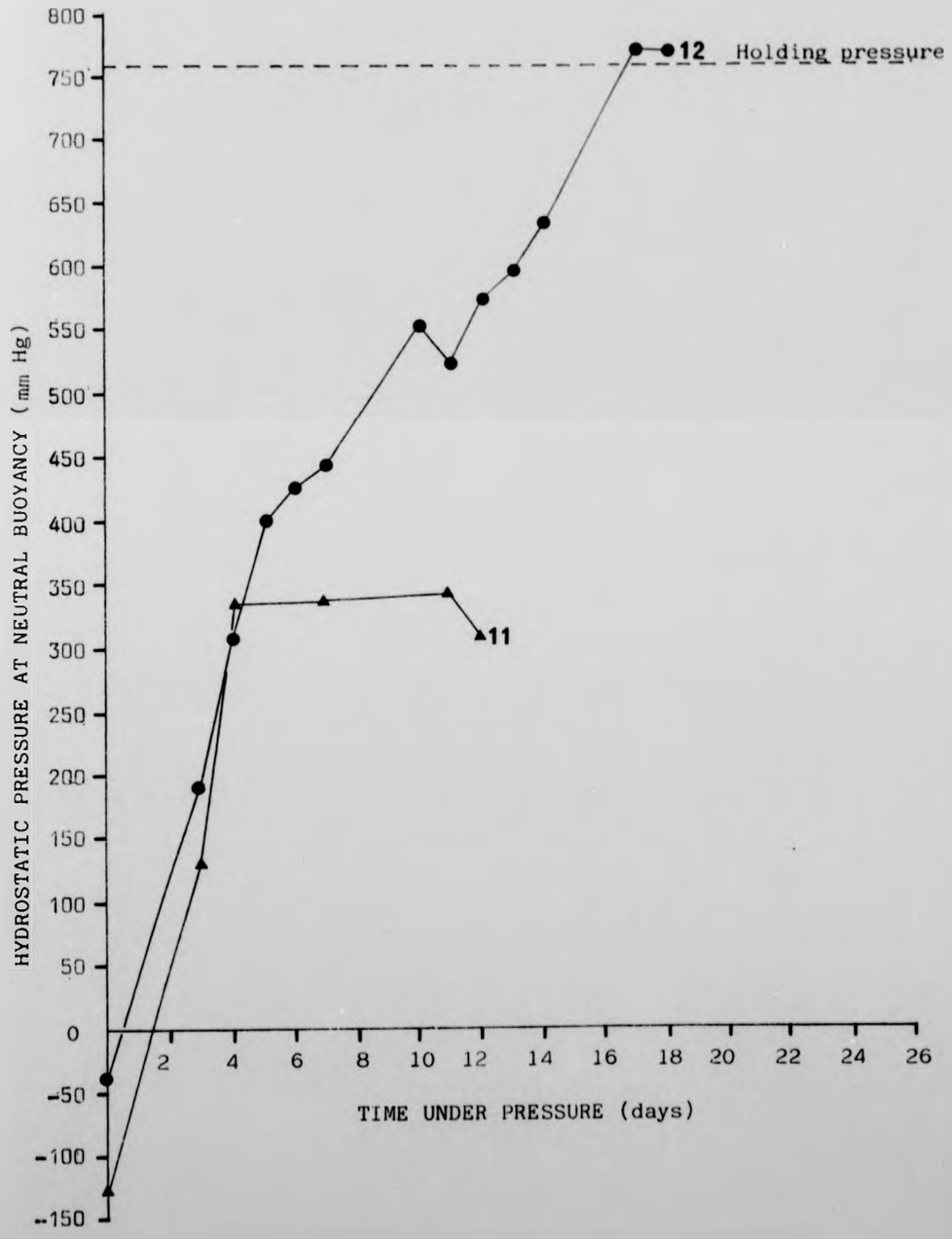


Figure 12 (f) 20°C

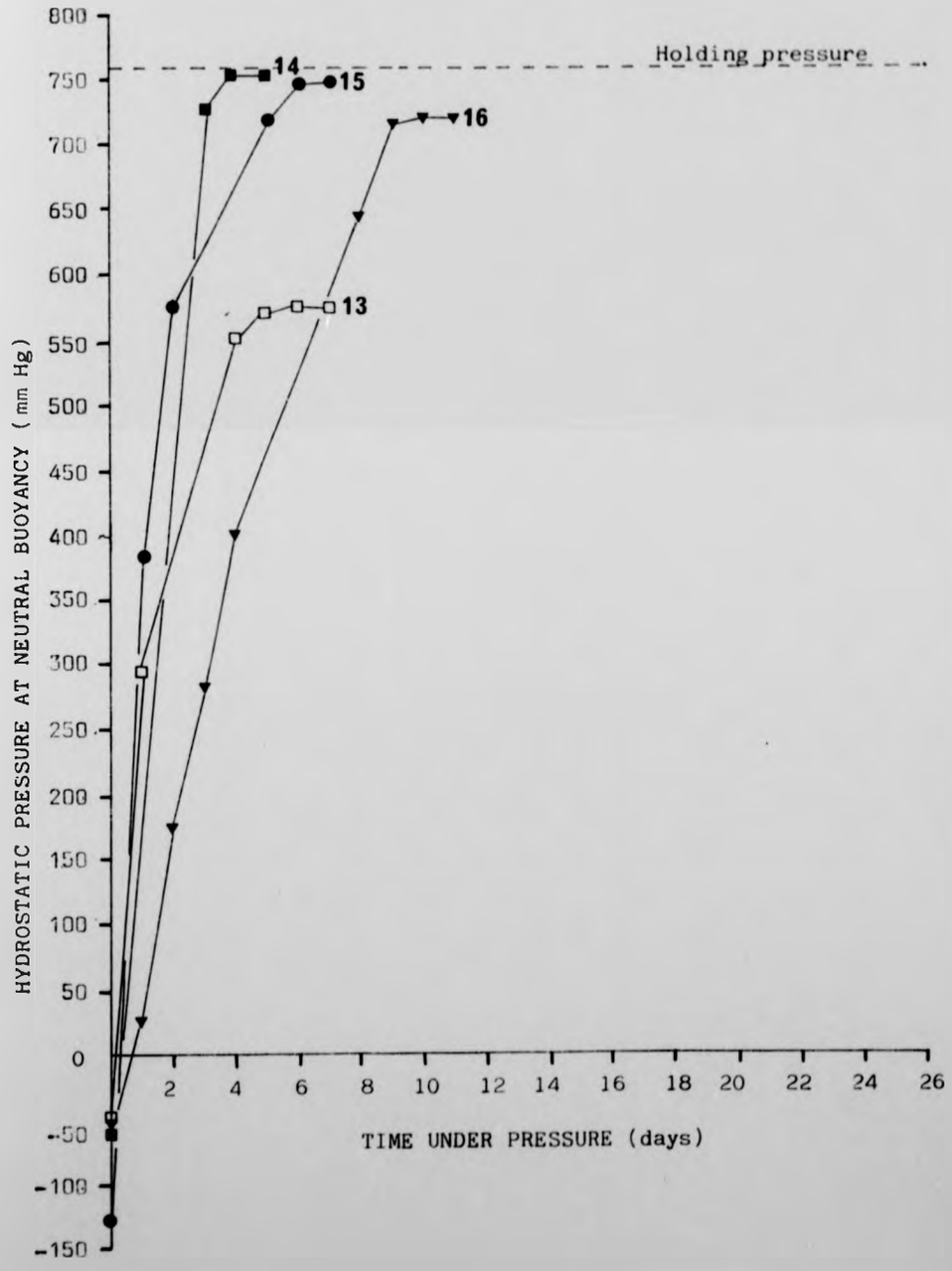


TABLE 4 Net gas secretion rates in pike, calculated from rates of buoyancy adjustment under increased hydrostatic pressure.

(Sample standard errors in parentheses).

Temperature (°C)	Fish No. (Fig. 12)	Mean gas secretion rate $cm^3 hr^{-1}$	$cm^3 kg^{-1} hr^{-1}$	Maximum gas secretion rate $cm^3 hg^{-1}$	$cm^3 kg^{-1} hr^{-1}$
10	4	0.01(0.003)	0.12(0.02)	0.02	0.21
	1	0.02(0.005)	0.17(0.04)	0.07	0.45
	2	0.05(0.009)	0.72(0.19)	0.07	1.12
	3	0.05(0.004)	0.74(0.10)	0.07	1.05
12	6	0.02(0.002)	0.18(0.02)	0.04	0.33
	5	0.04(0.003)	0.31(0.01)	0.05	0.34
14	8	0.02(0.003)	0.17(0.03)	0.04	0.35
	7	0.05(0.008)	0.36(0.07)	0.08	0.57
16	9	0.04(0.004)	0.12(0.01)	0.06	0.18
	10	0.04(0.020)	0.42(0.18)	0.12	1.14
18	12	0.02(0.003)	0.18(0.04)	0.04	0.44
	11	0.08(0.050)	0.22(0.15)	0.27	0.76
20	16	0.04(0.007)	0.33(0.06)	0.07	0.58
	14	0.06(0.035)	0.59(0.38)	0.10	1.06
	15	0.13(0.067)	0.74(0.38)	0.35	1.96
	13	0.15(0.080)	0.57(0.30)	0.36	1.34

secretion, calculated over the whole period of adjustment, range from $0.12cm^3kg^{-1}hr^{-1}$ (fish 4 and 9) to $0.74cm^3kg^{-1}hr^{-1}$ (fish 3 and 15), with a maximum observed rate of $1.96cm^3kg^{-1}hr^{-1}$ (fish 15). Two of the 16 pike adjusted to neutral buoyancy at pressures slightly above the holding pressure (fish 3 and 12), six adjusted to within 50mm Hg of the holding pressure (fish 2, 5, 10, 14, 15 and 16), two adjusted to within 100mm Hg of the holding pressure (fish 7 and 9), and the remaining six pike attained neutral buoyancy at pressures less than 100mm Hg below the holding pressure.

Changes in the composition of swimbladder gas during secretion under increased hydrostatic pressure were examined in five pike. The results are given in Table 5. Further changes in swimbladder gas composition in these fish after removal from the pressure chamber are shown in Figures 13(a) and 13(b). The swimbladders of all five pike contained substantially greater proportions of oxygen and carbon dioxide after secretion then before, with the mean oxygen content rising from 26.4% to 57.0%, and the mean carbon dioxide content from 3.1% to 6.7%. After removal from the chamber and during subsequent confinement in shallow tanks with no access to the surface, the proportions of oxygen and carbon dioxide in the swimbladder decreased with time.

Changes in the composition of swimbladder gas in pike which refilled their swimbladders in shallow tanks without access to the surface were determined using eight fish over 20 day periods. The oxygen and carbon dioxide values are plotted in Figure 14, and are corrected for the influence of gas remaining in the swimbladders after emptying them as fully as possible. A rapid and substantial increase in swimbladder oxygen and carbon dioxide, followed by a gradual reduction in the proportions of these gases was found to occur in these fish. 24 hours after emptying the swimbladders, the mean levels of oxygen and carbon dioxide present were 74.5% and 10.1% respectively. Thereafter, the

TABLE 5 Compositional changes in the swimbladder contents of pike during gas secretion under increased hydrostatic pressure.

Fish No.	Time under pressure (days)	Swimbladder volume (cm^3)	Gas volume secreted at N.T.P. (cm^3)	CO_2 (cm^3)	O_2 (cm^3)
15	7	12.3	14.1	+ 0.5	+ 9.3
10	10	7.2	7.9	+ 0.8	+ 6.8
11	12	24.8	14.2	+ 0.6	+10.4
12	18	6.8	7.3	+ 0.6	+ 6.7
4	22	5.3	5.0	+ 0.4	+ 4.2

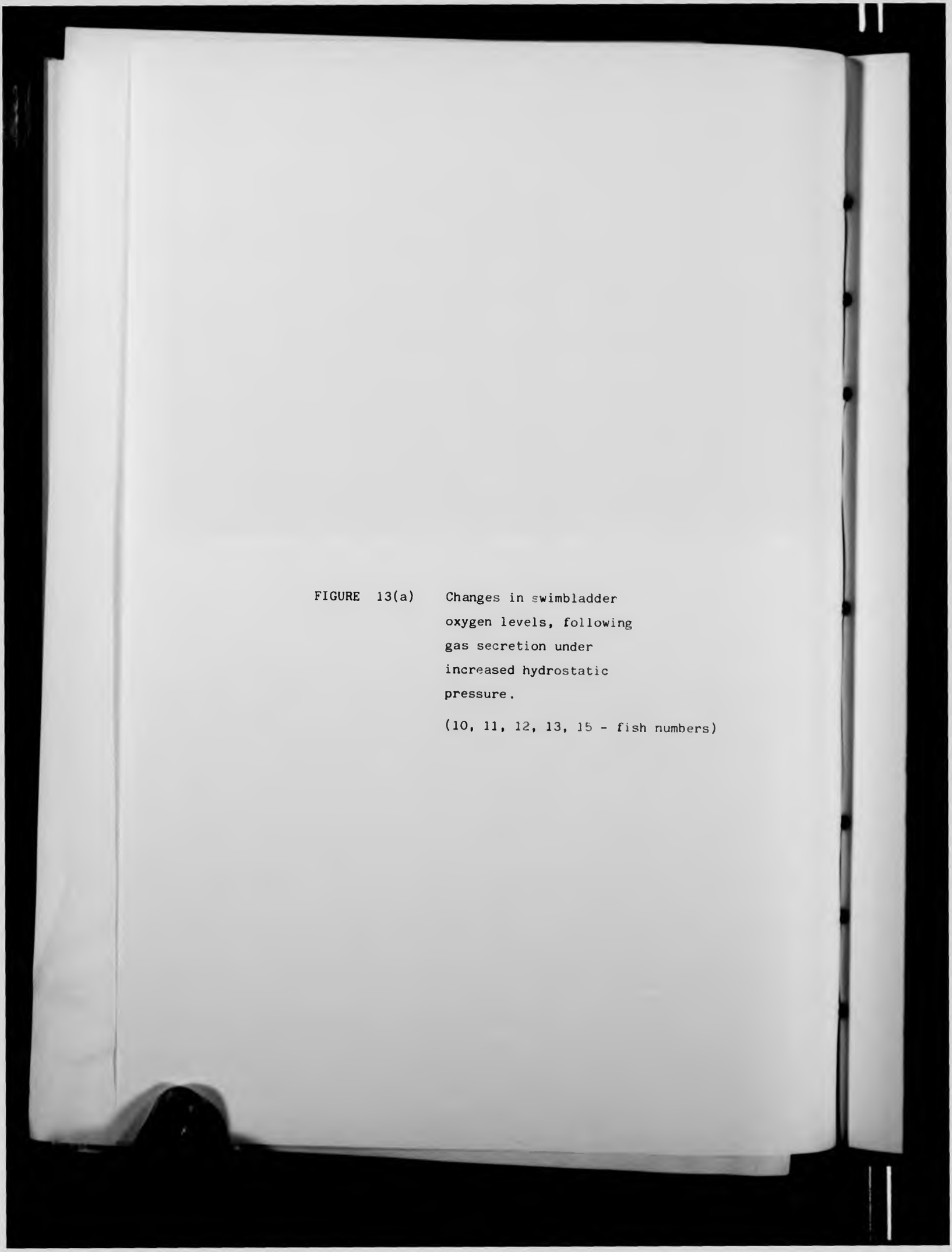
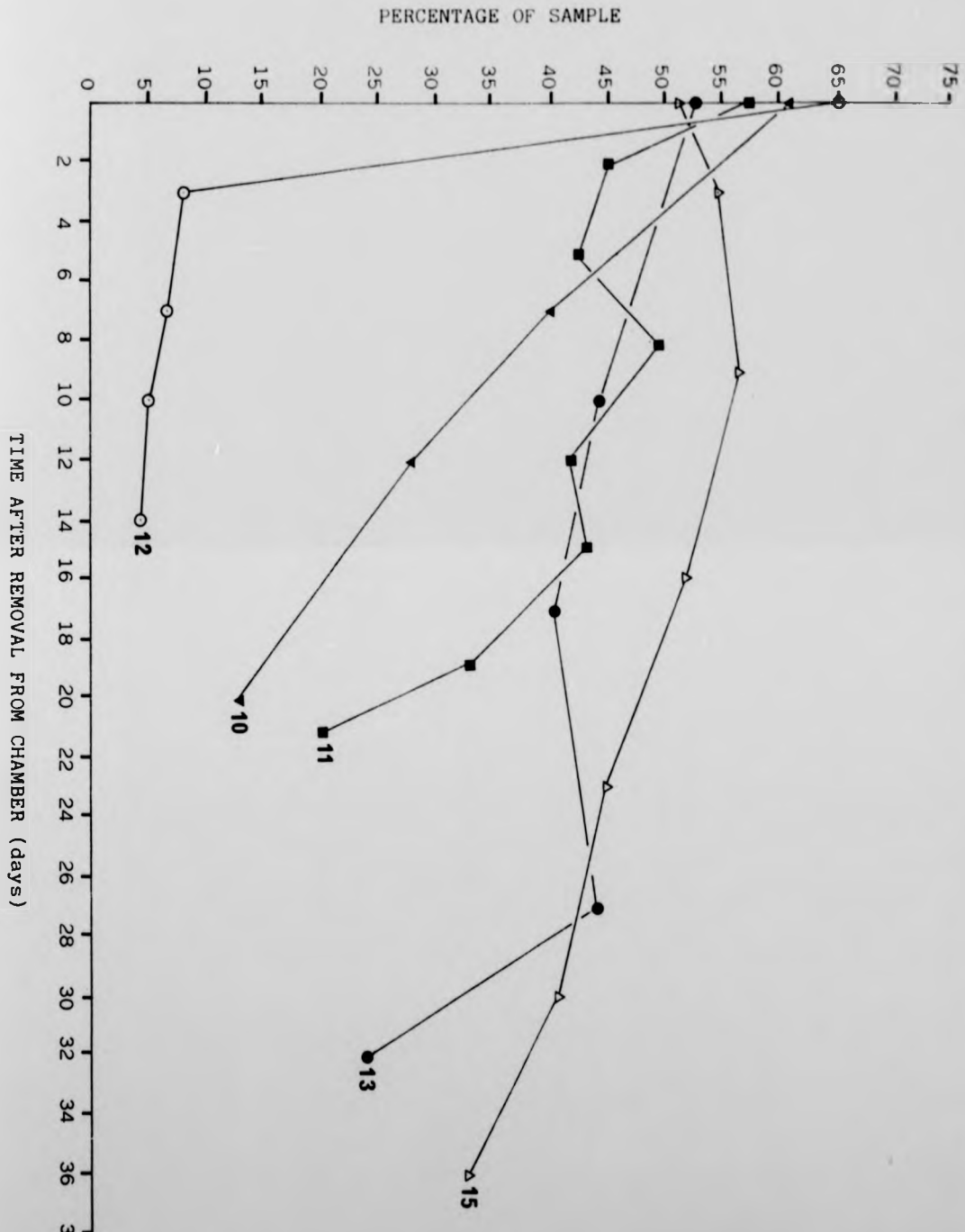


FIGURE 13(a) Changes in swimbladder oxygen levels, following gas secretion under increased hydrostatic pressure.

(10, 11, 12, 13, 15 - fish numbers)


PERCENTAGE OF SAMPLE
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
TIME AFTER REMOVAL FROM CHAMBER (days)
10
11
12
13
15

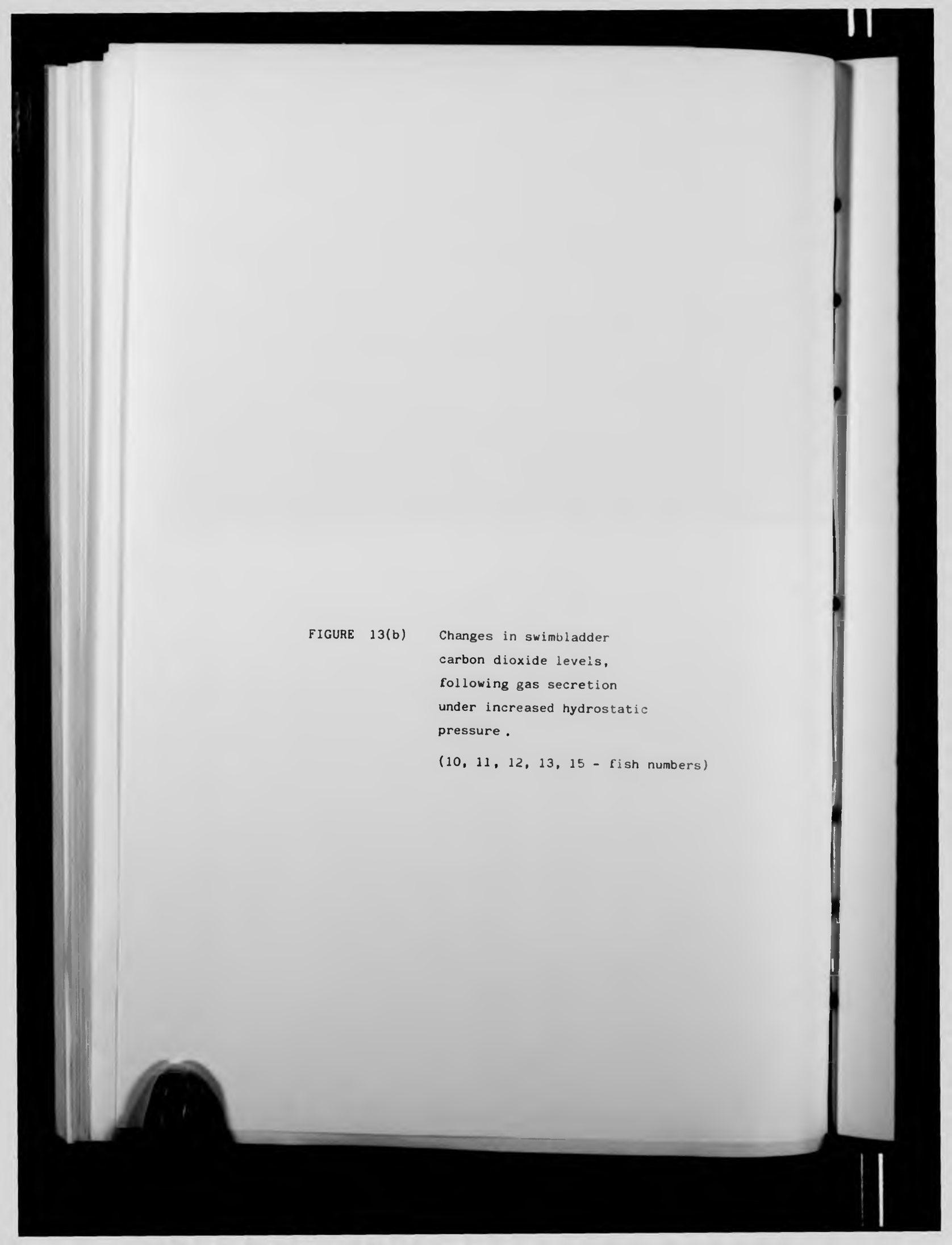


FIGURE 13(b) Changes in swimbladder carbon dioxide levels, following gas secretion under increased hydrostatic pressure.

(10, 11, 12, 13, 15 - fish numbers)

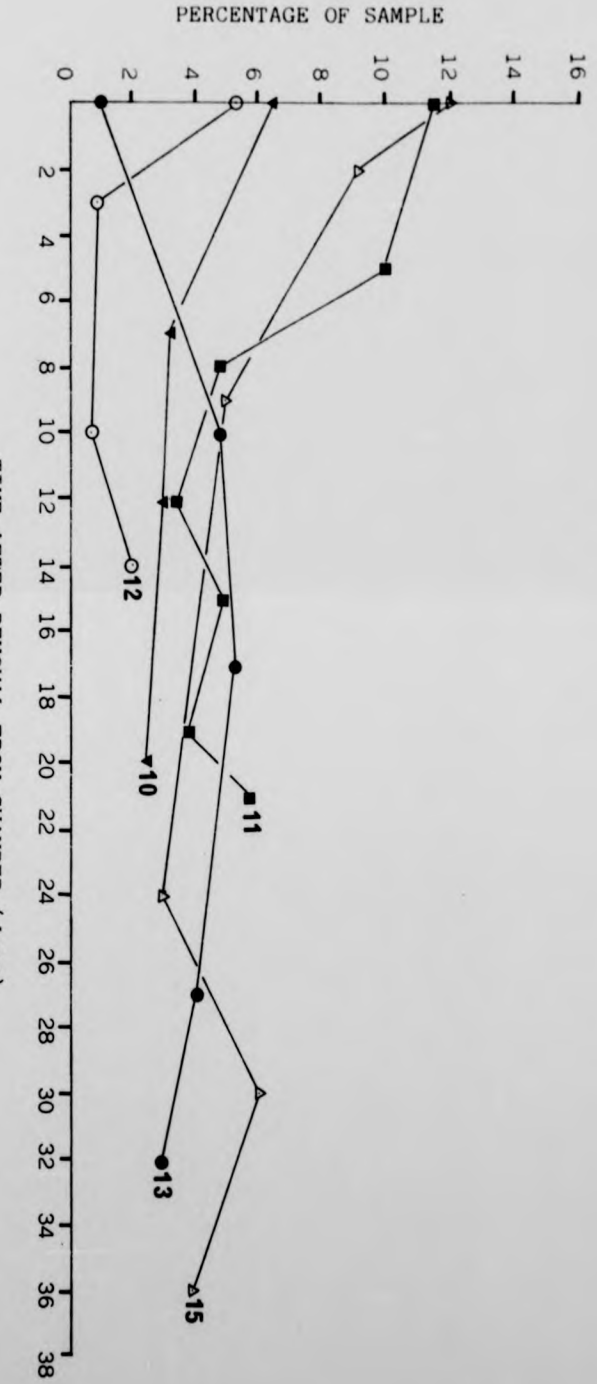
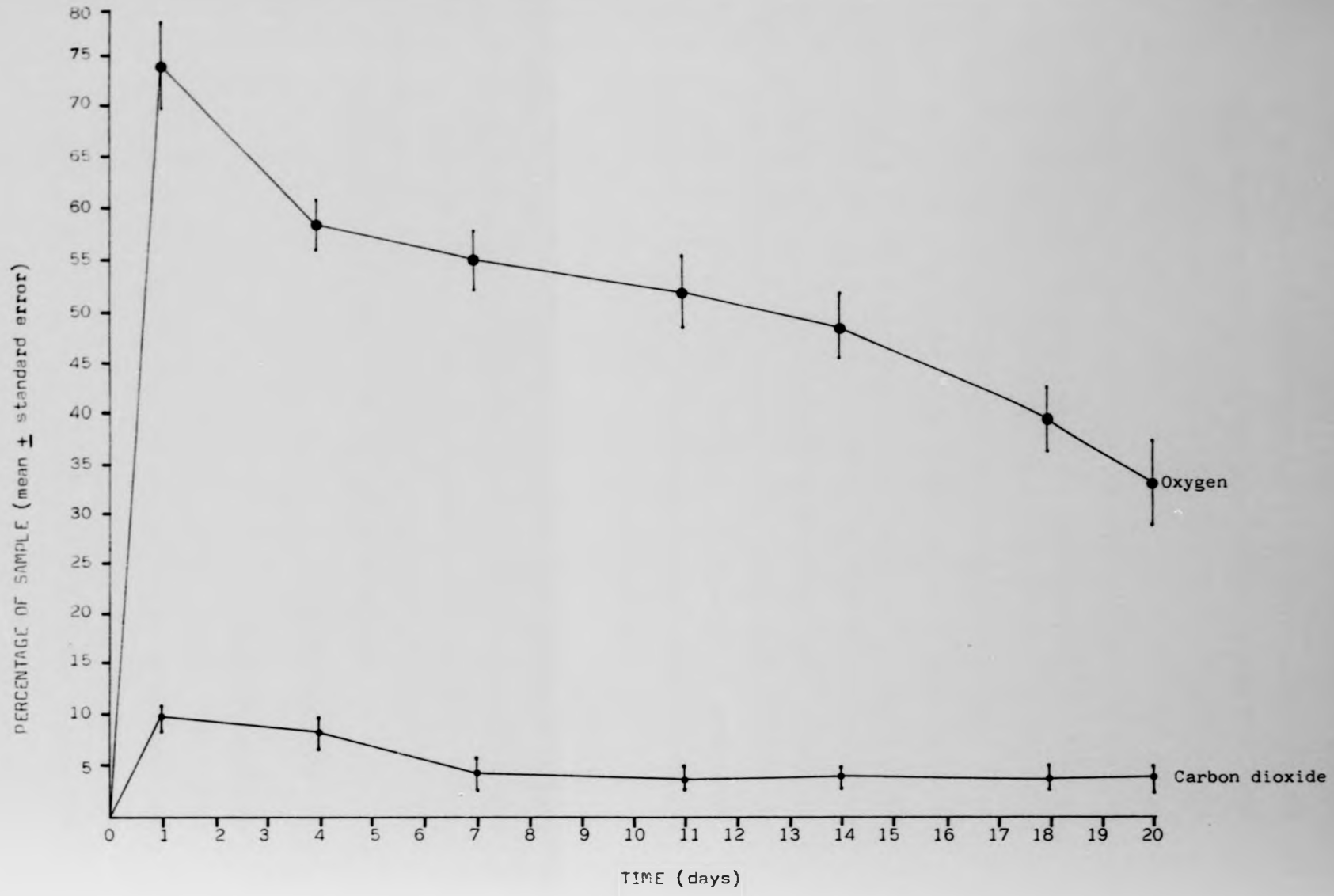

PERCENTAGE OF SAMPLE
0
2
4
6
8
10
12
14
16
TIME AFTER REMOVAL FROM CHAMBER (days)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
12
10
11
13
15

FIGURE 14 Changes in the composition of the swimbladder contents in pike which refilled emptied swimbladders at atmospheric pressure without access to the surface.

(n = 8)


PERCENTAGE OF SAMPLE (mean ± standard error)
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
TIME (days)
Oxygen
Carbon dioxide

proportion of oxygen decreased gradually during the whole sampling period, until at 20 days it occupied around 33.0% of the swimbladder contents. The proportion of carbon dioxide present also decreased to around 4.0% after 7 days, where it remained at a fairly constant level until 20 days, when sampling ceased.

(ii) Deflatory Mechanisms

(a) Pressure thresholds for gas release during decompression

Following adjustment of buoyancy by gas secretion in the pressure chamber, eight pike were decompressed from the holding pressure in a controlled manner to permit pressure thresholds for the release of gas from the swimbladder to be determined. The results are shown in Figures 15(a) to 15(h).

The reduction in hydrostatic pressure required to produce gas release is extremely variable, ranging from 3 to 800mm Hg, and does not appear to be related to size. Of the nine pike examined, only four released sufficient volumes of gas to render them negatively buoyant at pressures lower than the pressure at gas release. This occurred on only 22% of the occasions that gas was released by these fish, and the mean difference between the pressure at gas release and the new pressure of neutral buoyancy was only 13mm Hg (s.e $\pm$ 1.2mm Hg).

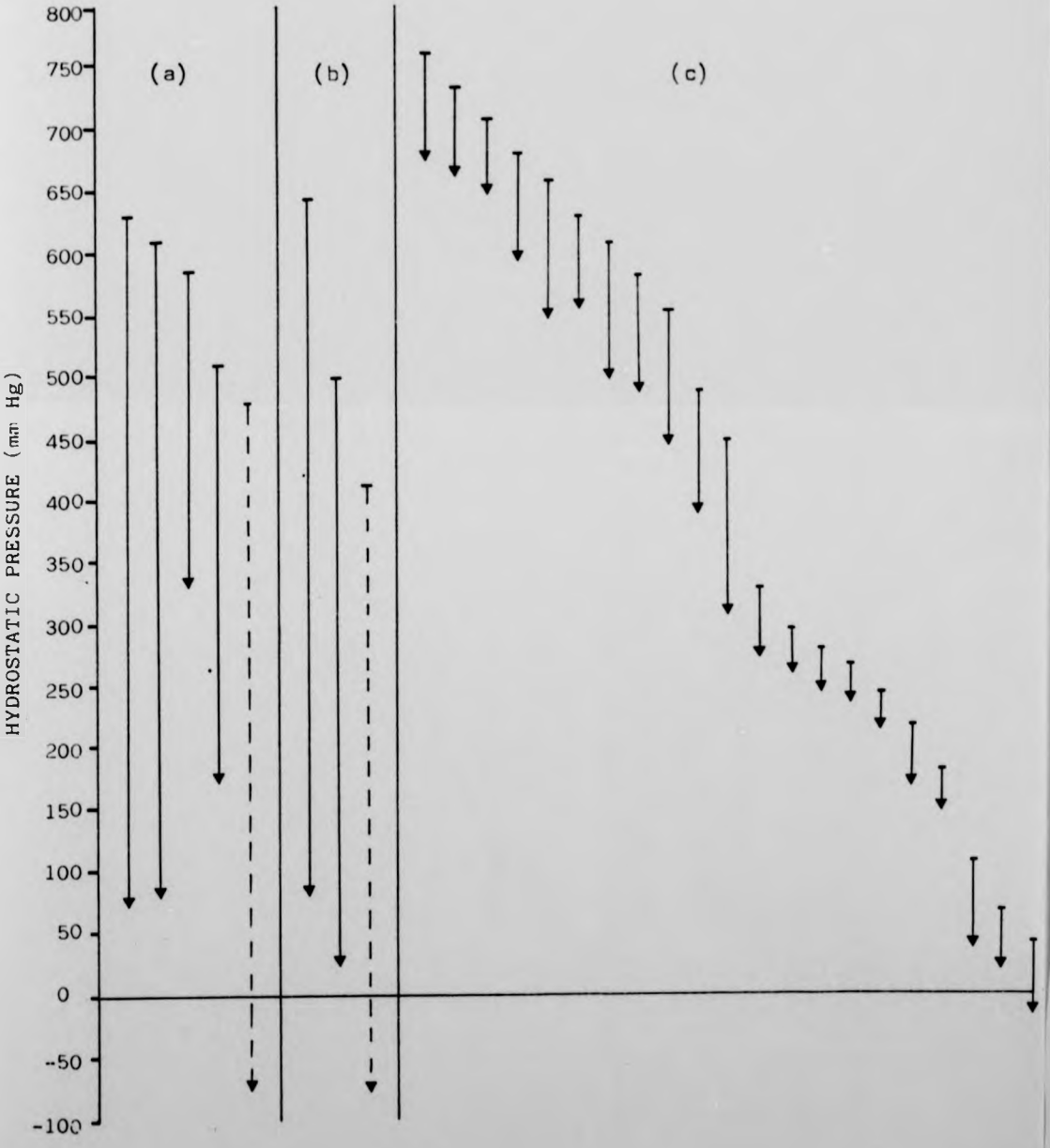
During decompression, fish exhibited a characteristic pattern of behaviour as the pressure was slowly reduced to and below successive pressures of neutral buoyancy. When the pressure of neutral buoyancy was reached, fish rose slowly from the floor of the chamber with the longitudinal body axis horizontal, and the fins folded back against the body surface. If the hydrostatic pressure was held static at the pressure of neutral buoyancy, two different responses were observed. The first of these involved the fish remaining almost motionless in the water column,making only ventilatory movements and very slight and

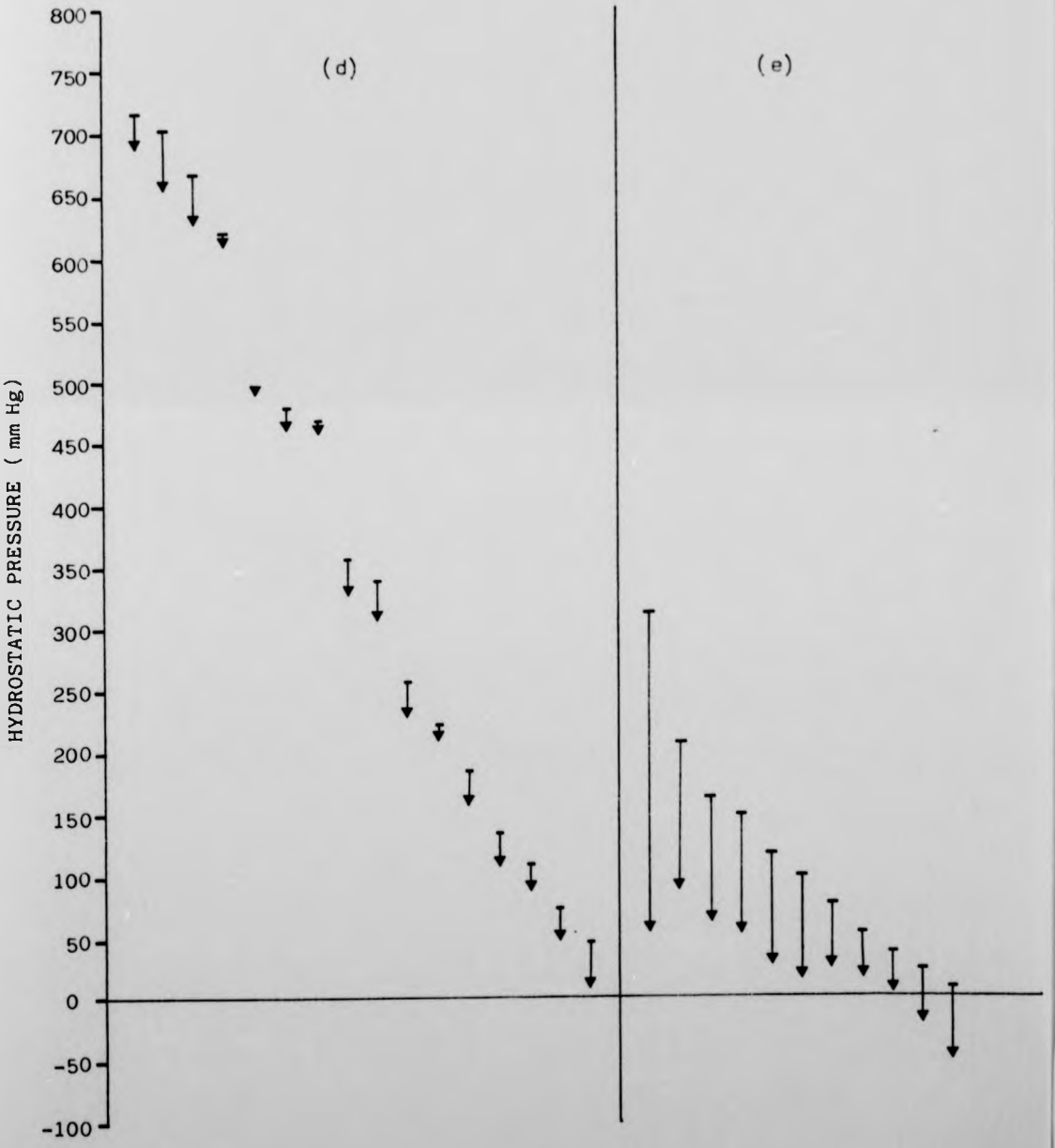
FIGURES 15(a) to 15(h) Pressure reductions required to produce gas release in pike adapted to increased hydrostatic pressure.

- = Pressure of neutral buoyancy.

▼ = Pressure at gas release.

(Broken lines indicate decompression to below atmospheric pressure without gas release).


800
750
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
-50
-100
HYDROSTATIC PRESSURE (mm Hg)
(a)
(b)
(c)


HYDROSTATIC PRESSURE (mm Hg)
800
750
700
650
600
550
500
450
400
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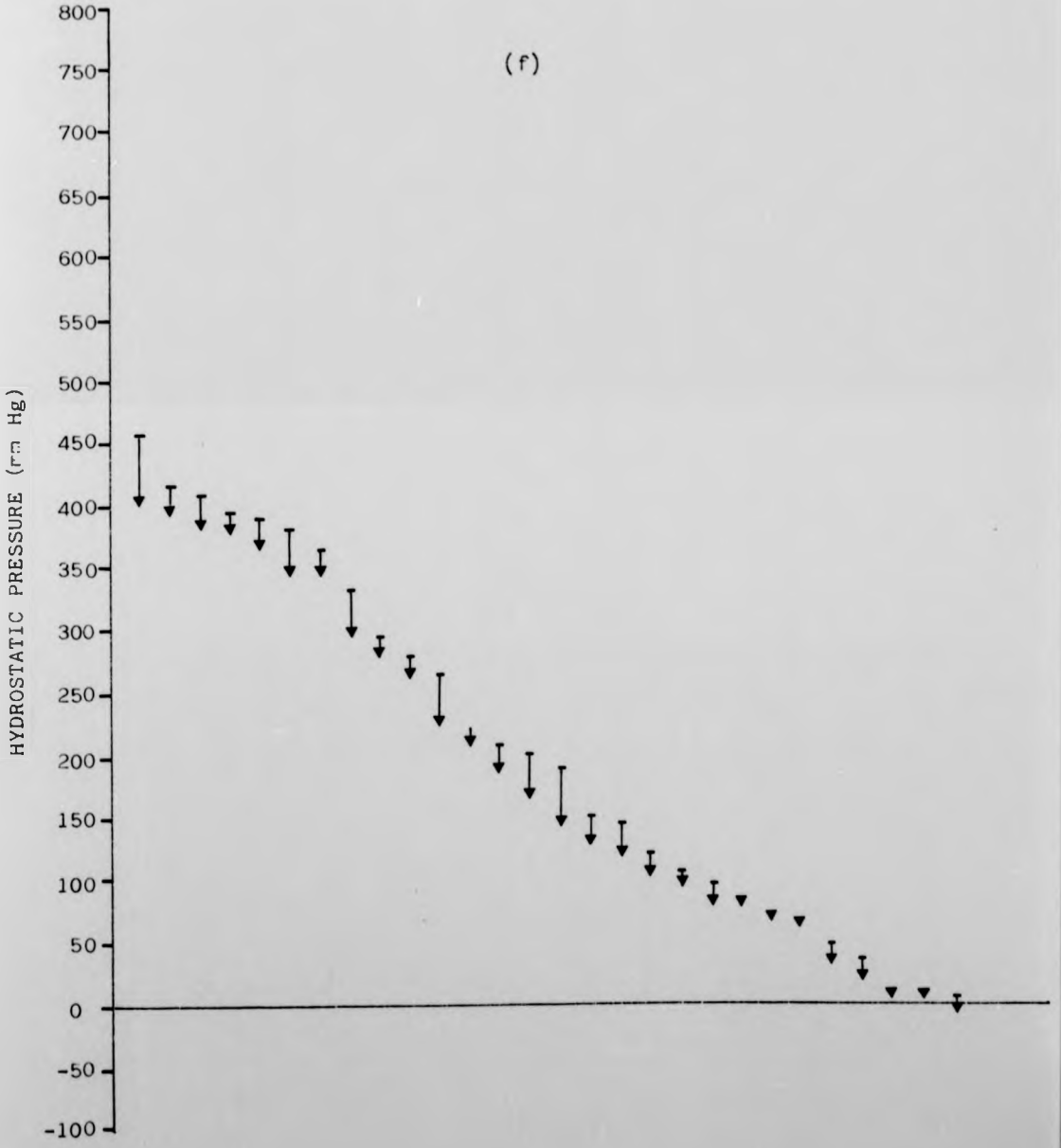
300
250
200
150
100
50
0
-50
-100
(d)
(e)


(f)
HYDROSTATIC PRESSURE (mm Hg)
800
750
700
650
600
550
500
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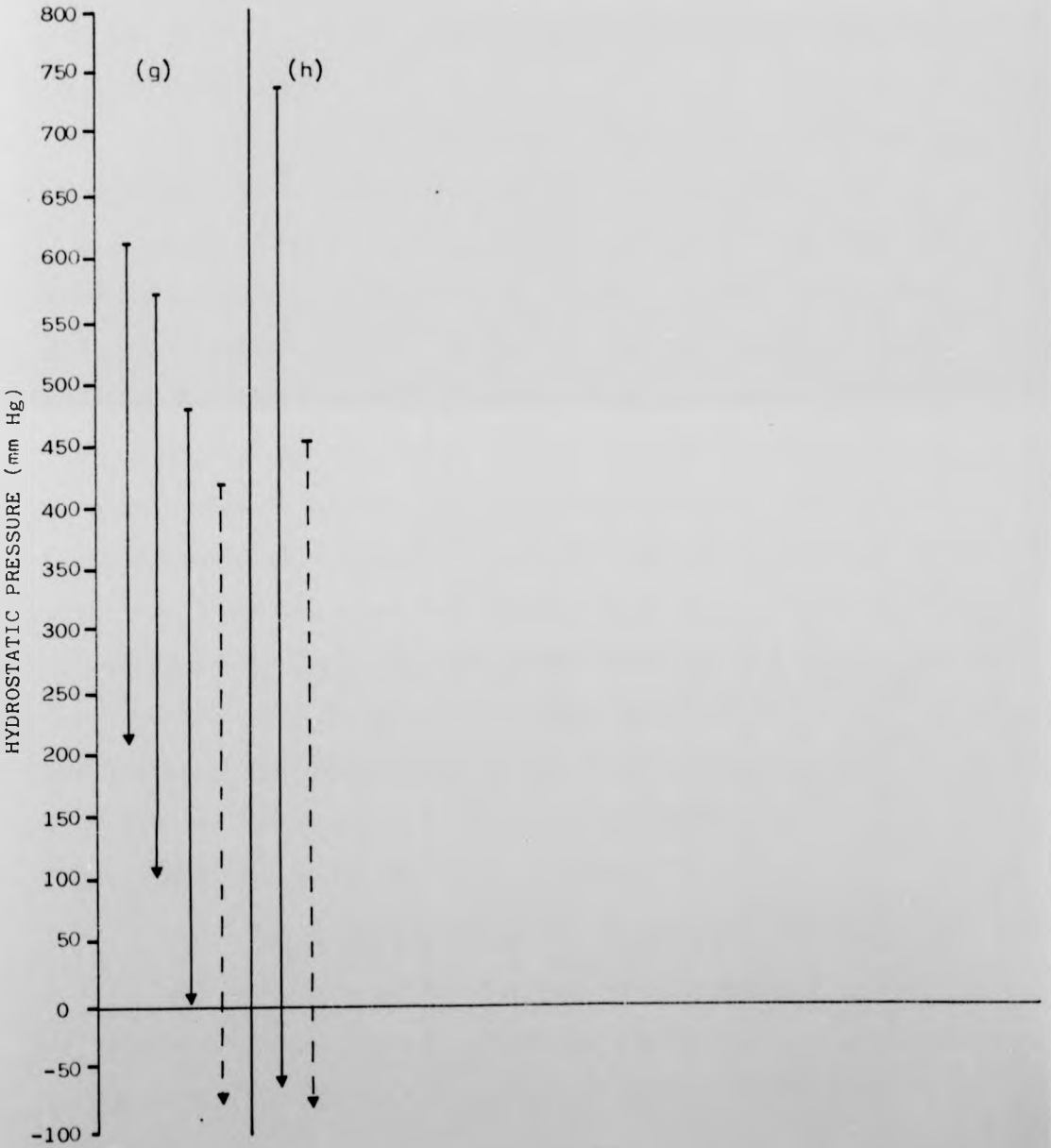
350
300
250
200
150
100
50
0
-50
-100


800
750
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
-50
-100
HYDROSTATIC PRESSURE (mm Hg)
(g)
(h)

occasional fin movements. In the second response, fish were observed to arch the body axis, with the head and tail downward, then sink slowly to the bottom of the chamber without making any discernible fin movements. Further decompression to approximately 30 to 40mm Hg below the initially observed pressure of neutral buoyancy was subsequently required to cause the fish to rise again.

As fish became increasingly more buoyant with further reduction in hydrostatic pressure, the body orientation was altered from the horizontal attitude observed at neutral buoyancy, and finning activity commenced to prevent or reduce upward movement. The body was inclined downwards at approximately 30^{o} to the horizontal, and alternate antero-posterior movements of the paired ventral fins, and lateral movements of the dorsal and anal fins began. As the reduction in hydrostatic pressure continued, the finning frequency increased until station could not be maintained by finning alone, and full downward swimming activity began. The transition from compensatory finning to full downward swimming occurred following a mean reduction in ambient pressure of 21% (s.e. $\pm$ 4.3%). After a mean pressure reduction of 44% (s.e. $\pm$ 6.0%), fish could no longer hold station by full swimming activity, and rose to the roof of the chamber. The release of gas bubbles occurred at various stages during the decompression procedure in different fish.

(b) Pressure thresholds for gas release in stunned fish

The increase in swimbladder gas pressure required to produce gas release in stunned fish was determined using nine pike, ranging in size from 60.0 to 256.4g. The results are given in Table 6 and in Figure 16.

In these stunned pike, the change in swimbladder gas pressure required to produce gas release increased in proportion to the size of the swimbladder.

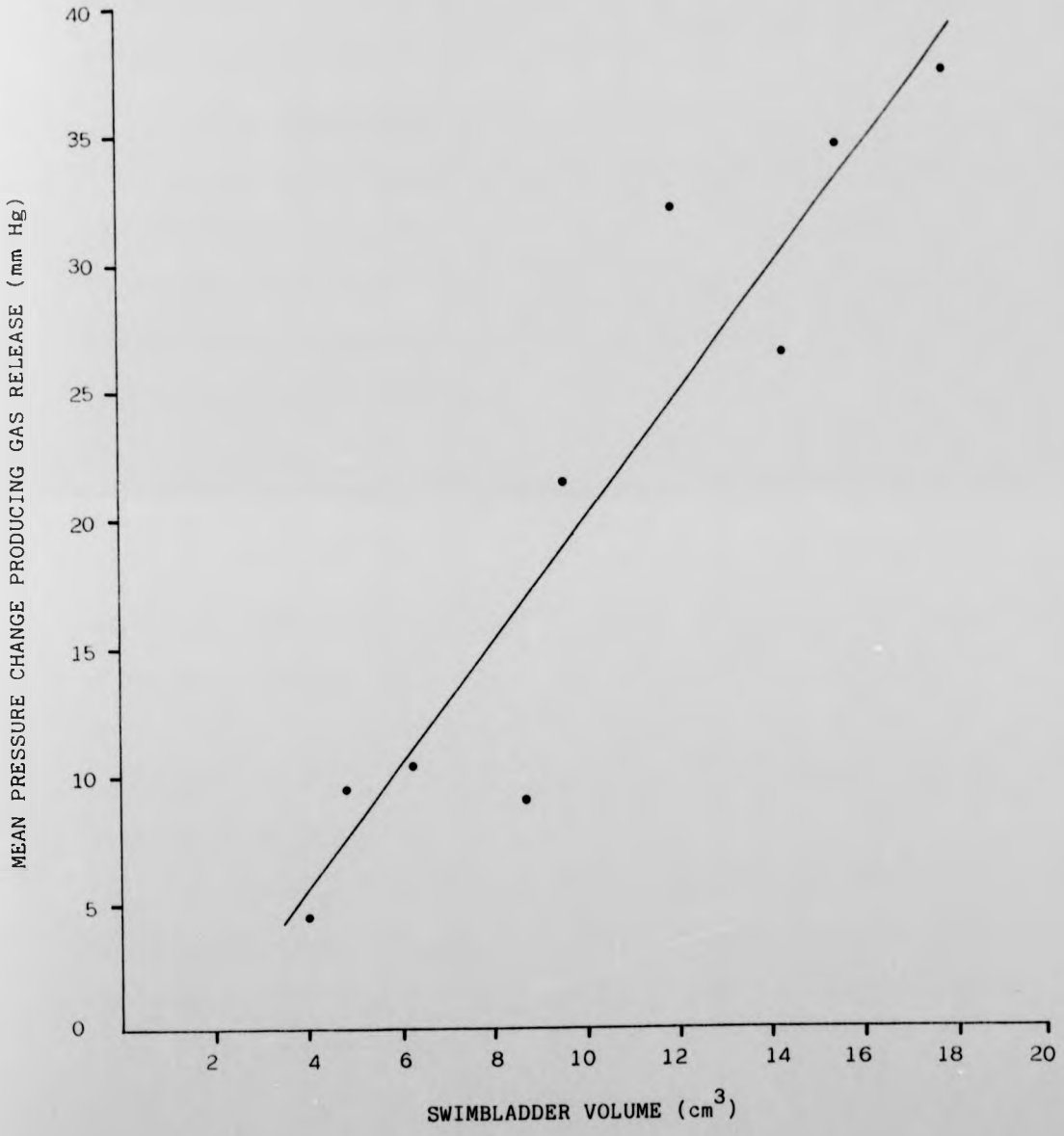
TABLE 6 Mean pressure thresholds for gas release as determined by injecting gas into the swim-bladders of stunned fish.

Swimbladder volume (cm^3)	Mean pressure increase producing gas release (mm Hg)	$\pm$ S.e.	Weber fraction $\left[\frac{\Delta P}{P} \times 100\right]$
4.0	4.4	0.1	0.6
5.0	9.5	0.5	1.2
6.3	10.3	0.4	1.3
8.8	9.0	0.3	1.2
9.6	21.5	3.0	2.8
12.0	32.0	3.5	4.2
14.3	26.5	3.4	3.5
15.5	34.5	2.8	4.5
17.8	37.5	3.8	4.9

FIGURE 16 The relationship between swimbladder volume and mean internal pressure increase required to produce gas release in stunned fish.

Regression line: $y = 2.4x - 4.4$

$r = 0.943$


MEAN PRESSURE CHANGE PRODUCING GAS RELEASE (mm Hg)
0
5
10
15
20
25
30
35
40
2
4
6
8
10
12
14
16
18
20
SWIMBLADDER VOLUME (cm^3)

The correlation coefficient between swimbladder volume and the increase in internal pressure producing gas release is significant at the 0.1% level. The largest observed increase in swimbladder gas pressure producing gas release was 37mm Hg.

(c) Oxygen diffusion

The rate of oxygen loss by diffusion through the walls of the swimbladder was measured in five freshly excised swimbladders. The results are given in Table 7. The oxygen permeability of the swimbladder tissue is expressed in terms of the diffusion constant of Krogh (1919), according to the formula:

$$K(O_2) = \frac{Q \cdot d}{A \cdot P \cdot t} \qquad (c)$$

where Q = volume of oxygen diffusing from the swimbladder at N.T.P. (cm^3), d = tissue thickness (μm), A = area of tissue over which diffusion occurs (cm^2), P = partial pressure difference of oxygen across the swimbladder wall (atm), t = time taken for Q cm^3 to diffuse across the swimbladder wall (minutes).

Swimbladder volumes and surface areas were calculated from measurement of major and minor semi-axes, making the assumption that the shape of the swimbladder approximated to that of a prolate spheroid, using the following formulae:

$$\text{Volume} = \frac{4}{3} \pi a.b \qquad (d)$$

$$\text{Surface area} = 2\pi b + 2\pi \frac{a.b}{E} . (\text{Sin}^{-1}E) \qquad (e)$$

where a = major semi-axis, b = minor semi-axis, and eccentricity

$$E = 1 - \left[\frac{b}{a}\right]^2 \qquad (f)$$

TABLE 7 Oxygen diffusion rates and $K(O_2)$ values for the pike swimbladder, determined at $13^{o}C$.

Swimbladder volume (cm^3)	Swimbladder surface area (cm^2)	Mean oxygen diffusion rate ($cm^3\ O_2 hr^{-1}$)	Mean tissue thickness (μm)	$K(O_2)$ $\left[\frac{cm^3 . \mu m}{atm.min.cm^2}\right]$	CO_2 after 24 hours (%)
4.4	21.9	0.14	200	0.021	<0.1
7.2	28.7	0.33	215	0.041	0.3
7.7	31.6	0.28	210	0.031	0.5
11.3	37.1	0.44	240	0.047	0.2
16.9	48.9	0.62	310	0.065	0.2
$\bar{x}$ = 9.5	$\bar{x}$ = 33.6	$\bar{x}$ = 0.36	$\bar{x}$ = 235	$\bar{x}$ = 0.041	$\bar{x}$ = 0.3
S.e. = 2.15	S.e. = 4.53	S.e. = 0.081	S.e. = 19.7	S.e. = 0.007	S.e. = 0.04

The mean tissue thickness for each of the swimbladders was calculated from measurement of six transverse sections, taken at regular intervals along the length of nitrocellulose-embedded swimbladders.

(iii) Buoyancy and Swimbladder Gas Pressure

(a) Excess internal pressure development under increased ambient pressure

The swimbladder gas pressure at various pressures of neutral buoyancy was determined in five pike with catheterized swimbladders, which adjusted their buoyancy under increased hydrostatic pressure. The results are given in Table 8.

An excess internal pressure was found to exist on all occasions that swimbladder gas pressure and pressure of neutral buoyancy were determined. The excess internal pressure in three of the five pike increased with the pressure of neutral buoyancy, while in the remaining two pike, it remained relatively constant at a mean level of 16mm Hg (s.e $\pm$ 1mm Hg).

(b, c) Stillwater and current experiments

The excess internal pressure and state of buoyancy in twenty pike held in still water, and ten pike held in a current of 30cm sec^{-1} are given in Tables 9(a) and 9(b) respectively.

All of the pike held in still water had excess internal pressures, and only two of the twenty were negatively buoyant after 14 days. The pike held in current also demonstrated excess internal pressures, but in contrast to those held in still water, were all negatively buoyant. The difference between the mean excess internal pressure of 107mm Hg for still water-adapted pike and 6mm Hg for current-adapted pike is significant at the 0.1% level.

TABLE 8 Excess internal pressure in pike with catheterized swimbladders, which secreted gas under increased ambient pressure.

Fish No.	Pressure of neutral buoyancy (mm Hg)	Swimbladder gas pressure (mm Hg)	Excess internal pressure (mm Hg)
1	86	89	3
	95	104	9
	126	139	13
	169	192	23
2	54	57	3
	69	76	7
	104	121	17
	173	194	21
3	163	183	20
	192	210	18
	212	233	21
4	42	52	10
	64	81	17
	105	121	16
	146	163	17
	169	181	12
5	58	66	8
	99	113	14
	106	122	16
	152	179	27

TABLE 9(a) Buoyancy and excess internal pressure in pike held in shallow stillwater tanks for ten to fourteen days at 13°C.

\+ = Positive buoyancy

o = Neutral "

\- = Negative "

Swimbladder volume (cm^3)	Excess internal pressure (mm Hg)	Buoyancy
1.6	192	+
3.1	75	o
3.4	45	+
3.7	230	+
3.8	216	o
5.1	168	o
6.2	83	o
6.3	140	+
6.4	87	o
6.5	131	o
6.5	144	+
7.3	75	o
8.0	105	o
8.6	112	o
8.7	68	+
9.4	55	o
10.2	30	-
11.9	71	o
12.6	74	o
15.6	40	-
$\bar{x}$ = 7.2	$\bar{x}$ = 107.0	
S.e. = 0.8	S.e. = 13.1	

TABLE 9(b) Buoyancy and excess internal pressure in pike held in a current of 30 $cmsec^{-1}$ for eleven to thirteen days at $13^{o}C$.

- = Negative buoyancy

Swimbladder volume (cm^3)	Excess internal pressure (mm Hg)	Buoyancy
5.6	6.5	-
7.0	9.0	-
7.2	8.0	-
7.9	5.0	-
8.2	2.5	-
8.6	5.5	-
9.0	8.0	-
10.3	4.0	-
10.8	7.0	-
12.0	8.5	-
$\bar{x}$ = 8.7	$\bar{x}$ = 6.4	
s.e. = 0.5	s.e. = 0.6	

(d) Changes in gas pressure in the exposed swimbladder

In all eight swimbladder preparations used, the initial gas pressure exceeded atmospheric pressure (Mean E.i.p. = 8mm Hg, s.e. $\pm$ 1mm Hg).

Adrenalin at a concentration of 10^{-5}g cm^{-3} produced a mean increase in gas pressure of 4.4mm Hg (s.e. $\pm$ 0.8mm Hg). The application of adrenalin at this concentration to the pneumatic duct area produced a drop in gas pressure to ambient in all but one case, where the internal pressure fell to 2.0mm Hg. Adrenalin at a concentration of 10^{-6}g cm^{-3} produced strong contractions in isolated longitudinal and circular tissue strips.

The application of acetylcholine to swimbladder preparations had no observable effect on gas pressure, even after pretreatment with Eserine. However, when solutions of 10^{-6}g cm^{-3} were applied to preparations contracted by adrenalin, a mean reduction in gas pressure of 3.0mm Hg (s.e. $\pm$ 0.5mm Hg) was noted. The application of acetylcholine solutions to the pneumatic duct area had no effect on gas pressure. Acetylcholine at concentrations of 10^{-7} to 10^{-4}g cm^{-3} produced no observable effect on tissue strips, even after pretreatment with Eserine, although it caused relaxation in strips contracted by adrenalin.

These results indicate that the smooth muscles of the swimbladder wall are capable of compressing the gas contained within the swimbladder by a very limited amount.

DISCUSSION

Possession of an internal gas-filled sac, which reduces the overall density of the tissues to close to that of their environment, is of undoubted advantage to those fish species which spend much of their time in the water column. The benefits conferred by such an organ are further increased by the ability to regulate its volume, by increasing and reducing the gas content, when circumstances require.

The traditionally accepted means by which the volume of gas in the swimbladder is regulated differ between physostomes and physoclists. Air-gulping and gas-spitting are attributed to the more 'primitive' physostomes, and the secretion and reabsorption of gas to the more 'advanced' physoclists. However, more recent research into the operation of the swimbladder in physostomes has shown that some species are capable of regulating the volume of gas in the swimbladder by means other than those described above. These additional capabilities have relieved them of much of the dependence on the surface formerly thought to exist in all physostomes.

It is clear from this study that the swimbladder of the northern pike is a complex organ, and that its volume may be manipulated in several ways to allow the pike to adapt successfully to changing conditions.

The ability to regulate buoyancy by manipulation of swimbladder volume is of particular importance to the pike in its feeding behaviour. The pike is a predatory species, and after attaining a length of 20cm or so, feeds almost exclusively on fish, including members of its own species. Its normal feeding behaviour involves much time being spent hovering either in the water column, or amongst aquatic vegetation, until a fish of suitable size comes close enough to be seized (Wheeler, 1969). The facility to hold station for long periods of time, using

only very slight compensatory and orientational fin movements, is, therefore, clearly of considerable benefit in the seeking and capture of prey.

This study has shown that pike tissue density and percentage swimbladder volume are similar to those of a number of other freshwater physostomes (Table 10). Alexander (1966) calculated that freshwater teleosts with tissue densities of between 1.06 and 1.09g cm^{-3} (Delaroche, 1809; Harden Jones and Marshall, 1953) would require swimbladders with percentage volumes of between 5.7 and 8.3 for neutral buoyancy. The pike swimbladder has a volume which lies approximately midway between these values.

The rapidity with which pike replaced lost swimbladder gas in shallow water by air-gulping is indicative of the relative efficiency of this method, and of the importance of attaining a state of neutral buoyancy in still water. Even when free access to the surface was temporarily delayed, air-gulping began almost immediately when access became available. It is interesting to note that with the removal of a fairly constant proportion of the swimbladder contents, the number of occasions on which air was gulped increased with the volume removed. This suggests that the amount of air passed into the swimbladder on each occasion when air was gulped was relatively independent of the size of the fish. This is a little surprising from a morphological viewpoint, but it may be that the rate-determining step in the refilling process is the passage of air through the pneumatic duct. It is possible that only air bubbles of finite size can be accommodated in the duct, regardless of fish size, and that this may govern the refilling rate.

The treatment to which the fish were subjected in this experiment was rather artificial, in that their swimbladders were deflated more than is likely to occur in their natural environment, and because the maximum distance which fish had to swim in order to reach the surface

TABLE 10 Tissue density and percentage swimbladder volume in some freshwater physostomes.

(Sample standard errors in parentheses)

* = Calculated from available data.

Species	Tissue density ($g\ cm^{-3}$)	% Volume (v/w)	% Volume (v/v)	n	Source
Esox lucius	1.077(0.004)	6.9 (0.3)	7.5 (0.3)	14	Present work
Oncorhynchus nerka					
Sockeye smolts	1.063(0.001)	5.9*	6.3*		Harvey, Hoar and Bothern (1968)
Kokanee smolts	1.055(0.004)	5.2*	5.5*		
Rutilus rutilus	--	--	9.7		Plattner (1941)
	--	--	9.9		Alexander (1959b)
Phoxinus phoxinus	--	--	6.6		Franz (1937)
	--	--	6.1		Alexander (1959b)
Carassius auratus	--	--	8.2		Plattner (1941)
	--	--	7.9		Alexander (1959b)

was only one metre. This air-gulping behaviour may, however, provide a clue to the response of pike in their natural environment after the loss of relatively small quantities of gas in shallow water. However, because of the gas volumes involved, and the associated problem of containment, it is most unlikely that air-gulping is used by pike adapting to neutral buoyancy at depths of more than a few metres. In order to illustrate the problems involved, let us consider the example of a 100g pike which is neutrally buoyant at the surface, but which requires to adapt to neutral buoyancy at a depth of ten metres. The swimbladder of this fish must occupy a volume of approximately $7cm^3$ at all depths in order to confer neutral buoyancy, but since the ambient pressure at ten metres is twice that at the surface, the volume of gas contained within it at the surface must also be doubled if the fish is to be neutrally buoyant at ten metres. Accommodation of the additional $7cm^3$ of air required for neutral buoyancy clearly poses an insurmountable problem for such a fish, especially since it appears that the pneumatic duct is not adapted for pumping gas into the swimbladder under pressure.

The composition of the gas in the swimbladders of pike which adapted to neutral buoyancy in shallow tanks with free access to the surface, provides some initial evidence that pike are not completely dependent on air-gulping as an inflatory mechanism, but are capable of gas secretion (Figure 11). The proportions of swimbladder oxygen and carbon dioxide in neutrally buoyant fish a few days after their swimbladders were emptied were significantly greater than the proportions of these gases in atmospheric air, and it seems reasonable to conclude from this that some oxygen and carbon dioxide had been accumulated by secretion. Jacobs (1934) found that the proportion of oxygen and carbon dioxide in the swimbladders of pike adapted to shallow tanks significantly exceeded ambient concentrations. Unfortunately, Jacobs does not specify how

long these fish had been held before gas samples were removed for analysis. This is important because the proportions of oxygen and carbon dioxide in the swimbladder decrease with time (Figures 11, 13(a), 13(b), 14).

The fact that gas secretion seems to be employed to assist in the reinflation of the swimbladder under such circumstances lends weight to the argument that air-gulping is only of limited use in buoyancy adaptation. The data shows that gas secretion was initiated even in situations where rapid swimbladder reinflation could be brought about by air-gulping.

The density and degree of development of the vessels of the blood vascular system, and the nature of the epithelium present in the anterior part of the pike swimbladder suggest very strongly that this is the area modified for the secretion of gas. Fahlen (1967, 1968) similarly concluded that the columnar epithelium and underlying vascular plexus in the swimbladders of the whitefish Coregonus lavaretus, and grayling Thymallus thymallus were involved in gas secretion. In physostomes which are incapable of gas secretion, the arrangement of blood vessels and the type of epithelium lining the swimbladder are very different to those described by Fahlen. In the rainbow trout, Salmo gairdneri, for example, the numerous capillaries which arise by ramification of the swimbladder blood vessels do not form 'microretia mirabilia', and the epithelium consists of ciliated and non-ciliated cells. Brooks (1970) proposed that the non-ciliated cells discharge a mucus-like material, which protects the epithelium from airborne particles, while the ciliated cells move the material and trapped particles along the swimbladder surface towards the pneumatic duct.

Conclusive evidence of the ability of pike to produce substantial changes in buoyancy by gas secretion comes from the experiments in which fish were confined under pressure, without access to an air-water interface.

Exposure to a hydrostatic pressure equivalent to that experienced at a depth of ten metres resulted in pike adapting to neutral buoyancy at pressures corresponding to depths of between 4.6 and 10 metres by gas secretion (Figures 12(a) to 12(f)). The mean net gas secretion rates, calculated from rates of buoyancy adjustment, range between 0.12 and 0.74$cm^3kg^{-1}hr^{-1}$, with a maximum observed rate of 1.96$cm^3kg^{-1}hr^{-1}$ (Table 4). These rates were calculated making the assumption that the gas pressure inside the swimbladder did not exceed the ambient pressure at neutral buoyancy, but since it is evident that excess internal pressures (E.i.p.) are developed during gas secretion, the calculated values are probably underestimates of the true net secretion rates. Although it is not clear whether fish secreting gas at depth develop E.i.p's of the magnitude found in some shallow-adapted pike, they are nevertheless capable of developing significant E.i.p's. Using the maximum E.i.p. of 27mm Hg found in pike secreting gas under pressure (Table 8) as a guide, a new estimate of 2.06$cm^3kg^{-1}hr^{-1}$ for maximum net secretion rate results. If pike can indeed develop E.i.p's equivalent to the maximum observed in shallow-adapted fish (230mm Hg - Table 9(a)) by gas secretion under pressure, the estimated maximum net secretion rate would rise to 2.84$cm^3kg^{-1}hr^{-1}$. This figure represents an increase of 45% over the initially calculated maximum rate. Even the lowest of these estimates compares very favourably with gas secretion rates demonstrated in other studies on physostomes. Jacobs (1934) found that Esox required between five and 17 days to refill partially emptied swimbladders in shallow tanks. Evans and Damant (1929) showed that Rutilus rutilus could adjust to neutral buoyancy by gas secretion following gas removal in three to five days, and Wittenberg (1958) found that Carassius auratus could refill partially emptied swimbladders in five to seven days. Overfield and Kylstra (1971) quantified gas secretion rates in C. auratus, based on

rates of buoyancy adaptation in a hyperbaric chamber after gas removal at 0.18 to 0.48$cm^3 kg^{-1} hr^{-1}$. The calculated net secretion rates for the pike also compare well with the rates demonstrated by many physoclists. McNab and Meecham (1971) found that the sunfish Lepomis macrochirus secreted gas at between 1.36 and 1.65$cm^3 kg^{-1} hr^{-1}$ when the swimbladder was deflated by 50%, and Blaxter and Tytler (1973) calculated that the saithe, Pollachius virens secreted gas at 1.67$cm\ kg^{-1} hr^{-1}$ when exposed to a pressure increase from 1 to 2 ATA.

It would be interesting to discover how environmental pressures greater than those applied in the pressure chamber in the present study influence gas secretion rates in the pike, and how secretion rates in an enclosed chamber compare with those in free-swimming pike exposed to similar pressures. Telemetric tags, such as those developed to transmit information on hydrostatic pressure and swimming activity, could be used to provide valuable data on buoyancy adjustment in the field, assuming that a relatively long tag-life could be achieved.

When pike under pressure adjusted their buoyancy by gas secretion, the oxygen and carbon dioxide levels inside the swimbladder increased dramatically (Table 5). Analysis of the swimbladder contents before and after secretion showed that the newly-secreted gas contained around 80% oxygen and 7% carbon dioxide. The swimbladders of pike which replaced lost gas by secretion in shallow water were found to have similarly high oxygen and carbon dioxide contents, with around 75% oxygen and 12% carbon dioxide being present in the early stages of gas secretion (Figure 14).

The nature of the changes in the composition of swimbladder gas during secretion in physoclists is well documented (Jacobs, 1932; Fänge, 1953; Fänge and Wittenberg, 1958; Wittenberg, Schwend and Wittenberg, 1964, etc.). Fänge et al. (1958) showed that in the toadfish Opsanus tau, newly-secreted gas contained up to 95% oxygen and up to

18% carbon dioxide, and Wittenberg et al (1964) showed that newly-secreted gas in the bluefish Pomatomus saltatrix contained 65 to 85% oxygen and 17 to 37% carbon dioxide.

The temporal reduction in the proportions of oxygen and carbon dioxide, and the increase in the proportion of nitrogen present in the swimbladders of pike undoubtedly occurred as a result of the existence of diffusion gradients across the swimbladder wall, coupled with differences in the oxygen, carbon dioxide and nitrogen permeability. One beneficial consequence of the gradual replacement of oxygen and carbon dioxide by nitrogen is that, with time, the overall rate of loss of the swimbladder contents by simple diffusion will decrease. The results of Kutchai and Steen (1971) and Denton et al. (1972) indicate that in the common eel Anguilla anguilla, the $K(O_2)/K(N_2)$ ratio is approximately 16:1. If a ratio of this order of magnitude exists for the pike swimbladder wall, then the rate of gas loss in the long term will be substantially lower if the swimbladder contains a high proportion of nitrogen instead of oxygen.

Possession of a swimbladder with a pneumatic duct, which permits the rapid release of gas, would appear to be advantageous because of the potential risk of being carried to the surface if the fish were to exceed the upper limit at which compensatory swimming movements can be employed to hold position. Although such a problem is not likely to be encountered at depth, fish which are adapted to neutral buoyancy in the upper ten to fifteen metres of the water column are exposed to this risk. Harden Jones (1952) showed that the physoclist Perca fluviatilis was unable to maintain station by swimming when exposed to a reduction in ambient pressure of only 32%. The corresponding figure for the pike used in the present study was 44% (Section ii(a)).

If the pneumatic duct in Esox functioned as an automatic safety

valve during decompression, one would expect gas to be released from the swimbladder with percentage reductions in ambient pressure of a similar magnitude to those found when the pressure of the swimbladder gas was artificially increased in stunned fish (Table 6, Figure 16). However, when pike were decompressed from the pressure to which they had adapted, swimbladder gas was not always released with relatively small reductions in pressure (Figures 15(a) to 15(f)). In several cases, gas was retained even after a reduction in pressure beyond the point where fish were capable of holding station by full swimming activity. When gas was eventually released, it was seldom released in sufficient quantity to reduce the buoyancy by a significant amount, so that even after gas release, fish often remained positively buoyant.

It is interesting to note that many of the pike caught in water between ten and fifteen metres deep appeared to release only small quantities of gas during ascent, since after being transferred into polythene holding bins, they often experienced great difficulty in remaining upright because of distention of the swimbladder. However, this problem was usually only temporary, and the excess gas was vented within ten minutes or so of capture.

This retention of swimbladder gas shows that the pneumatic duct sphincter in the pike is not automatically forced open when the swimbladder gas pressure exceeds the ambient pressure by a small amount, but that a considerable degree of control can be exercised over the tonus of the sphincter muscles.

The influence of stress on the ability to retain swimbladder gas is evident from Figures 15(d) and 15(f). These fish were inadvertantly startled during the early stages of decompression, and show much lower pressure thresholds for gas release than the other fish examined. These observations, and the gas release produced by topical application of

adrenalin to the pneumatic duct, substantiate the hypothesis that a reduction in the tonus of the sphincter results when blood catecholamines increase under conditions of stress.

The "gasspuckreflex" occasionally observed when pike adapted to shallow water became distressed, as distinct from the controlled release of gas during decompression, is probably also brought about as a result of increased levels of blood catecholamines. Application of adrenalin to the walls of the swimbladder alone produced very small increases in internal pressure, while simultaneous application to the swimbladder walls, and to the pneumatic duct produced gas release (Section iii (d)). When the "gasspuckreflex" occurs in the pike, the excess internal pressures found in the swimbladder will doubtless assist in the ejection of gas. These findings are in keeping with the results of Franz (1937), who suggested that a similar situation exists in cyprinids, and Harvey et al. (1968), who showed that gas-spitting occurred in salmonids even without the assistance of an excess internal pressure.

In addition to the controlled release and active expulsion of swimbladder gas via the pneumatic duct, loss of gas will be incurred as a result of simple diffusion across the swimbladder wall. Because of the relatively high partial pressure of oxygen found inside the swimbladder during gas secretion, loss of oxygen appears to be a potential problem for the pike. However, the oxygen permeability constant determined for the swimbladder wall of Esox shows that it forms a relatively effective barrier to rapid oxygen loss (Table 11).

The physical basis for the relatively impermeable nature of swimbladder tissue has been described by Denton et al. (1972), who showed that the inclusion of purine crystals substantially reduces the surface area available for diffusion, and increased the length of the diffusion path. The mean purine content found in the pike swimbladder wall compares

TABLE 11 The oxygen permeability constants for the swimbladder walls of some fish.

Species	$k(O_2)$ ($cm^3 \mu m/atm\ min\ cm^2$)	Source
Esox lucius	0.0410	Present work
Anguilla anguilla	0.0106	Kutchai and Steen (1971)
Conger conger	0.0010	Denton et al. (1972)
Ceratoscopelus maderensis	0.0672	Ross (1976)
Pollachius virens	0.0534	Ross (1977)
(Frog connective tissue)	0.1150	Krogh (1919)
(Water)	0.3400	Hüfner (1897)

Erratum. For line 18 please read :

.. a depth of ten metres, the initial rate of oxygen loss with no excess internal pressure would be ..

well with the levels found in other physostomes (Table 12), and is of a similar magnitude to the mean content of 47$\mu g\ cm^{-2}$ found in the swimbladder of the vertically migrating marine physoclist Pollachius virens (Ross, 1977). The very significantly higher purine levels present in the pike swimbladder wall in the area of the secretory complex will tend to reduce the localized loss of gas by diffusion, and may thus contribute to more efficient inflation of the swimbladder by gas secretion.

Using the example of a 140g pike, which has a swimbladder with a volume of 9.5cm^3, surface area of 33.6cm^2, mean wall thickness of 235μm, and a $K(O_2)$ of 0.041cm^3 μm/atm min cm^2, it is possible to estimate the rate of loss of oxygen from the swimbladder. Assuming that no excess internal pressure exists, and using the mean PO_2 value of 0.74 atm, calculated from data collected from refilling experiments in shallow water, and a tissue PO_2 of 0.20 atm, the estimated initial rate of oxygen loss at the surface would be 1.36$cm^3kg^{-1}hr^{-1}$. If an excess internal pressure of the magnitude of the maximum observed in shallow water adapted pike was developed, this estimate would rise to 1.77$cm^3kg^{-1}hr^{-1}$. At a depth of ten metres, the initial rate of oxygen pressure would be 3.22$cm^3kg^{-1}hr^{-1}$, and with the maximum observed E.i.p. the rate would rise to 4.00$cm^3kg^{-1}hr^{-1}$.

These estimated rates of gas loss by diffusion, considered together with the maximum net rates of gas secretion calculated previously, permit estimates of the maximum gross secretion rate to be made. Assuming that no E.i.p. was developed during buoyancy adaptation from the surface to a depth of ten metres, the maximum gross secretion rate would be 1.96 + 3.22 = 5.18$cm^3kg^{-1}hr^{-1}$. With an E.i.p. equivalent to the maximum observed the maximum gross secretion rate would increase to 2.84 + 4.00 = 6.84 $cm^3kg^{-1}hr^{-1}$.

In the light of these estimates, it becomes clear that the

TABLE 12 The purine content of the swimbladder wall, and the depth of occurrence of some freshwater physostomes.

(* - sample standard error)

Species (n)	Purine content ($\mu g\ cm^{-2}$)	Depth range (m)	Source
Esox lucius (5)	43.0 (4.9*)	0 - 15	Present work
Salmo trutta (2)	24.0	0 - 15	Ross (1977)
Scardinius erythrophthalmus (2)	14.0	0 - 5	Ross (1977)

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secretory complex in the pike swimbladder is not merely one of low capacity, such as those present in the swimbladder of some other physostomes, but has the potential to bring about gas secretion at quite considerable rates.

The nature of the stimuli which are responsible for initiating the inflatory and deflatory reflexes in pike is unclear. Copeland (1952) suggested that in physoclists, the inflatory reflex is initiated by stimulation of proprioceptors in the muscles of the pectoral fins, which occurs as a result of fin movement. However, since pike secreted gas even as they lay on the floor of the pressure chamber, this source of stimulation is unlikely to be responsible. It is possible that proprioceptors, present in the swimbladder walls may be responsible, although no experimental evidence to support or disprove this proposal was collected during the course of the present study. Fänge (1953, 1966) and Qutob (1962) both conclude that gas secretion does not depend on such proprioceptive stimuli. The remaining possibility is that various exteroceptive stimuli, arising from the eyes, balance organs, etc., initiate the inflatory reflex (Harden Jones, 1957). Exteroceptive stimuli may also be responsible for producing gas release in unstressed pike. However, in the case of pike confined in the pressure chamber, any upward movement was limited to less than 20cm, so it seems improbable that these were responsible. Proprioceptive stimuli, arising for example through changes in tension in the swimbladder wall may be responsible (Harden Jones and Marshall, 1953; Qutob, 1962). Further work is required to identify the stimuli which initiate the inflatory and deflatory reflexes in the pike, and indeed in teleosts in general.

Although a state of neutral buoyancy is of undoubted advantage to many fish in still water, it is likely to be distinctly disadvantageous to fish in flowing water, since it requires the expenditure of additional

effort to maintain position, and increases the probability of being swept away by the current.

The majority of pike held in still water were found to have either neutral or slight positive buoyancy, while all of the pike exposed to a moderate current were found to be negatively buoyant (Tables 9(a), 9(b)). These findings differ from those of Gee et al. (1974) who showed that a shift from near neutral to positive buoyancy occurred when pike were exposed to a current. However, these differing results are not necessarily incompatible, since Gee et al. suggest that the increased buoyancy in current found in the young pike used in their study was "... a mechanism for displacing these fish downstream to lakes". Larger pike, such as those used in the present study presumably have no such requirement for dispersal by current.

The state of negative buoyancy, and the significantly lower mean excess internal pressure found in pike held in a current, suggest that the buoyancy is reduced by gas loss, either via the pneumatic duct in the short term, or as a result of diffusion through the swimbladder walls in the longer term. The occasionally observed release of gas bubbles via the opercula points to the use of the "gasspuckreflex" as the principal deflatory mechanism under such circumstances. However, the existence of an excess internal pressure in the swimbladder of negatively buoyant pike (Table 9(b)) is indicative of the ability to bring about volume changes by compression of the swimbladder contents. Such a phenomenon has been shown to occur in the physoclists Lagodon rhomboides (McCutcheon, 1958, 1962) and Gadus morhua (Sundnes and Gytre, 1972). Further evidence of the ability of pike to produce buoyancy changes by compression of the swimbladder contents comes from the observation that fish decompressed to their pressure of neutral buoyancy were capable of making transient reductions in buoyancy (Section ii (a)).

Compression of the swimbladder contents can be brought about in two possible ways. Firstly, since the swimbladder wall contains circular and longitudinal smooth muscle fibres (Corning, 1888; Rauther, 1923; present work), and has contractile properties (Czermak, 1850; present work), this may be used to exert pressure on the swimbladder gas. However, experimental evidence suggests that the degree to which volume changes could be produced by this means are likely to be very limited, and would not adequately explain the observed transient changes in buoyancy. The second way in which swimbladder volume could be reduced involves the use of the body wall musculature. The clearly discernible arching of the longitudinal body axis observed during decompression experiments (Section ii (a)), suggests that pressure exerted by the muscles of the body wall, coupled with the influence of a relatively inextensible swimbladder wall may be responsible for producing small buoyancy changes. The benefits of an ability to make small changes in buoyancy by direct manipulation of the swimbladder volume, to a fish which depends largely on accurate buoyancy control for the stalking and capture of prey are clear.

This study set out to investigate the means by which the gas content of the swimbladder in the northern pike, Esox lucius, changed under different conditions, and to assess the relative importance of the various mechanisms by which buoyancy can be regulated by manipulation of swimbladder volume. The results of the various investigations show that the pike is capable of employing the well-developed secretory complex present in its swimbladder to adapt to neutral buoyancy at depths greater than those permitted by air-gulping alone. They further show that pike are capable of secreting gas at rates comparable to those demonstrated by some physoclists. Swimbladder deflation in pike was shown to occur as a result of the controlled release of gas via the pneumatic duct

during decompression; the ejection of gas on exposure to a water current, and under conditions of stress; and as a consequence of the diffusion of gas through the swimbladder wall. Diffusion losses are, however, reduced by the presence of purine crystals. In addition, pike demonstrated an ability to manipulate the volume of the swimbladder to a small degree in the short term, probably primarily as a result of the action of the muscles of the body wall.

In light of these findings, it becomes clear that the physostomous swimbladder of the pike is a complex organ, which permits successful adaptation under conditions of changing environmental pressure, and contributes substantially to the pike's success as a predator.

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APPENDIX 1 - Composition of Acid Rinsing Solution

H_2SO_4	1.00 cm^3
Na_2SO_4	55.60 g
Glycerol	21.00 cm^3
Distilled water	400.00 cm^3

In use, 40 mg $K_2Cr_2O_7$ added per 50 cm^3 of solution

APPENDIX 2 - Composition of Burnstocks Physiological Saline (Burnstock, 1958)

NaCl	7.25 g
KCl	0.38 g
$NaH_2PO_4.H_2O$	0.41 g
$NaHCO_3$	1.00 g
$MgSO_4.7H_2O$	0.23 g
$CaCl_2.2H_2O$	0.23 g
D-Glucose	1.00 g
Distilled water	1000.00 cm^3

$\Delta T(^oC) = -0.58$

II