Respiration Physiology (1978) **34**, 153–170

GILL VENTILATION IN THE STURGEON, *ACIPENSER TRANSMONTANUS*: UNUSUAL ADAPTATIONS FOR BOTTOM DWELLING

W. W. BURGGREN[1]

Department of Zoology, University of British Columbia, Vancouver, B.C. V6T 1W5, Canada

Abstract. Measurements of branchial cavity water pressures and flow patterns, arterial blood P_{O_2} and pH, and oxygen utilization and uptake have been made in undisturbed, free swimming sturgeon, *Acipenser transmontanus*. Although the jaws are degenerate and the oral apparatus is highly modified for feeding, gill ventilation is nonetheless powered by a buccal force pump and an opercular suction pump common to most bony fishes. The reduced spiracles play little or no role in gill ventilation.

In sturgeon in which water intake through the ventrally located mouth was experimentally eliminated, a condition which may often develop when these fish forage in mud and sand on river substrates, effective ventilation of the gills was maintained with water drawn into the branchial cavities in a retrograde fashion solely through permanent openings in the upper regions of the opercular slits. O_2 uptake and transport also remained at control levels. It is suggested that this unusual alternative mode of gill ventilation in the sturgeon represents an important respiratory adaptation to bottom dwelling and feeding.

Arterial blood — Gill ventilation
Chondrostei — Mechanics of breathing

The evolution of the ray-finned fishes was heralded by the appearance of the Chondrostei in the lower Devonian. The highly successful Teleost fishes have descended from this founding order of the actinopterygians. However, the Chondrostei reached their zenith during the Carboniferous and the sturgeon, the paddlefish, and the bichir, *Polypterus*, and its relatives are the only Chondrostei which have survived to present times. These fishes have retained little in common with their extinct paleoniscid ancestors. The sturgeon, for example, is generally considered to be highly degenerate, having lost the characteristically thick palaeoniscid scales

Accepted for publication 25 March 1978

[1] The author was a recipient of a joint NRC–Killam Postdoctoral Fellowship during the course of this investigation.

as well as most of the ossification of the skeleton (Romer, 1970). One of the more striking morphological differences between the sturgeon and its large-jawed, heavily toothed predacious ancestors is the degeneration of the oral apparatus. The jaws of the sturgeon are weak and toothless. The tubular mouth and fleshy lips form an evertable organ which, in combination with the broad spade-like snout, allows the sturgeon to sift through the mud and detritus of river and lake bottoms and then suck in the small invertebrates upon which it feeds.

Because of the extreme ventral position of the highly modified suctoral mouth, as well as the foraging nature of feeding in an often silty substrate, the inhalation of water through the mouth for the purpose of gill ventilation in the sturgeon will introduce a variable amount of detritus into the branchial chamber. Many bottom feeding fishes have long, interdigitating gill rakers to protect the gills and prevent them from becoming clogged. This then necessitates a periodic transient reversal of water flow in the branchial cavity (coughing) to clean the gill rakers. A more unusual anatomical arrangement occurs in the skates and rays, which are also bottom dwelling, where the ventrally located mouth does not serve for branchial water intake when the fish is resting on the substrate. Instead, the gills are ventilated with presumably cleaner water drawn in the dorsally located spiracles (Hughes, 1960a).

Unlike almost all other bony fishes, the sturgeon has also retained patent spiracular openings. Yet, the extent to which they serve to draw in water for branchial ventilation is currently unknown, as is to what extent the respiratory function of the highly modified oral apparatus has become subservient to the demands of feeding in the sturgeon. The object of the present investigation was to investigate processes of gill ventilation and respiration in the sturgeon *Acipenser transmontanus* in order to (1) determine if morphological and physiological adaptations for bottom dwelling are evident and (2) to compare gill ventilation in this ancient fish with that in more recently evolved Teleosts.

Methods

Ten white sturgeons (*Acipenser transmontanus*) weighing between 0.80 and 1.10 kg (mean weight 0.90 kg) were used in this investigation. All fish were caught in fresh water on hook and line in the lower reaches of the Fraser River in British Columbia, and transported to large holding tanks containing flowing well water. The sturgeons were maintained in these tanks for a minimum of 5 weeks before experimentation was begun, at which time any wounds to the mouth suffered during capture were completely healed. All experiments were performed at 15.0 °C $\pm \frac{1}{2}$ °C, unless otherwise indicated.

PHOTOGRAPHIC AND X-RAY ANALYSIS OF VENTILATORY MOVEMENTS

Ventilatory movements in individual fish resting in a clear plexiglass container of water were photographed from above and through the sides and bottom of the container. Both black and white super 8 mm cine film and 35 mm single frames were taken. X-ray cine films (16 frames/sec) of branchial water flow during gill ventilation in unanaesthetized sturgeons were made during periods when the inhalent and/or exhalent water was marked with minute amounts of a radio-opaque dye, sodium diatrizoate (Hypaque). Dye was introduced into the ventilatory water stream via a cannula placed externally to the lips, spiracle or opercular slit or via a cannula advanced through the mouth into the buccal or opercular cavity.

OXYGEN CONSUMPTION MEASUREMENTS

Individual fish were placed within a respirometer constructed from a 70 cm long piece of PVC pipe 12 cm in internal diameter (total volume approximately 8 l). One end of the respirometer contained an inlet for air-equilibrated water at 15 °C, the other end contained a water outlet. Sufficient mixing of water within the respirometer was assumed to be provided by the high velocity of the introduced water and by the fish's own ventilatory and locomotor movements. Water flow rate through the respirometer (1.10–1.12 l/min) was determined at the time of each oxygen consumption measurement by making timed water collections. Oxygen tensions of water entering (P_{O_2} 150–155 mm Hg) and leaving (P_{O_2} 130–140 mm Hg) the respirometer were measured with a water-jacketed Radiometer O_2 electrode and Radiometer 27 gas analyzer. The respirometer walls were translucent, and auditory stimuli were minimal. $\dot{V}_{O_2}$ measurements were made every 15 min during the experiment, and only those values which together in a series reflected a steady-state were utilized in calculations. This condition was usually not achieved until fishes had been left undisturbed in the apparatus for at least 2–4 hours.

Oxygen consumption during different swimming speeds was measured in a Brett (1964) respirometer maintained at 10 °C. Details of construction and operation of the particular apparatus used in these experiments have been given elsewhere (Kiceniuk, 1975; Jones and Schwarzfeld, 1974). Fish were placed in the respirometer for 12–15 hours before measurements were made. Oxygen consumption was measured during 10-min periods of swimming at known speeds, and 30-min rest periods were allowed between each speed increment.

BRANCHIAL HYDROSTATIC PRESSURE MEASUREMENTS

Sturgeons were anaesthetized for cannulation with MS 222 (1 : 10,000). Chronic cannulation of the anterior region of the buccal cavity was made ventrally through

the thin layer of skin posterior to the mouth forming a narrow portion of the buccal cavity floor. Chronic opercular cavity cannulation was through the dorsal and/or ventral regions of the cleithrum (see arrows and legend in Plate 1). PE90 polyethylene cannulae were used in all instances, and were secured in position as described by Smith and Bell (1964).

Fish were then allowed 24 h to recover from the effects of anaesthesia before measurements were begun. In addition to chronic cannulation, in some experiments the tips of hand-held cannulae were manoeuvred around the gill slits and into the opercular cavities, down the spiracles, or advanced between the gill arches into the buccal cavity.

Cannulae were attached to either water filled E and M P-1000 or Biotronics BT-70 pressure transducers, whose outputs were recorded on a rectilinear E and M Physiograph 6 chart recorder. Because of the very small pressure oscillations being measured, extreme care was taken to establish accurate zeros and water calibration heads, and transducer calibration was regularly and often performed. No corrections for the kinetic component of the total fluid pressure energy were made (see Holeton and Jones, 1975), though the chronic buccal and opercular cannulation sites and techniques used tended to measure 'side pressures' and so minimized errors from this source.

Pairs of thin copper wires insulated but for the tips were implanted into the operculae and the lips of each fish. The copper electrodes were attached to a Biocom 2991 Impedance Convertor writing out on the chart recorder and were used to monitor mouth and opercular opening and closure associated with ventilatory movements.

EXPIRED WATER AND BLOOD GAS ANALYSIS

Water being expired from the opercular cavity of the sturgeon was drawn into a syringe from a carefully determined position under the velum at the lower edge of the opercular slit where almost all ventilatory water is expired. (Precise locations of the exhalent water streams along the opercular slit are described in the Results). Arterial blood was sampled from freely swimming sturgeon. Sampling was via a cannula (PE 90) tipped with the shaft of a 21 ga. Huber point chronically implanted under MS 222 anaesthesia into the dorsal aorta through the roof of the mouth. The dorsal aorta cannula, which was filled with heparinized saline, was led out through the top of the opercular slit and secured for 10 cm along the dorsal surface of the fish with sutures. Expired water P_{O_2} and dorsal aorta blood P_{O_2} and pH were determined with a Radiometer pHM 71 acid–base analyzer and the appropriate Radiometer electrodes. Blood O_2 content was determined according to Tucker's method (1967). Blood withdrawn for P_{O_2} measurement was returned into the dorsal aorta and 10–15 samples taken over 1–2 days produced no significant changes in hematocrit.

Results

Plate 1 presents a photographic analysis of respiratory movements in *Acipenser transmontanus* during a single ventilatory cycle, while records of hydrostatic pressure changes in the buccal cavity and lower regions of the opercular cavity as well as mouth and opercular movements during several successive ventilatory cycles are presented in fig. 1. Phasing terminology of buccal and opercular events as described for teleost fishes by Hughes and Shelton (1958) has been adopted.

Opercular abduction during phase 1 (plate 1) produced an increase in opercular cavity volume and the development of a pressure approximately 1.0 cm H_2O below ambient hydrostatic pressure in the lower region of the opercular cavity. The floor of the buccal cavity was lowered during this phase producing a buccal pressure approximately 0.5 cm H_2O below ambient hydrostatic pressure (fig. 1). Buccal volume thus also increased and water entered the open mouth. Although the opercular suction pump predominated during phase 1, the pressure gradient driving water flow across the gill sieve (buccal minus opercular pressure) remained quite small compared to other phases in the ventilatory cycle (fig. 1).

The very small paired spiracles (1–2 mm diameter in 1 kg fish) enter into the dorsal region of the buccal cavity and open externally approximately half ways between the eye and the top of the opercular slit. The external openings are partially occluded by a small projection of tissue which rhythmically varies in position during the ventilatory cycle. During opercular abduction in phase 1 the projection was partially withdrawn and the small spiracular openings were fully opened. Since buccal pressure at this time was lower than ambient hydrostatic

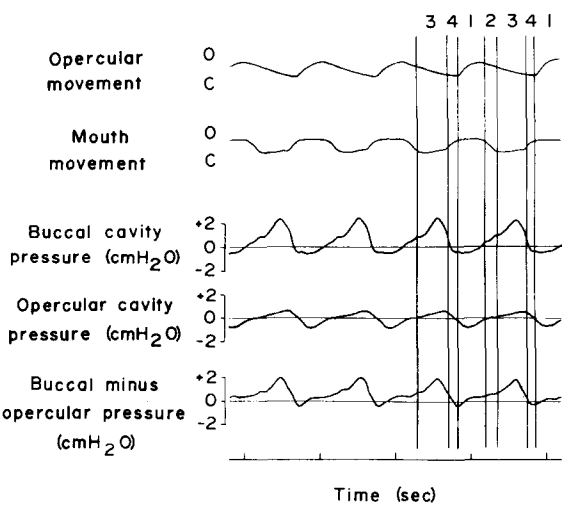

Fig. 1. Ventilatory movements and branchial hydrostatic pressures recorded during several successive ventilatory cycles in a freely swimming *Acipenser transmontanus*. Opercular and mouth opening (O) and closing (C) are indicated, as are the phases of the last 2 ventilatory cycles.

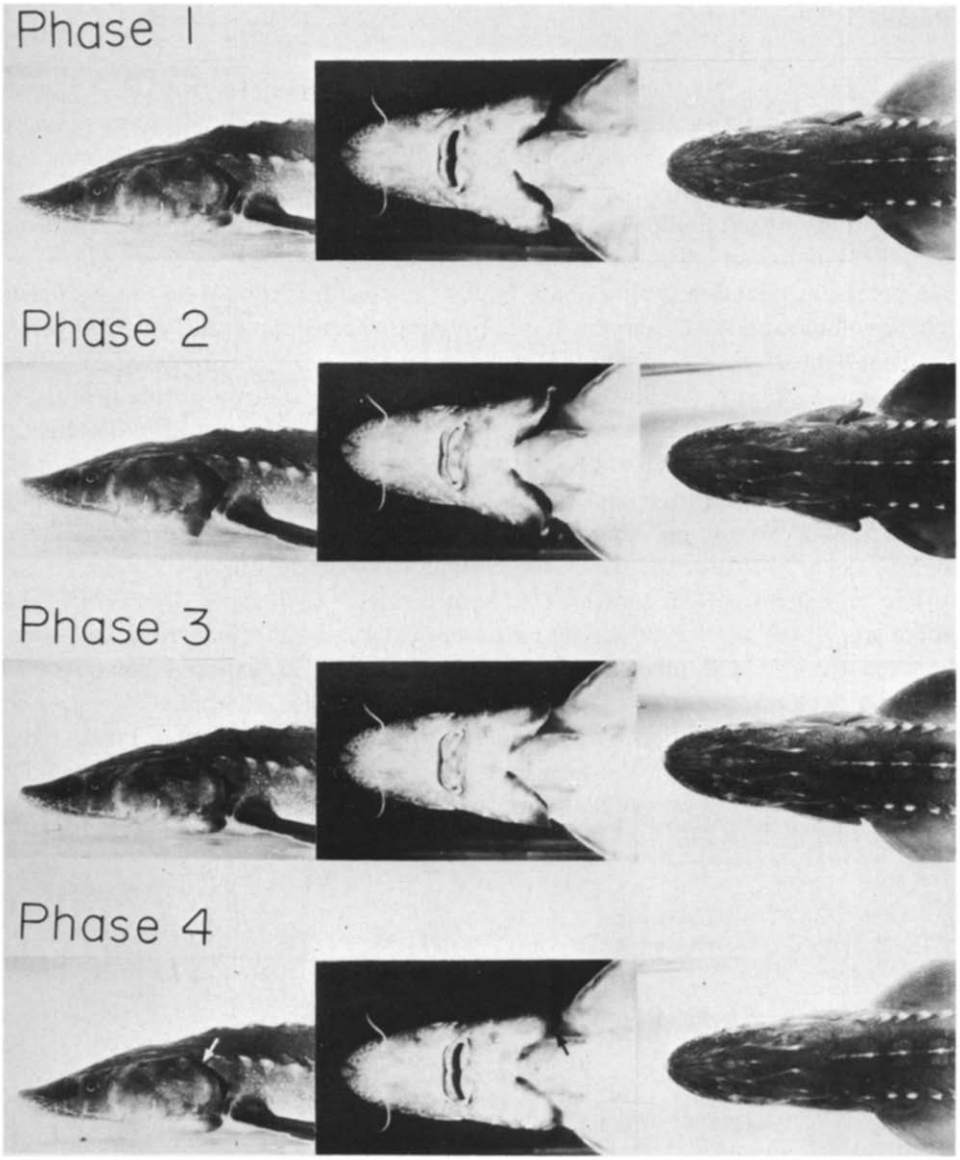

Plate 1. Lateral, ventral and dorsal views of the head of *Acipenser transmontanus* during a single ventilatory cycle. The width of the head at the level of the mouth of this sturgeon is approximately 8 cm. See the text for a description of the phasing of the ventilatory movements. The white arrow in the lateral view, phase 4, indicates the site of chronic cannulation of the upper region of the left opercular cavity. The black arrow in the ventral view, phase 4, indicates the site of chronic cannulation of the lower region of the left opercular cavity.

pressure in the buccal cavity (fig. 1), a pressure gradient tending to draw into the spiracles existed.

Phase 2 consisted of a transitionary period in which the operculae reached the limits of their outward excursion and begin to adduct and the mouth rapidly closed (plate 1). With the onset of phase 3 buccal pressure became elevated to over 2.0 cm H_2O as the floor of the buccal cavity rose and buccal cavity volume decreased (plate 1, fig. 1). Since the mouth was firmly closed during this phase water was propelled from the buccal cavity through the gill sieve into the opercular cavity. The operculae continued to close (plate 1), a very small positive opercular cavity pressure developed, and water was exhausted out the gill slits. It was during the buccal force pumping of phase 3 that the largest pressure gradient (approximately 2.0 cm H_2O) occurred from the buccal to the opercular cavities (fig. 1), and potentially the phase during which the greatest movement of water across the gills could occur. During phase 3 the external spiracle openings were occluded to varying degrees in different fishes. Since buccal pressure was high during this phase, a pressure gradient driving water flow out the spiracles existed, but with the complete closing or extreme narrowing of the spiracle openings to only 0.5–1.0 mm spiracle channel resistance was probably very high, producing functional occlusion.

Phase 4 consisted of a second transitionary period in which the mouth began to open (plate 1) and pressures in the buccal and opercular cavities fell rapidly towards 0. A reversed hydrostatic pressure gradient from the opercular to the buccal cavity existed during phase 4, but it was usually less than 0.3 cm H_2O and very brief (fig. 1).

The generation of significant pressures below ambient hydrostatic pressure in the opercular cavity was perhaps unexpected, for one of the most unusual and striking morphological features of the ventilation system of *Acipenser* is the failure of the velum, the membranous flap of tissue on the trailing edge of each operculum, to form a functional valve to seal the opercular cavity from the exterior when the operculae are abducting during phase 1. In fact the velum is progressively reduced dorsally to the extent that the tops of gill arches 2 and 3 are always clearly visible in *Acipenser* throughout the ventilatory cycle. This is clearly evident even in the lateral view of phase 4 in plate 1, which depicts the period in the ventilatory cycle when the operculae are maximally closed. Interestingly, the permanently exposed regions of the gills are darkly pigmented so that the filaments carrying oxygenated blood, which are bright red deeper in the opercular cavity, blend in with the dark grey skin of the sturgeon's back, presumably for the purposes of camouflage. This anatomical arrangement of the velum, as well as the above mentioned phasing of buccal and opercular movements, was also found to be the case for a very large female *Acipenser* 336 kg in weight and 3.45 m in length which was observed in captivity over a 24-h period.

That the uppermost region of the opercular cavity is never functionally isolated from the external environment must have important implications both to the effectiveness of the opercular suction pump and to the patterns of water flow over the gills. A series of more detailed water pressure measurements were thus undertaken

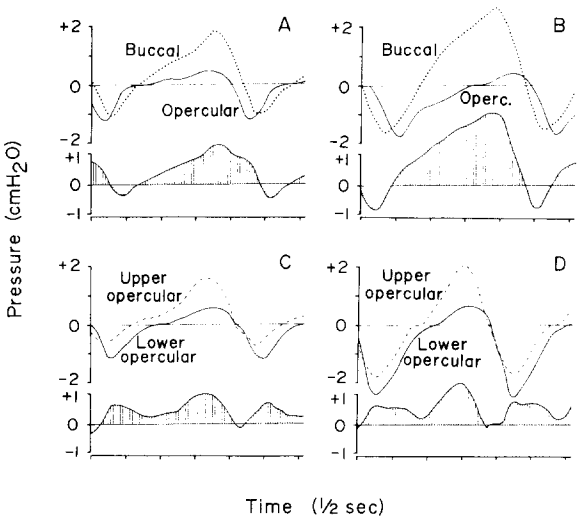

Fig. 2. Buccal and opercular hydrostatic pressures recorded during single ventilatory cycles in a freely swimming *Acipenser transmontanus*. Also indicated by hatched lines are pressure differentials between each set of pressure records. In A and B buccal pressure and the pressure recorded at the lower opercular chronic cannulation site are presented, while in C and D pressures in the upper and the lower regions of the opercular cavity appear. Pressures were recorded both when buccal water intake was freely occurring (A and C) and when all buccal water intake was eliminated and strictly opercular water intake served to ventilate the gills (B and D).

to reveal any regional pressure variations in the opercular cavity. Figure 2A presents superimposed records of buccal cavity pressure and of opercular cavity pressure recorded from a chronically implanted cannula located at the point indicated by the black arrow in the ventral view of phase 4, plate 1. A nearly continuous pressure gradient from the buccal cavity to the lower region of the opercular cavity was evident in this fish as in all others. Figure 2C presents superimposed records of pressures measured simultaneously in the opercular cavity of the same sturgeon both at a point 1 cm from the top of the opening of the gill slit (white arrow in lateral view of phase 4, plate 1) and at the same chronic opercular cannulation point as in fig. 2A on the underside of the fish 1 cm from the bottom of the gill slit. During 95% of the ventilatory cycle pressure in the uppermost regions of the opercular cavity exceeded that in the lower regions by as much as 1.0 cm H_2O. Pressure in the upper opercular region reached a maximum of 2.0 cm H_2O during phase 3, while pressure developed in the lower portion of the opercular cavity never exceeded approximately 0.5 cm H_2O. The development of pressures lower than ambient hydrostatic pressure during abduction of the operculae was much more pronounced in the lower regions than in the uppermost region of the opercular cavity of *Acipenser*, and consequently the pressure differential between these two sites was also maintained during the opercular suction phase (fig. 2C). These large regional differences in opercular cavity pressure development were also demonstrated

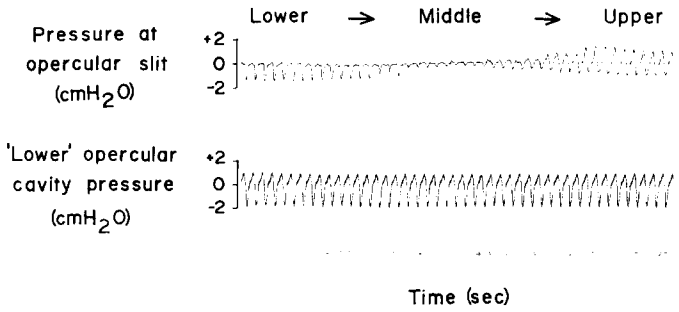

Fig. 3. Opercular pressures recorded during many successive ventilatory cycles in a freely swimming *Acipenser transmontanus*. The lower trace is the hydrostatic pressure recorded from a chronically implanted cannula. The upper trace is the pressure recorded from a cannula whose tip was manoeuvred from the bottom to the top of the gill slit.

by slowly manipulating the tip of a hand-held cannula from the bottom through to the top of the opercular cavity, maintaining the tip at a depth of approximately 2–4 mm into the opercular slit. Pressures predominantly below ambient hydrostatic pressure were recorded at the opercular slit in the lower regions of the opercular cavity corresponding to the region of the operculum where the velum is most complete (fig. 3). Midway along the gill slit pressure oscillations greatly decreased, but as the cannula was advanced towards the top of the opercular cavity pressure pulsatility returned. However in the uppermost regions of the opercular cavity pressures below ambient hydrostatic pressure were diminished and a much more highly positive pressure component than in the lower regions was now evident.

A pressure differential between widely separated upper and lower points in the opercular cavity does not *a priori* imply significant water flow along this pressure gradient for the two regions could be functionally separated by a high resistance pathway through the holobranchs, for example. Hence, experiments were designed to determined directions of water flow in the buccal and opercular cavities. Radio opaque dye injected into the ambient water near the mouth was drawn into the buccal cavity during phase 1 and within 2–3 ventilatory cycles was flushed through all of the gill arches and then exhausted almost exclusively via the middle and lower regions of the gill slits. Water flow was quite laminar (though pulsatile) in the buccal cavity until passage through the gill sieve, beyond which localized regions of turbulent flow within the opercular cavity were quite apparent. When radio opaque dye was injected near the external spiracular openings, a very small inward flow of water through the spiracles was apparent during phase 1. No direct attempt was made to quantify spiracle water intake, but based on spiracle diameter and water velocity observations, it must certainly have constituted much less than 1% of total gill water flow in *Acipenser*.

Dye injected into the ambient water approximately 2–5 mm away from the very top of either the left or right gill slit of *Acipenser* was drawn into the opercular cavity through the opening at the top of the gill slit in a pulsatile fashion during oper-

cular abduction. The dye was then flushed in a ventral direction through only the most distal filaments of the gill arches (mostly arches 2 and 3), and then within 1 ventilatory cycle was exhausted from the opercular cavity via the middle or mainly the bottom regions of the gill slit. Dye injected into the water 2–5 mm outside the middle or lower regions of the gill slits was either directly swept away in the exhalent water stream during phase 3, or was drawn in a turbulent eddy a cm or less into the lower regions of the opercular cavity during phase 1 and then exhausted out the opercular slit without making direct contact with the distal gill filaments.

In an attempt to assess the potential in *Acipenser* for gill ventilation to be maintained strictly with water taken in at the top of the gill slits during opercular abduction, experiments were performed in fish in which all buccal water intake was prevented. Two experimental approaches were taken. Surprisingly, sturgeon resting quietly in an experimental tank usually showed no outward response to having fine sand slowly poured over their snout until the forward portion of their head below the eyes, as well as their mouth was completely buried. The sand was fine enough to provide a very large resistance to water flow through it, so all water flow in through the mouth to the buccal cavity was terminated upon burial of the mouth in sand. A second more severe approach, which also produced no apparent disturbance to the fish, was to sew the lips tightly shut with 2–3 interrupted sutures of silk thread. Ventilatory movements of the operculae as well as movements of the buccal floor continued nearly unabated in both situations, though the amplitude of the opercular excursions was greater when buccal water intake was eliminated. Figures 2B and 2D present records of buccal pressure and pressure in the upper and lower regions of the opercular cavity during ventilation with the mouth experimentally sealed. Fundamental branchial pressure relationships, such as the existence of a pressure gradient driving water from the buccal to the opercular cavity (fig. 2A compared to fig. 2B) and an elevated pressure at the top compared to the middle or bottom of the opercular cavity (fig. 2C compared to fig. 2D) remained unchanged when buccal water intake was eliminated. However, branchial pressures were much more pulsatile when the gills were only being ventilated with water drawn in through the top of the opercular slits (figs. 2B and 2D). Moreover, the pressure differentials between the upper and lower recording sites and between the buccal and opercular cavities were elevated during experimental periods of opercular generated gill ventilation.

Whereas only a very small stream of radio-opaque dye was drawn into the upper region of the gill slit and rapidly passed over a few distal gill filaments during control ventilation, a large dye stream was drawn into the upper region of the opercular cavity and passed in an anterior direction in a discrete laminar stream against the dorsal-lateral body wall when buccal water intake was prevented. This water stream did not immediately ventilate the gill filaments, but rather passed forward into the buccal cavity. Once into the buccal cavity this water was directed back through and between the filaments of all eight gill arches and into the opercular cavities,

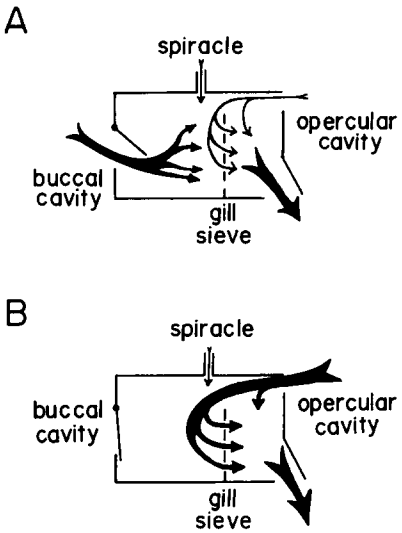

Fig. 4. Diagrammatic summary of gross water flow patterns through the buccal and opercular cavities of *Acipenser* (A) during unrestricted buccal water intake and (B) when buccal water intake is eliminated.

then exhausted out the lower regions of the opercular slits. Again, dye placed outside the middle or lower regions of the gill slit was very briefly drawn, if at all, only a cm or less into the lower regions of the opercular cavity during outward opercular movement. Active ventilation of the gill arches in the absence of any buccal water intake was also demonstrated by advancing a cannula between the lips of the mouth and then injecting a bolus of dye into the anteriorly sealed buccal cavity. 5–10 movements of the operculae were usually sufficient to completely flush all of the dye from the buccal cavity across the gills and into the opercular cavity. The minute inward flow of water through the spiracles during phase 1 remained unchanged during conditions of strictly opercular water intake.

Figure 4 presents a diagrammatic summary of gross water flow patterns through the buccal and opercular cavities during buccal and during opercular water intake in *Acipenser*, as revealed by pressure recordings, visible dye injection, and cine X-rays.

Records of heart rate and ventilatory movements recorded in *Acipenser transmontanus* during the two different patterns of branchial water intake are presented in fig. 5. Heart rate during predominantly buccal water intake when the mouth was free to rhythmically open and close ranged from 25–35 beats/min at 15 °C in resting sturgeon. Heart rate fluctuated little in undisturbed individuals, though fell markedly immediately following a cough (beginning of fig. 5B). Upon gently covering the lower portion of the head and the mouth with sand and so stopping buccal water intake, heart rate decreased immediately by 20–50%, while ventilatory frequency decreased by 5–15% (figs. 5A and 5C). In many sturgeon, heart rate became more irregular during mouth occlusion, and after several cycles at the lower

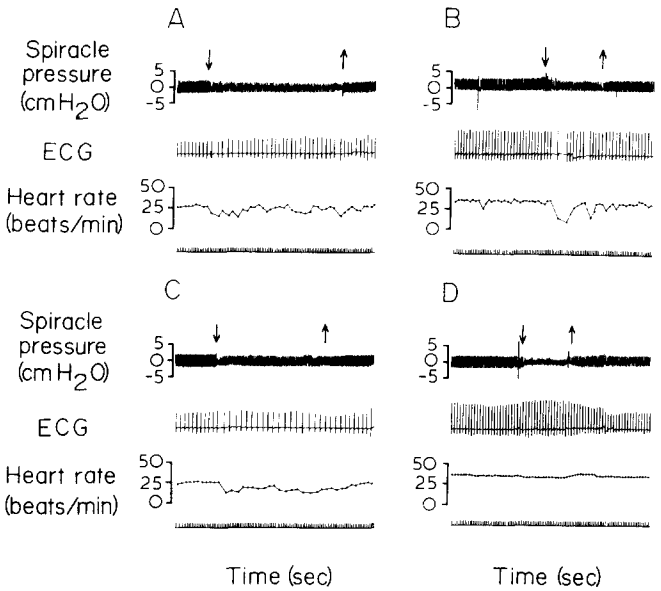

Fig. 5. Heart rate responses to the elimination of buccal water intake by burial of the mouth in sand in 2 unrestrained *Acipenser transmontanus*. A cannula was placed in the spiracle to provide an indication of ventilatory frequency. The downward arrows indicate the time at which burial of the head (but for the opercular slits) with sand occurred. The upward arrows indicate the point at which the fish spontaneously lifted its head out of the sand and reverted to buccal water intake. Representative responses in a fish (A) in normoxic water and (B) in hyperoxic water (P_{IO_2} = 400 mm Hg) are presented. Cardiac responses in a different fish in normoxic water (C) before and (D) after the injection of atropine are also shown.

heart rate began to rise back towards control levels (fig. 5C). If the fish spontaneously swam up out of the sand or was lifted from the bottom heart rate returned to former levels. Bradycardia could also be elicited by reaching beneath a resting sturgeon and simply holding its lips together. Mechanical stimulation of the barbels, eyes or lateral line produced little or no heart rate change. This reflex slowing of heart rate was apparently not related to asphyxia developing from the elimination of buccal water flow, for a bradycardia still occurred when the P_{O_2} of the inhalent water was elevated to 400–500 mm Hg (fig. 5B). Atropine (0.75 mg/kg body weight) injected intra-muscularly produced an increase in heart rate above control levels and completely blocked all heart rate changes associated with mouth occlusion (fig. 5D).

Table 1 presents a summary of respiratory data in *Acipenser transmontanus* at 15 C during periods of predominantly buccal water intake and during periods when strictly opercular intake of water served to ventilate the gills. Mean values for each fish were calculated from a minimum of at least five measurements in each condition, and these values were then used to calculate overall means. Data from 9 *Acipenser* are included, though any particular parameter was only measured in 5 fish. Also indicated are the significance levels as determined with a Student's t-test of the differences between conditions.

TABLE 1
Respiration in *Acipenser transmontanus* at 15 C

	Buccal water intake	Opercular water intake	Significance level of difference
$P_{I_{O_2}}$ (mmHg)	152 ± 3	150 ± 4	NS*
$P_{E_{O_2}}$ (mmHg)	105 ± 12	107 ± 9	NS
% util	30 ± 9	28 ± 7	NS
$\dot{V}g$ (ml/kg/min)	421 ± 98	425 ± 66	NS
$\dot{V}_{O_2}$ (ml O_2/kg/h)	57.3 ± 13.9	52.3 ± 8.3	NS
$P_{DA_{O_2}}$ (mmHg)	94 ± 5	84 ± 5	$P < 0.025$
$C_{DA_{O_2}}$ (vol. %)	7.04 ± 1.66	7.05 ± 1.46	NS
$[H^+]$ (nm/l)	14.40 ± 1.80 (pH 7.84)	14.40 ± 2.30 (pH 7.84)	NS

* Not significant, *i.e.* $P > 0.10$
Mean values ± 1 SD are given. Data are from 9 fish.

Expired oxygen tension ($P_{E_{O_2}}$), oxygen utilization (% utilization), and gill ventilation volume (Vg) were nearly identical during buccal and during strictly opercular water intake. Dorsal aorta oxygen tension ($P_{DA_{O_2}}$) was significantly reduced when buccal water intake was eliminated, but dorsal aorta blood oxygen content ($C_{DA_{O_2}}$) and hydrogen ion concentration $[H^+]$ were not. Overall, steady-state oxygen consumption ($\dot{V}_{O_2}$) fell very slightly from 57.3 ± 13.9 to 53.3 ± 3.8 ml O_2/kg/h when buccal water intake was prevented and only opercular water intake occurred, but this change was not significant ($P > 0.10$).

In order to assess whether $\dot{V}_{O_2}$ during strictly opercular water intake could also be maintained in the exercising as well as the resting sturgeon, $\dot{V}_{O_2}$ was measured in 4 fish during swimming at varying speeds. Figure 6 indicates a representative experiment. $\dot{V}_{O_2}$ at 10 °C during buccal water intake increased from approximately 35 ml O_2/kg/h during rest up to a maximum of approximately 100 ml O_2/kg/h at a swimming speed of 1.05 body lengths/sec. In most sturgeon, a further slight increase in swimming speed could be achieved for a 10-min period without the benefit of an increase in $\dot{V}_{O_2}$ (fig. 6). No sturgeon could maintain a swimming speed of greater than approximately 1.2 body lengths/sec during a test period when normal buccal water intake occurred. After sewing the lips shut and providing a further 2–12 h rest period, $\dot{V}_{O_2}$ over the first half of the swimming speed range of each fish was maintained at levels very similar to those measured during buccal water intake. However, $\dot{V}_{O_2}$ at a speed of 0.6–1.0 body length/sec began to fall significantly below

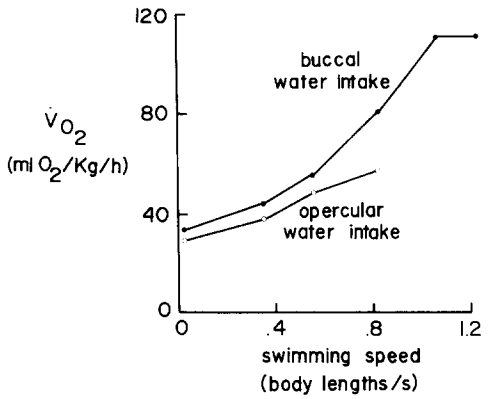

Fig. 6. Oxygen consumption (10 C) at different swimming speeds expressed in body lengths/sec in a representative experiment in *Acipenser transmontanus* (fork length 62 cm). The relationship during both buccal water intake (solid lines) and strictly opercular water intake (dotted lines) is presented.

levels of $\dot{V}_{O_2}$ maintained during buccal water intake at the same speed, and in fact, the maximum speed which could be maintained for a 10-min period during strictly opercular water intake was approximately 2/3 of that maintained during buccal water intake.

Discussion

Ventilation of the gills of *Acipenser* with water drawn in through the mouth is achieved by synchronous movements of both the buccal cavity floor and the opercula. The gross sequencing of these movements in the sturgeon is similar to that described for both Teleost fishes (Hughes and Shelton, 1958; Hughes, 1960b; Saunders, 1961; Hughes and Umezawa, 1968) and Elasmobranchs (Teichman, 1959; Hughes, 1960a; Hughes and Ballintijn, 1965). The general arrangement in these fishes consists of the generation of large positive pressures anterior to the gill sieve by a buccal force pump, whose action alternates with that of an opercular suction pump generating pressures below ambient hydrostatic pressure posterior to the gills. Water flow will follow the resulting pressure gradient across the gills, though at least in some fish branchial water flow is very pulsatile and because of the considerable inertia of water in the branchial cavity flow may lag signficantly behind pressure gradients (Holeton and Jones, 1975). While pressure/flow relationships have not been examined in detail in *Acipenser*, the tracing of movements of radio-opaque dye added to the inhalent water has revealed that a continuous flow of water from the buccal to the opercular cavities of *Acipenser* occurs during all but the late stages of phase 4 and the earliest stages of phase 1 when a small negative buccal/opercular pressure gradient develops (figs. 1, 2).

Although patent spiracular canals in *Acipenser* exist between the sides of the buccal cavity and the external environment, their diameter of only 1–2 mm in 1 kg

fish is minute compared to the dimensions of the other branchial channels. The relatively high resistance of the very narrow spiracle channels along with the valve-like action of the projections of tissue guarding the external spiracle openings obviates significant spiracle water flow in terms of overall gill ventilation volumes during any phase of the ventilation cycle. A very small pseudobranch lines the internal opening of the spiracle canal in *Acipenser*, and the maintenance of a very slight spiracular water flow could be important if the pseudobranch were the location of chemoreceptors, as occurs in some teleost fish (Laurent and Rouzeau, 1972). A somewhat similar arrangement of pseudobranchs and spiracles to that in *Acipenser* exists in the elasmobranch *Squalus acanthias*, but they seem to play no role in the responses of this selachian to hypoxia (Satchell, 1961).

Although the upper regions of the opercular cavities are permanently open to the ambient water in the sturgeon, a still significant pressure below ambient hydrostatic pressure during phase 1 and an active generation of a considerable positive pressure during phase 3 nonetheless occurred in the upper regions of the opercular cavities (fig. 2). During opercular abduction, when the opening at the top of the opercular slit was large, a pressure gradient tending to drive water from the outside into the top of the opercular cavity, and then in turn to the middle and lower regions of the opercular cavity thus existed. Radiography experiments subsequently demonstrated that the great majority of filaments were ventilated with water drawn in through the mouth, but an undetermined proportion of water originally inhaled at the top of the opercular slits actually served to ventilate some of the distal gill filaments. Hence, that amount of energy expended by the muscles powering opercular abduction on drawing water in at the tops of the gill slits was not totally ineffectively applied in terms of filament ventilation achieved. The active generation during phase 3 of a considerable pressure in the upper regions of the opercular cavities maintained the positive pressure gradient from top to bottom of the opercular cavities, as well as caused a reversal of the gradient formerly drawing water into the top of the opercular slits. The expulsion of water out of the opercular cavities during phase 3 was thus facilitated by positive pressure generation in the upper regions of the opercular cavities.

In the resting sturgeon with unimpaired buccal water intake the gill filaments were hence ventilated in part with water drawn in the spiracles (almost negligible), in the top of the opercular slits, and, by far the greatest extent, in through the mouth. Additional water drawn into the opercular cavity via the top of the opercular slit which did not come into contact with the gill filaments before being exhausted from the opercular cavity constituted a considerable anatomical water shunt or dead space, and in combination with ventilation/perfusion inequalities in other regions of the gill filaments likely contributed to relatively low O_2 utilization values of 30%. Nonetheless, dorsal aorta blood P_{O_2} was sufficiently high to produce full blood oxygen saturation during unimpaired buccal water intake (see Burggren and Randall, 1978, for a detailed discussion of oxygen uptake and transport in *Acipenser transmontanus*).

Acipenser demonstrated a remarkable capacity to maintain gill ventilation at control levels in the face of an elimination of buccal water intake. Pressure relationships between the standard buccal and lower opercular cannulation sites showed no striking changes during strictly opercular water intake (figs. 2A and 2B). This suggests that the two basic ventilatory pumping units in *Acipenser* – the buccal force pump and the opercular section pump – are evoked in both situations to power gill ventilation. Mouth resistance must be much lower than that of the water path at the top of the opercular slit during normal water intake, since little dye flows in the top of the opercular slit compared to in the mouth under these circumstances. Yet, the lack of an increase in oxygen consumption with the maintenance of $\dot{V}g$ during strictly opercular water intake suggests that during strictly opercular intake upper opercular resistance must fall to a level similar to that of the mouth during buccal water intake. Some dimensional changes of the opercular cavity and gill sieve must thus occur with the switch from one pattern of water intake to the other, though these have yet to be documented.

Tidal ventilation of a gas exchange organ with a dense medium such as water is generally considered to be prohibitive in terms of metabolic cost. The lamprey is the only fish which normally displays such an arrangement (Hughes, 1963). Gill ventilation is not truly tidal during opercular water intake in the sturgeon since water moving forward into the buccal cavity passes dorsally over the tops of the gills, but in returning back to the opercular cavities it flushes directly through the gill filaments (fig. 4). Nonetheless, a situation homologous to tidal ventilation in terms of energy expenditure can exist in *Acipenser* when the mouth is occluded. Gill ventilation with water drawn into the opercular slits necessitates first acceleration of the water in the opercular cavity as it is drawn dorsally in an anterior direction, then deceleration of the water once in the buccal cavity, and finally acceleration of the water back through the gill sieve (fig. 4), all of which demands extra metabolic energy expended by the ventilatory pumps compared to the unidirectional water flow occurring during buccal water intake. The total cost of ventilation usually represents less than 10% of the total O_2 uptake of resting fishes which have been examined (See Shelton, 1970; Jones and Schwarzfeld, 1974), and the added cost of gill ventilation in terms of $\dot{V}_{O_2}$ resulting from the transition to opercular water intake in the sturgeon may be masked by either normal small fluctuations in $\dot{V}_{O_2}$ or by a compensating decrease in oxygen demand of some other organ system.

The small but significant reduction in dorsal aorta P_{O_2} which developed during strictly opercular water intake (table 1) probably reflects the development of lamellar ventilation/perfusion inequalities (*i.e.* a slight increase in 'physiological dead space') under these circumstances. While a bradycardia of an often transient nature developed in *Acipenser* with the switch from opercular to buccal water intake, cardiac output was not determined, and whether a compensatory increase in stroke volume occurred during strictly opercular water intake is unknown. However, $\dot{V}g$ is independent of the site of water intake, and the maintenance of blood O_2 saturation, blood hydrogen ion concentration and total $\dot{V}_{O_2}$ (table 1) suggests that the matching

of the volume of blood perfusion and the volume of water ventilating the gills of the sturgeon may be somewhat similar during both buccal and opercular water intake. Grigg (1970) has reported that another bottom dwelling fish, the Port Jackson shark, has a limited ability to ventilate its gills with water drawn in the first of its five pairs of gill slits, though the respiratory effectiveness of this ventilatory pattern remains to be clearly demonstrated for this shark.

Afferent and efferent neural pathways responsible for the bradycardia during opercular water intake in the sturgeon were not established, though the response is certainly cholinergically mediated. Asphyxia was not the stimulation for this bradycardia (fig. 5B), and in any event unlike many other fishes which show a marked bradycardia during hypoxia (see Randall, 1970), *Acipenser transmontanus* shows very little reduction in heart rate in all but the most extreme conditions of hypoxia (Burggren and Randall, 1978). That the act of simply holding the sturgeon's lips shut immediately produces a bradycardia may reflect the involvement of mechanoreceptors in some region of the buccal cavity.

Strictly opercular water intake in *Acipenser* can effectively maintain gill ventilation and hence $\dot{V}_{O_2}$ when an exercise stress is placed upon the ventilatory system, although ultimately with increasing exercise $\dot{V}_{O_2}$ cannot meet the oxygen demands of the tissues and the swimming speed of the fish becomes limited (fig. 6) compared to fish ventilating their gills with water drawn into the mouth. The presence or absence of ram ventilation in the two situations may have played a role in the breakdown of gas exchange at high swimming speeds during strictly opercular water intake in *Acipenser*, but the ventral location of the recessed mouth well back from the leading edge of the fish as well as the comparatively low swimming speeds probably reduce the importance of ram ventilation in the sturgeon compared to other fishes.

The ability of the sturgeon to alternate between buccal and opercular water intake for the purposes of gill ventilation without compromising O_2 uptake represents a highly effective and flexible adaptation for an aquatic life style involving bottom dwelling and feeding. *Acipenser* in its natural habitat will probably often encounter protracted periods when the ventrally located mouth is either resting on or actually submerged beneath a mud or sand substrate. Because gill ventilation can still be maintained under these circumstances, the sturgeon has been able to utilize a feeding style in a region of the environment inaccessible to the vast majority of fishes which are unable to suspend buccal gill ventilation during feeding.

Acknowledgements

The author wishes to thank Dr. K. Hammond of the Vancouver General Hospital for supplying the radiography materials and making the cine X-rays. Gil Hewlett and Billy Wong of the Vancouver Public Aquarium generously provided all of the fish used in this study.

References

Brett, J. R. (1964). The respiratory metabolism and swimming performance of young sockeye salmon. *J. Fish. Res. Board Canada* 21: 1183–1226.

Burggren, W. W. and D. J. Randall (1978). Oxygen uptake and transport during hypoxic exposure in the sturgeon *Acipenser transmontanus*. *Respir. Physiol.* 34: 279–291.

Grigg, G. C. (1970). Use of the first gill slits for water intake in a shark. *J. Exp. Biol.* 52: 569–574.

Holeton, G. F. and D. R. Jones (1975). Water flow dynamics in the respiratory tract of the carp (*Cyprinus carpio* L.). *J. Exp. Biol.* 63: 537–549.

Hughes, G. M. and G. Shelton (1958). The mechanism of gill ventilation in three freshwater teleosts. *J. Exp. Biol.* 35: 807–823.

Hughes, G. M. (1960a). The mechanism of gill ventilation in the dogfish and skate. *J. Exp. Biol.* 37: 11–27.

Hughes, G. M. (1960b). A comparative study of gill ventilation in marine teleosts. *J. Exp. Biol.* 37: 28–45.

Hughes, G. M. (1963). Comparative Physiology of Respiration. London, Heinemann.

Hughes, G. M. and C. M. Ballintijn (1965). The muscular basis of the respiratory pumps in the dogfish (*Scyliorhinus canicula*). *J. Exp. Biol.* 43: 363–383.

Hughes, G. M. and S. Umezawa (1968). On respiration in the dragonet *Callionymus lyra* L. *J. Exp. Biol.* 49: 565–582.

Jones, D. R. and T. Schwarzfeld (1974). The oxygen cost to the metabolism and efficiency of breathing in trout (*Salmo gairdneri*). *Respir. Physiol.* 21: 241–254.

Kiceniuk, J. W. (1975). Some aspects of exercise physiology in fish. Master's Thesis, University of British Columbia.

Laurent, P. and J. D. Rouzeau (1972). Afferent neural activity from pseudobranch of teleosts. Effects of P_{O_2}, pH, osmotic pressure and Na^+ ions. *Respir. Physiol.* 14: 307–331.

Randall, D. J. (1970). The circulatory system. In: Fish Physiology. Vol. 4, edited by W. S. Hoar and D. J. Randall. London, Academic Press.

Romer, A. S. (1970). The vertebrate body. London, W. B. Saunders Co.

Satchell, G. H. (1961). The response of the dogfish to anoxia. *J. Exp. Biol.* 38: 531–543.

Saunders, R. L. (1961). The irrigation of the gills of fishes. I. Studies of the mechanism of branchial irrigation. *Can. J. Zool.* 39: 639–653.

Shelton, G. (1970). The Regulation of Breathing. In: Fish Physiology. Vol. 4, edited by W.S. Hoar and D. J. Randall. London, Academic Press.

Smith, L. S. and G. R. Bell (1964). A technique for prolonged blood sampling in freeswimming salmon. *J. Fish. Res. Board Canada* 21: 711–717.

Teichmann, H. (1959). Über den Atemmechanismus bei Haifischen und Rochen. *Z. Vergleich. Physiol.* 41: 449–455.

Tucker, W. A. (1967). Method for oxygen content and dissociation curves on microliter blood samples. *J. Appl. Physiol.* 23: 410–414.