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ABSTRACT

Although the majority of the sea lamprey's (*Petromyzon marinus*) life cycle is spent as a burrow-dwelling larva, or ammocoete, surprisingly little is known about intermediary metabolism in this stage of the lamprey's life history. In this study, larval sea lampreys (ammocoetes) were vigorously exercised for 5 min, and their patterns of metabolic fuel depletion and replenishment and oxygen consumption, along with measurements of net whole-body acid and ion movements, were followed during a 4–24-h postexercise recovery period. Exercise led to initial five- to sixfold increases in postexercise oxygen consumption, which remained significantly elevated by 1.5–2.0 times for the next 3 h. Exercise also led to initial 55% drops in whole-body phosphocreatine, which was restored by 0.5 h, but no significant changes in whole-body adenosine triphosphate were observed. Whole-body glycogen concentrations dropped by 70% immediately following exercise and were accompanied by a simultaneous ninefold increase in lactate. Glycogen and lactate were quickly restored to resting levels after 0.5 and 2.0 h, respectively. The presence of an associated metabolic acidosis was supported by very high rates of metabolic acid excretion, which approached 1,000 nmol g^{-1} during the first 2 h of postexercise recovery. Exercise-induced ion imbalances were also rapidly alleviated, as initially high rates of net Na^+ and Cl^- loss ($-1{,}200$ nmol g^{-1} h^{-1} and $-1{,}800$ nmol g^{-1} h^{-1}, respectively) were corrected within 1–2 h. Although larval sea lampreys spend most of their time burrowed, they are adept at performing and recovering from vigorous anaerobic exercise. Such attributes could be important when these animals are vigorously swimming or burrowing as they evade predators or forage.

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Introduction

The sea lamprey (*Petromyzon marinus*) spends most of its life burrowed in the substrate of streams as a suspension-feeding larva known as an ammocoete (Beamish and Potter 1975; Young et al. 1990). After 3–7 yr, ammocoetes undergo a complex true metamorphosis that prepares them for the freely swimming, parasitic phase of their life cycle (see Youson 1997 for review). Metamorphosis is characterised by the development of an oral disk as well as a reorganisation of the liver, gastrointestinal tract, and kidneys. A notable reorganisation of the gills also takes place, as the lampreys switch from a unidirectional to a tidally ventilated gill. Following the parasitic phase, the sexually mature adults migrate upstream, spawn, and then die (Beamish and Potter 1975). Few studies have determined whether these structural and lifestyle differences are reflected by changes in the intermediary metabolism of lampreys. Thus, the main objective of this study was to determine if there are differences in the metabolic makeup of ammocoetes and adult lampreys that influence their respective abilities to perform and recover from vigorous anaerobic exercise.

In recent years, several studies have demonstrated that adult (spawning phase) sea lampreys are adept at performing and recovering from vigorous anaerobic exercise, as characterised by their ability to rapidly replenish muscle fuel stores (Boutilier et al. 1993) and to correct postexercise acid-base disturbances (Tufts 1991; Wilkie et al. 1998). These characteristics are likely important during upstream spawning migration, when adult lampreys may undergo brief periods of intensive, anaerobic swimming as they encounter rapids or other obstacles.

In contrast to adult lampreys, much smaller ammocoetes are often considered "sedentary" or "sluggish" animals (e.g., Lewis 1980; Holmes and Lin 1994) due to their burrow-dwelling lifestyle, poor swimming ability (Holmes and Lin 1994), and very low resting metabolic rates (Potter and Rogers 1972). The low P_{50} of ammocoete haemoglobin is considered an adaptation that allows them to withstand low concentrations of dissolved oxygen in the interstitial water of the stream substrate (Potter et al. 1970; Bird et al. 1976). This high haemoglobin-oxygen affinity would also make it difficult for them, however, to un-

load oxygen to tissues when metabolic demands increase during sustained activity or during recovery from vigorous anaerobic activity. Indeed, the reduction in haemoglobin-oxygen binding affinity following metamorphosis is thought to suit the more active lifestyle of parasitic and adult lampreys by promoting more efficient oxygen delivery to working tissues (Bird et al. 1976).

Although it is presumed that these physiological characteristics reflect the burrow-dwelling lifestyle of ammocoetes, no study has attempted to compare the exercise physiology of ammocoetes with freely swimming adult lampreys. In this study, ammocoetes were vigorously exercised for 5 min, and their patterns of fuel depletion and metabolic recovery were followed over 24 h to determine how their responses to vigorous anaerobic exercise and their patterns of postexercise recovery differed from adult lampreys. We hypothesized that fuel depletion and metabolite buildups would be less pronounced and that the relative rates of postexercise recovery would be more prolonged in ammocoetes due to differences in their haemoglobin-oxygen binding characteristics, body size, gill design, and lifestyle. Accordingly, the patterns of postexercise oxygen consumption, metabolic acid excretion, metabolite elimination, and fuel replenishment were monitored for 4–24 h following the 5-min exercise bout.

Material and Methods

Experimental Animals and Setup

Sea lamprey (*Petromyzon marinus*) larvae (ammocoetes; $N = 69$, mean weight $= 2.7 \pm 0.1$ g) were collected using pulsed-DC electrofishing (Smith Root model 12 B) from the Cole Branch River, New Brunswick, Canada, in May of 1998 and 1999 and transported back to Mount Allison University in well-aerated, insulated containers. The animals were then transferred to 200-L holding containers receiving aerated well water ($T = 11$°C, hardness = 4.0 mEq as $CaCO_3$, pH = 7.6, $[Na^+] = 2.0$ mmol L^{-1}, $[Cl^-] = 2.9$ mmol L^{-1}, titration alkalinity = 2.3 mmol L^{-1}) at 400 mL min^{-1}, where they quickly burrowed into the sandy substrate (10-cm depth) lining the bottom of their holding container. The animals were held for a minimum of 1 mo before experiments and were maintained with a weekly feeding of brewers yeast (2 g yeast per ammocoete; Holmes and Youson 1994). All animals were cared for in accordance with Canadian Council on Animal Care guidelines.

Respirometry

Both static and flow-through respirometry were used in this study. Static respirometry permitted us to supply the ammocoetes with burrowing substrate (aquarium cotton), which was intended to calm the animals (thigmokinesis; Rovainen and Scheiber 1975) and was therefore a better representation of each animal's resting (preexercise or routine) metabolic rate. Although it was not possible to use cotton substrate in the flow-through respirometers (see below), this design allowed us to monitor Mo_2 (rate of oxygen consumption) during the early stages (5, 15, 30 min) of postexercise recovery, when postexercise Mo_2 was likely to be greatest (Scarabello et al. 1991*a*, 1991*b*).

Determinations of Mo_2 using the static respirometers were based on drops in water Po_2 (oxygen partial pressure) over selected time intervals (e.g., 0–1 h, 1–2 h) using standard methodology (see Cech 1990 for review). The static respirometers comprised a 215-mL jar, covered by a black mesh screen to maintain low light conditions for these negatively phototactic animals (e.g., Potter and Rogers 1972). A large rubber stopper containing two external three-way valves served as the airtight lid of the respirometer. The valves were attached to two 16-gauge needles, which were connected to lengths of polyethylene tubing (PE200) inside the respirometer. The animals rested on a mesh screen, located about 2 cm above the respirometer floor, or readily burrowed into 0.5 g of diffuse aquarium cotton. A small (1.0 × 0.5-cm) stir bar was positioned beneath the plastic mesh stage of the respirometer to ensure that the chamber water was always thoroughly mixed in the presence and absence of cotton substrate.

At each sampling period, either during rest or following exercise, the Po_2 of water-saturated air (1 mL) was initially measured, after which two 1-mL water samples were withdrawn from the static respirometer via the three-way valves using a 1-mL gas-tight Hamilton syringe. The first 1-mL sample was discarded to ensure that dead-space water in the sample tubing was not analysed, while the second sample was used for actual water Po_2 determination. As the water samples were removed, they were replaced by an equal volume of water injected into the chamber via the second sampling port. Each measurement of water Po_2 was then followed by a second determination of the Po_2 of water-saturated air. When water Po_2 dropped below 100 Torr (13.3 kPa), the water in the respirometer was gently aerated for 5 min to replenish water Po_2 before the next Mo_2 measurement.

Flow-through respirometers were used to instantaneously monitor postexercise Mo_2 patterns in a subset of nine ammocoetes. Burrowing substrate was not provided, however, because preliminary dye experiments indicated that it interfered with water flow through the respirometers. The flow-through respirometers were constructed from a 15-cm length of opaque PVC tubing fitted with rubber stoppers at either end, along with a three-way stopcock at the outflow of the device. Water flow through the respirometers was maintained at a rate of 0.25–0.30 L h^{-1} using a Gilson Minipuls peristaltic pump. Determinations of Mo_2 were based on the drop in water Po_2 as it passed through the respirometer, where the Po_2 of inflowing and outflowing water was determined on 1-mL samples withdrawn with a gas-tight Hamilton syringe. Typical inflow Po_2's

were about 130 Torr, while expired Po_2 decreased by about 10 Torr in resting fish and by up to 30 Torr in exercised animals.

Experimental Protocol

Approximately 1 wk before experiments, subsets (10–12) of uniformly sized ammocoetes were transferred to a 25-L isolation chamber, which contained clean silica sand as burrowing substrate and water at a temperature of 15°C 24 h before static respirometry experiments. Six ammocoetes were then selected at random, bathed in a tetracycline solution for 1 h before being transferred to tetracycline-treated individual respirometers receiving 15°C water at a rate of approximately 50 mL min^{-1}, and left to adjust to their surroundings overnight. Tetracycline treatment minimized bacterial contamination of the small-volume respirometers, which could have confounded Mo_2 determinations due to microbial respiration. It is unlikely that tetracycline treatment altered patterns of respiration, as Mo_2 was stable in resting animals during preliminary experiments.

Following an initial determination of resting Mo_2 over 4 h, ammocoetes ($N = 36$) were taken from their respirometers and vigorously exercised for 5 min by chasing in a 20-cm-diameter bucket containing water at 15°C and a depth of approximately 15 cm. The animals were then quickly returned to their respirometers, and postexercise Mo_2 was determined over 0–0.5 h, 0.5–1 h, 1–2 h, 2–3 h, 3–4 h, and after 24 h. An identical exercise protocol was used for determination of postexercise Mo_2 of ammocoetes using flow-through respirometry, which allowed us to resolve how Mo_2 was altered at precise points of postexercise recovery (e.g., 5, 15, 30, 60, 90, 120, 180, and 240 min following exercise). Pretreatment of the flow-through respirometers with tetracycline was not necessary, however, as these devices were sterilized in an autoclave before each experiment. Chasing was chosen as the method of exercise because it facilitated comparison with earlier studies conducted on similarly chased adult sea lampreys (Boutilier et al. 1993; Wilkie et al. 1998) and because it is known to reliably deplete key anaerobic fuel stores such as adenosine triphosphate (ATP), phosphocreatine (PCr), and glycogen in fish (Milligan 1996).

For the analysis of whole-body fuel stores and metabolites, subsets of ammocoetes ($N = 6$) were sampled at rest, immediately following exercise (0 h), and after 0.5, 2, 4, and 24 h following exercise. Since prior anaesthetisation is very effective for preserving metabolites in fish muscle (Wang et al. 1994*b*), ammocoetes were anaesthetised in 2.0 g L^{-1} of tricaine methanesulfonate (Syndel) buffered with 4.0-g L^{-1} $NaHCO_3$ for 5 min before being quick-frozen with precooled aluminum tongs and plunged into liquid nitrogen. The frozen carcasses were then stored at −80°C until processed for biochemical analyses.

Vigorous anaerobic exercise leads to high rates of net metabolic acid excretion and ion (Na^+, Cl^-) losses (Wood 1988). Thus, postexercise net acid (J_{net}^{H+}) and ion (J_{net}^{Cl-}, J_{net}^{Na+}) movements (fluxes) were measured in a subset of 13 ammocoetes, which had been subjected to the same 5-min exhaustive chasing protocol described above, using the protocol described by Wilkie et al. (1998) for adult sea lampreys. The only difference between the latter study and these experiments was that smaller (150-mL) flux chambers, constructed from darkened, 200-mL plastic specimen containers (described in detail by Wilkie et al. 1999), were used to analyse net acid and ion movements across the body surface of ammocoetes before and after exercise. As J_{net}^{H+} is the sum of the total ammonia excretion (J^{Amm}) and the titratable-acid (J^{TA}) flux across the body surface to the water, 20-mL water samples were taken at regular intervals in resting (preexercise conditions) ammocoetes or following exercise (0–0.5 h, 0.5–1 h, 1–2 h, 2–4 h, 8–10 h, 10–12 h). The titratable alkalinity, which was used to calculate J^{TA}, was then determined on 10-mL aliquots of water, and the remainder was then frozen for later determination of water ammonia, Na^+, and Cl^- concentrations (see Wilkie et al. 1998 for further details).

Analytical Techniques

Tissues used in the analyses of metabolic fuel stores and metabolites were processed by grinding the whole bodies of ammocoetes to a fine powder under liquid nitrogen, followed by extraction in four volumes of 7% perchloric acid, containing 1 mmol L^{-1} EDTA (ethylenediaminetetraacetic acid). The acidified extract was continuously mixed at 4°C for 5 min and then centrifuged at 10,000 *g* for 2 min (e.g., Kieffer et al. 1994). The resulting supernatant was then drawn off and neutralized with 1.5 volumes of 2 mmol L^{-1} KOH containing 0.4 mmol L^{-1} imidazole and 0.4 mmol L^{-1} KCl. This slurry was then quickly mixed and centrifuged at 10,000 *g* for 30 s, and the supernatant was transferred to 1.5-mL centrifuge tubes, which were stored at −80°C. The samples were subsequently analysed for ATP, PCr, creatine, glycogen, lactate, and pyruvate using established enzymatic assays (see Boutilier et al. 1993; Wilkie et al. 1997 for further details). Whole-body glycogen was determined on neutralized extracts digested in five volumes of 30% KOH and analysed using amyloglucosidase and hexokinase (Hassid and Abraham 1957). Whole-body water was determined by oven drying 100 mg of powdered carcass to constant weight at 80°C.

The maximal activity of lactate dehydrogenase (LDH) in muscle was also determined in a subset of six ammocoetes as an index of this animal's anaerobic potential (see Castellini and Somero 1981). Accordingly, animals were killed with an overdose of buffered MS222 and skinned, and the tails immediately posterior to the cloaca were removed and minced in a solution of ice-cold homogenisation buffer (composition: 50 mmol L^{-1} Hepes, 1 mmol L^{-1} EDTA, 5 mmol L^{-1} dithiothreotol, pH 7.4) and then homogenised for 30 s using a PowerGen model 125 tissue homogeniser. The slurry was then centrifuged at 10,000 *g* for 20 min at 4°C, and maximal LDH activity was determined at 15°C using 10 mL of the supernatant added to 2.0 mL of

assay medium containing 100 mmol L^{-1} Hepes, 1 mmol L^{-1} KCN, 0.17 mmol L^{-1} NADH (nicotinamide adenine dinucleotide, reduced), and 2 mmol L^{-1} pyruvate at a pH of 7.5 (see Stewart and Driedzic 1986 for additional details). The tail was chosen for LDH analysis because 90% of its wet weight is muscle (Wilkie et al. 1999).

The measurement of water titratable alkalinity, ammonia, and Cl^- concentrations closely followed methods described in Wilkie et al. (1998), while water Na^+ concentrations were measured using flame photometry at the laboratories of Environment Canada, Moncton, New Brunswick.

Calculations and Statistics

When Mo_2 was determined using static respirometry, it was based on the decrease in water Po_2 during each measurement period (see Cech 1990 for detailed review). The determinations of Mo_2 using flow-through respirometry were based on differences between the Po_2 of inflowing versus outflowing water using the well-known Fick principle. Preliminary experiments using dye (1-mL Evans Blue Dye) indicated that less than 1 min was required for water to be completely cleared from the flow-through respirometers, making it unnecessary to use a time lag correction factor (see Cech 1990).

If the slope of the logarithmic regression line relating body mass to resting Mo_2 is less than unity, Mo_2 must be corrected for differences in body mass (Lewis 1980). In this study, the experimentally determined allometric relationship for ammocoetes (mass range = 1.3–3.9 g), was described by

$$\log Y = 0.0479 + 0.826 \log X, \tag{1}$$

where Y represents Mo_2 in μmol h^{-1} and X is the body mass in grams ($P = 0.008$, $r = 0.559$, SEM = 0.238, $N = 21$). Using the slope of this relationship (mass exponent), 0.826, Mo_2 was corrected using the following formula:

$$\text{corrected } Mo_2 = \frac{Mo_2}{(\text{standard mass})(\text{actual mass/standard mass})^{0.826}}, \tag{2}$$

where Mo_2 is in micromoles per hour per animal (μmol h^{-1}) but corrected Mo_2 is expressed in micromoles per gram per hour (μmol g^{-1} h^{-1}), and the standard mass is 2.6 g, or the mean mass of animals used in the respirometry experiments.

Whole-body metabolite concentrations are expressed as micromoles per milliliter body water (μmol mL^{-1}), after dividing the wet whole-body concentrations (μmol g^{-1} wet wt) by the total body water (mL whole-body water g^{-1} wet wt).

Estimates of J^{Amm}, J^{Cl-}_{net}, and J^{Na+}_{net} were calculated according to

$$J^X = \frac{([x]_i - [x]_f)(V)}{(\text{actual mass})(\Delta T)}, \tag{3}$$

where $[x]$ is the concentration (μmol L^{-1}) of total ammonia, Cl^-, or Na^+ in the water of the chamber at the beginning (i) and end (f) of a measurement period; V is the volume of the chamber; and ΔT represents the duration of the measurement period (see Wilkie et al. 1998 for further details). By convention, negative J^X values represent net outward movements of ammonia or ions, while positive values indicate net inward movement.

The J^{TA} of lampreys, used to estimate the net acid flux, was based on the difference between the titratable alkalinity of water samples at the beginning and end of a flux period as previously described (Wood 1988; Wilkie et al. 1998). As above, positive and negative J^H_{net} values represent net inward and outward (excretion) acid movement, respectively.

All data are expressed as the mean ± 1 SEM. Unpaired t-tests were used to identify significant differences between the mean Mo_2's of burrowed and unburrowed ammocoetes during the same measurement period. Repeated-measures ANOVAs were used to determine whether there was significant variation between resting and postexercise Mo_2 values and net ion or net acid movements. One-way ANOVAs were used to look for differences between whole-body fuel and metabolite levels between resting and exercised animals. In instances where significant variation was observed, the statistical significance of mean resting versus postexercise values were determined using the Tukey-Kramer posttest. The limit of significance was at the $P < 0.05$ level.

Results

Static Respirometry

The resting Mo_2 of burrowed ammocoetes was about 40%–50% lower than rates measured in unburrowed ammocoetes, fluctuating around 1.0 μmol O_2 g^{-1} h^{-1} and 2.0 μmol O_2 g^{-1} h^{-1}, respectively (Fig. 1*A*) and exhibited no significant variation over the course of a 48-h experiment designed to determine if Mo_2 fluctuated diurnally (data not shown). The 5 min of vigorous exercise were characterised by short bursts of rapid swimming and powerful undulatory movements of the ammocoetes' trunk as they were chased. Following exercise, the ammocoetes were unresponsive to further stimuli and appeared incapable of further rapid swimming and were therefore considered exhausted. When cotton substrate was present, the ammocoetes slowly burrowed into this media and remained still for the duration of the recovery.

During the first 30 min of postexercise recovery, Mo_2 approached 4.5 μmol O_2 g^{-1} h^{-1} in both the burrowed and unburrowed ammocoetes. Notably, the 4.5-fold increase in the Mo_2 of the burrowed ammocoetes was significantly greater than the 2.3-fold increase observed in the unburrowed animals (Fig.

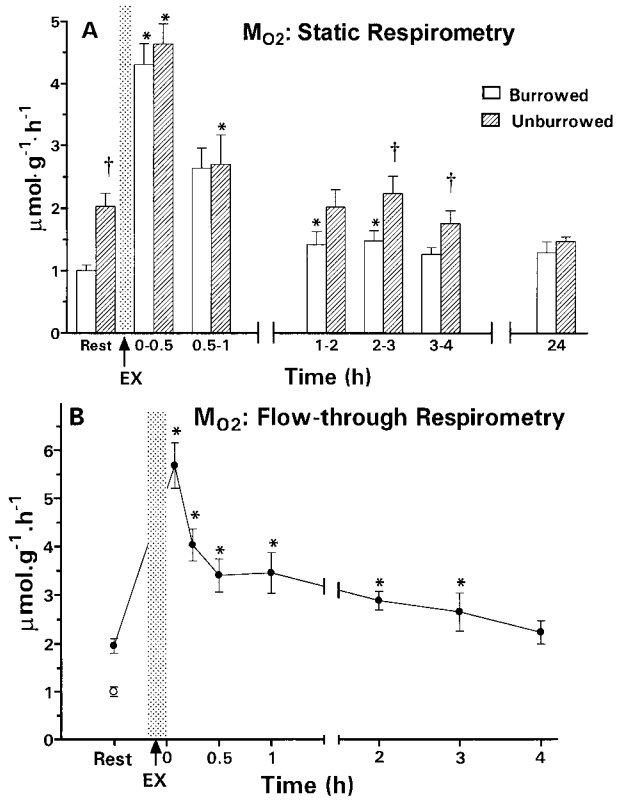

Figure 1. Changes in the oxygen consumption rates (Mo_2) of ammocoetes of the sea lamprey (*Petromyzon marinus*) following 5 min of vigorous chasing as measured using (*A*) static respirometry or (*B*) flow-through respirometry. *A*, Open and hatched bars represent the Mo_2 of burrowed ($N = 8$–21) and unburrowed ammocoetes ($N = 6$–10), respectively. *B*, All ammocoetes ($N = 9$) were unburrowed (*filled circles*), but note that the resting Mo_2 of burrowed ammocoetes is included for reference (*open circle*). The vertical stippled bar represents the period of exercise (*EX*). All data expressed as the mean ± 1 SEM. Asterisks indicate statistically significant values from resting (control) rates, while daggers represent statistically significant differences between the Mo_2 of burrowed versus unburrowed ammocoetes during the same sample period ($P < 0.05$).

1*A*). Between 0.5 and 1 h, Mo_2 remained significantly elevated, by about 2.5-fold in the burrowed ammocoetes, at which time the Mo_2 of the unburrowed animals approached preexercise levels (Fig. 1*A*). The Mo_2 did not approach preexercise levels until 3 h in the ammocoetes provided with burrowing substrate (Fig. 1*A*).

Flow-Through Respirometry

Consistent with the data described for unburrowed ammocoetes examined using static respirometry, the preexercise Mo_2 of ammocoetes examined with flow-through respirometry was about 2 μmol O_2 g^{-1} h^{-1} (Fig. 1*B*). Immediately following exercise (5 min), Mo_2 approached 6 μmol O_2 g^{-1} h^{-1} but had declined by about 30% after 15 min of postexercise recovery. Notably, these immediate postexercise values were about sixfold greater than resting levels measured in the burrowed animals using the static respirometry setup (Fig. 1*B*, *open circle*). As with the static setup, Mo_2 remained significantly elevated for the first 3 h of postexercise recovery before returning to preexercise levels by 4 h (Fig. 1*B*).

Postexercise Acid and Ion Movements

Postexercise acid-base and ion disturbances, based on determinations of net ammonia, acid, and ion losses, were very pronounced but short-lived in ammocoetes. Over the first 30 min following exercise, J^{Amm} and J^{TA} approached −220 nmol g^{-1} h^{-1} and −600 nmol g^{-1} h^{-1}, respectively (Fig. 2*A*, 2*B*), leading to net acid excretion rates (J^{H+}_{net}) of −860 nmol g^{-1} h^{-1} during this time (Fig. 2*C*). By 1–2 h, both J^{Amm} and J^{TA} were not significantly different from resting rates (Fig. 2*A*, 2*B*), but J^{H+}_{net} did not approach resting levels until 2–4 h (Fig. 2*C*).

There were also accompanying ionoregulatory disturbances as characterised by marked elevations of net Cl^- and Na^+ loss rates of −1,800 and −1,200 nmol g^{-1} h^{-1}, respectively (Fig. 3*A*, 3*B*). These net Cl^- and Na^+ losses were short-lived, as the net ion fluxes approached control levels after 1.0–2.0 h (Fig. 3*A*, 3*B*). Although net Cl^- losses exceeded Na^+ losses during the first 30 min, there was no significant elevation of the predicted net acid excretion rates (predicted $J^{H+}_{net} \approx J^{Cl-}_{net} - J^{Na+}_{net}$; Wood 1988) following exercise (Fig. 3*C*).

Tissue Fuels, Metabolites, and LDH Activity

Resting whole-body ATP concentrations in burrowed ammocoetes were about 4 μmol mL^{-1} (Fig. 4*A*), while PCr concentrations approached 27 μmol mL^{-1} (Fig. 4*B*) with a corresponding creatine charge (PCr/[creatine + PCr]; Schulte et al. 1992) of approximately 0.81 (Fig. 4*C*). Although exercise led to a notable 55% drop in PCr and a 43% lower creatine charge, there was little change in the whole-body ATP concentrations of ammocoetes (Fig. 4). By 0.5 h, the PCr concentrations and creatine charge were restored to resting levels, fluctuating between 25 and 30 μmol mL^{-1} and 0.77 and 0.80, respectively, for the rest of the experiment (Fig. 4*B*, 4*C*).

Whole-body glycogen concentrations were approximately 17 μmol mL^{-1} in resting ammocoetes but dropped by 70% immediately following vigorous exercise (Fig. 5*A*). There was a rapid replenishment of glycogen stores, however, which approached resting levels after only 30 min of postexercise recovery (Fig. 5*A*). The drops in whole-body glycogen were accompanied by a simultaneous ninefold increase in whole-body lactate, which exceeded 8 μmol mL^{-1} immediately following exercise (Fig. 5*B*). This lactate load was rapidly eliminated, however. By 30 min, 60% of the accumulated load had been

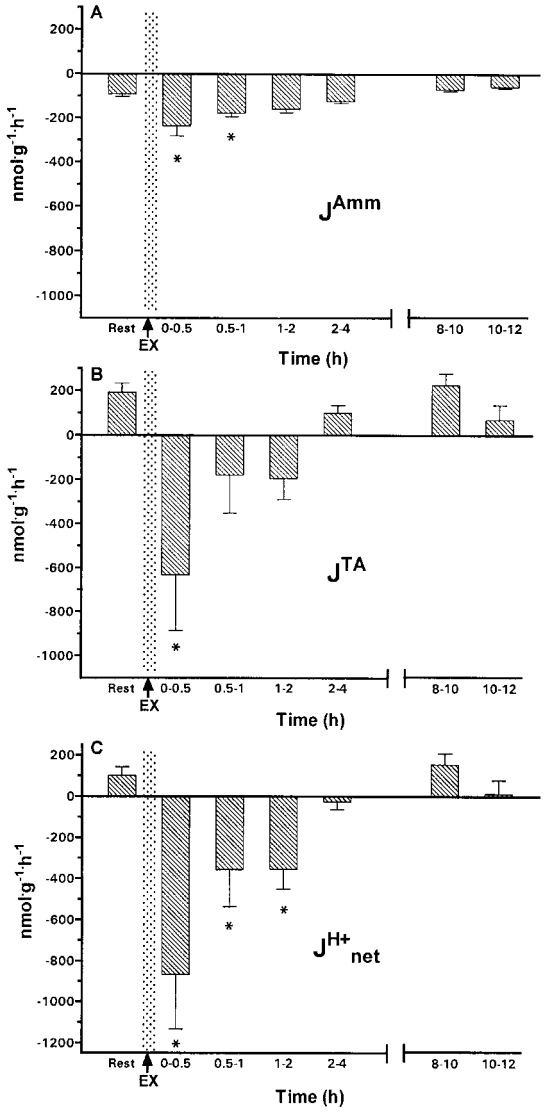

Figure 2. Changes in (*A*) ammonia excretion rate (J^{Amm}), (*B*) titratable acid movements (J^{TA}), and (C) net metabolic acid flux (J^{H+}_{net}) by ammocoetes ($N = 13$) of *Petromyzon marinus* following 5 min of vigorous exercise. Upward-facing bars (positive values) represent net inward movement of titratable acid or metabolic acid. Downward-facing bars (negative values) represent net outward movements of ammonia, titratable acid, or metabolic acid. The vertical stippled bars represent periods of exercise (*EX*). All data expressed as the mean ± 1 SEM. Asterisks indicate statistically significant values from resting (pre-exercise) rates ($P < 0.05$).

eliminated, and by 2 h, lactate concentrations had returned to resting levels (Fig. 5*B*). Whole-body pyruvate was 40% higher and remained elevated for 2 h following exercise (Fig. 5*C*). The maximal LDH activity in muscle was 351 ± 20 ($N = 6$) μmol min^{-1} g^{-1} wet tissue.

Discussion

Postexercise Oxygen Consumption

As predicted, the lower resting Mo_2 of burrowed versus unburrowed ammocoetes indicates that burrowing substrate tended to calm the animals (Potter and Rogers 1972; Rovainen and Scheiber 1975). Compared with other similarly sized fish, however, the resting or routine metabolic rates of ammocoetes are very low (see Lewis 1980 for review). Ammocoetes primarily feed on detritus, but unlike other detrivorous fishes, ammocoetes have a simple, unspecialised digestive tract, which leads to very slow rates of digestion (Sutton and Bowen 1994). Thus, the ammocoetes' low metabolic rate is likely related to their suspension-feeding lifestyle and slow rates of nutrient assimilation. Despite these characteristics, ammocoetes are able to elevate their metabolic rate markedly as they correct exercise-induced physiological disturbances. In other words, despite their very low resting metabolic rate, ammocoetes have a relatively wide aerobic scope.

The marked increase in Mo_2 following exercise in burrowed and unburrowed ammocoetes is consistent with the five- to sevenfold increase in ventilation reported by Rovainen and Scheiber (1975) following similar periods of vigorous swimming. The elevated Mo_2 also conforms with the excess post-exercise oxygen consumption (EPOC) commonly observed in teleosts such as sockeye salmon (*Oncorhynchus nerka*; Brett and Groves 1979), fingerling rainbow trout (*Oncorhynchus mykiss*; Weiser et al. 1985; Scarabello et al. 1991*a*, 1991*b*, 1992; Gonzalez and McDonald 1994) and cod (*Gadus morhua*; Reidy et al. 1995). The relative increase of postexercise Mo_2 was generally greater in ammocoetes (four- to sixfold above resting levels), however, than relative increases reported in teleosts such as trout (two to three times; Scarabello et al. 1991*a*, 1991*b*). A comparison of EPOC, however, may be a more informative means to compare ammocoetes with teleosts because it reflects the overall metabolic costs of recovery from vigorous exercise (Wood 1991).

EPOC was 8.3 μmol O_2 g^{-1} over 4 h of postexercise recovery in ammocoetes, based on measurements made using flow-through respirometry and resting Mo_2 in cotton. This value is comparable to the EPOC (11.2 μmol O_2 g^{-1}) measured in fingerling rainbow trout over 6 h following a similar bout of vigorous exercise (Scarabello et al. 1991*b*). These findings suggest the metabolic costs associated with vigorous exercise and recovery in burrow-dwelling ammocoetes were likely comparable to those of freely swimming, active teleosts such as the rainbow trout, despite their very different lifestyles.

Based on their postexercise physiology, it is likely that EPOC in ammocoetes was associated with the restoration of high energy phosphagens, increased cardiorespiratory demands, the correction of ion and acid-base balance, the replenishment of glycogen reserves, and the clearance of lactate, as it appears to be in trout (Wood 1991; Scarabello et al. 1992). The actual

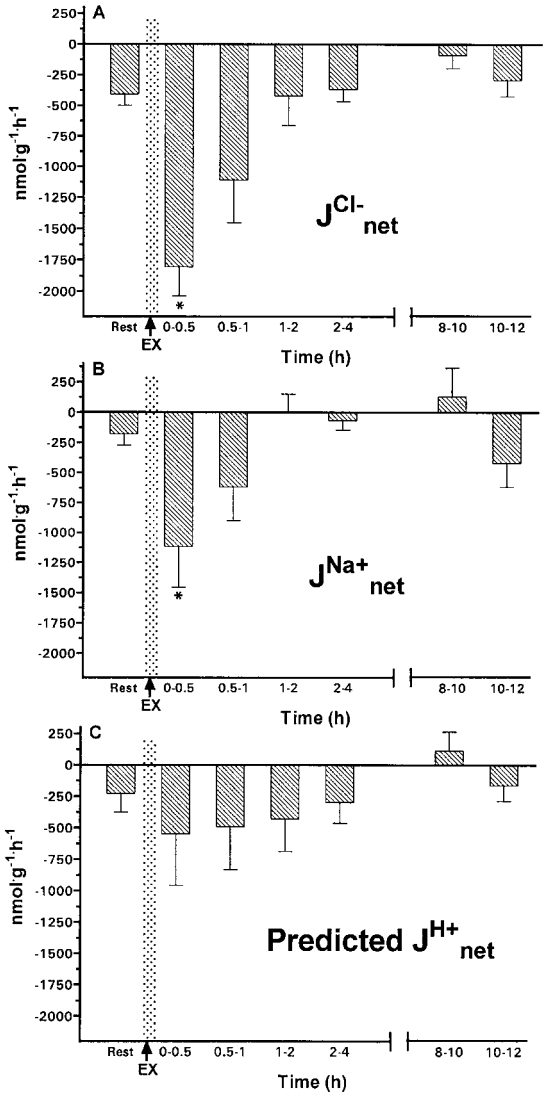

Figure 3. Changes in (*A*) net Cl^- movements ($J^{Cl^-}_{net}$), (*B*) net Na^+ movements ($J^{Na^+}_{net}$), and (*C*) the predicted net acid-excretion rates (predicted $J^{H^+}_{net} \approx J^{Cl^-}_{net} - J^{Na^+}_{net}$) measured across the body surface of ammocoetes ($N = 13$) following 5 min of vigorous chasing. Upward-facing bars (positive values) represent net inward movement. Downward-facing bars (negative values) represent net outward movement. The vertical stippled bar represents the period of exercise (*EX*). All data expressed as the mean ± 1 SEM. Asterisks indicate statistically significant values from resting (preexercise) rates ($P < 0.05$).

contributions that each of these indices made to EPOC in ammocoetes still requires further, careful examination, however. Since metamorphosis is accompanied by profound changes in diet, habitat, body structure, and the switch to a freely swimming lifestyle, it would also be very informative to conduct similar experiments on parasitic and adult lampreys. Regardless, the ammocoetes' wide aerobic scope and patterns of EPOC suggests that they are well suited for vigorous aerobic or anaerobic activities.

Acid-Base and Ionoregulation

Postexercise acid-base regulation has been studied in adult sea lampreys (Boutilier et al. 1993; Wilkie et al. 1998), but nothing is known about this process in ammocoetes. This study demonstrates that ammocoetes, like adult lampreys, had a very high capacity to unload metabolic acid following vigorous, anaerobic exercise, as the total excreted approached 1,000 nmol g^{-1} over the first 2 h of recovery. These observations also suggest the presence of a considerable postexercise acidosis in the muscle, which was also indicated by greater concentrations of whole-body lactate immediately following exercise (see below).

Compared with trout (Wood 1988), the postexercise elevations of metabolic acid excretion in ammocoetes and adult lampreys (Wilkie et al. 1998) were much greater but relatively short-lived, which suggests lampreys are able to correct internal acid-base disturbances more quickly following vigorous exercise. This interpretation is supported by the adult lamprey's more rapid correction of extracellular and intracellular muscle pH (Boutilier et al. 1993) as well as by the ammocoetes' rapid elimination of whole-body lactate (see below), which would be expected to follow the recovery of internal acid-base balance during postexercise recovery (Milligan 1996). In ammocoetes, an ability to rapidly correct internal acid-base status could be crucial when they are forced to undergo repetitive bouts of intensive exercise, such as swimming or burrowing to escape predators.

The greater chloride versus sodium losses following exercise (Fig. 3*A*, 3*B*) may shed light on the mechanisms used by ammocoetes to rapidly excrete metabolic acid following exercise. As dictated by the strong ion difference theory and the constraints of electroneutrality, net Cl^- or Na^+ losses are generally accompanied by simultaneous acid excretion (equivalent to base uptake) or base-equivalent excretion (equivalent to acid uptake), respectively (Wood 1988). Thus, greater net Cl^- versus Na^+ losses would accompany net H^+ excretion. In fact, when the actual (measured) $J^{H^+}_{net}$ (Fig. 2*C*) was plotted against the predicted $J^{H^+}_{net}$ (predicted $J^{H^+}_{net} = J^{Cl^-}_{net} - J^{Na^+}_{net}$; Fig. 3*C*) following exercise, a significant correlation was observed ($y = 0.526x - 191.5$; $P = 0.02$). Thus, the overall mechanism of postexercise acid excretion in ammocoetes resembles the strategies used by adult lampreys (Wilkie et al. 1998) and rainbow trout (Wood 1988). In ammocoetes and adult lampreys, however, it appears that an ability to elevate quickly and substantially net chloride versus sodium losses following exercise is the key factor that explains their ability to rapidly excrete metabolic acid during recovery from vigorous, anaerobic exercise.

In general, metabolic acid production during anaerobic exercise positively scales with body size in active fish such as the rainbow trout (Goolish 1991; Ferguson et al. 1993) and brook

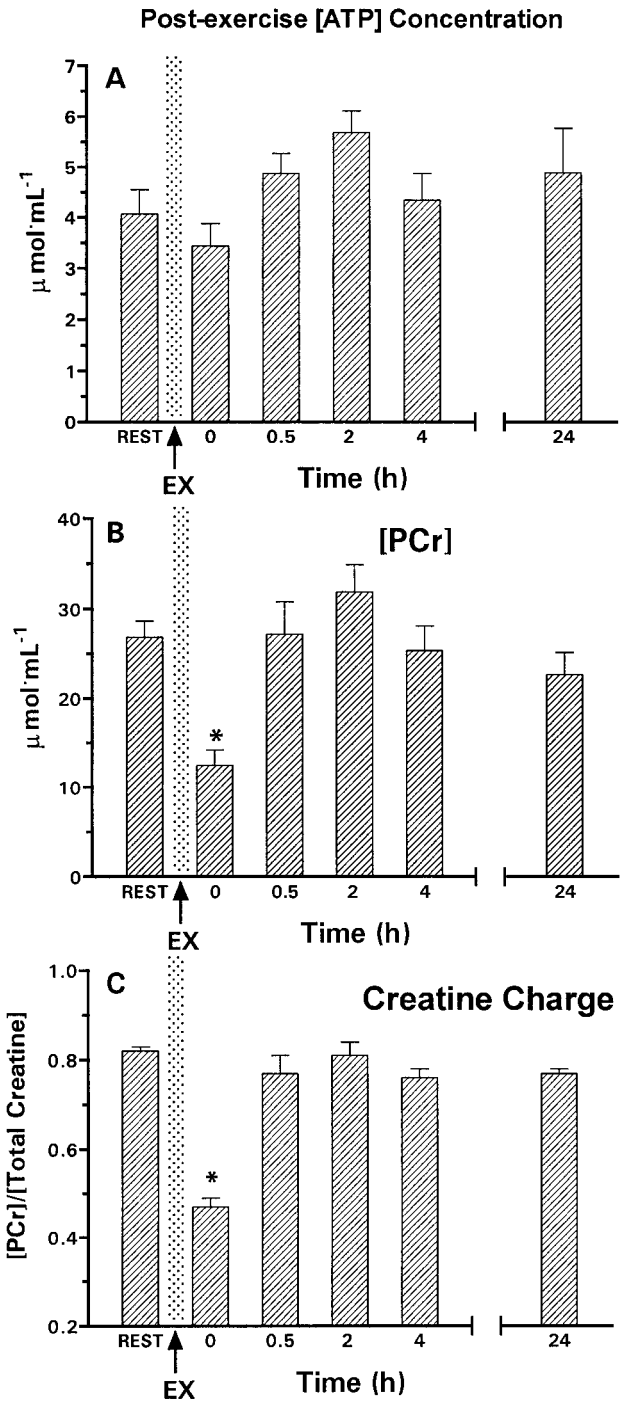

Figure 4. Changes in the whole-body concentrations of (*A*) adenosine triphosphate (*ATP*), (*B*) phosphocreatine (*PCr*), and (C) creatine charge (ratio of PCr : total creatine) of ammocoetes following 5 min of vigorous chasing. The vertical stippled bar represents the period of exercise (*EX*). All data expressed as the mean ± 1 SEM ($N = 6$ at each sample period). Asterisks indicate statistically significant differences between resting and postexercise concentrations ($P < 0.05$).

trout (*Salvelinus fontinalis*; Kieffer et al. 1996). This relationship is probably due to the greater anaerobic energy demands that larger fish are forced to contend with during burst swimming (Goolish 1991). Based on these observations, exercise-induced metabolic acid production would be expected to be greater in the much larger, freely swimming adult lampreys (mass ~260 g) than in burrow dwelling ammocoetes (mass ~2.7 g) that were exercised in an identical manner. It would also follow that net acid excretion should be higher in the adults. However, the total acid excreted over 2 h was 40% greater in ammocoetes (967 nmol g^{-1} body wt vs. 684 nmol g^{-1} body wt; Wilkie et al. 1998). Thus, the allometric relationship pertaining to greater metabolic acid production in larger fish during anaerobic exercise (Goolish 1991; Ferguson et al. 1993) may not apply to different stages of the lamprey life cycle. One possible explanation is that ammocoetes may have to rely heavily on anaerobic glycolysis while swimming or burrowing, which would be reflected by greater metabolic acid production and excretion. Regardless, measurements of muscle intracellular pH are required in future studies to confirm whether the postexercise metabolic acid load of ammocoetes is comparable to that of adult lampreys.

Postexercise Phosphagen and Carbohydrate Metabolism

Vigorous anaerobic exercise is accompanied by increased ATP hydrolysis, leading to an elevated free adenosine diphosphate (ADP)/free ATP ratio. This results in a shift of the creatine phosphokinase (CPK) reaction toward PCr dephosphorylation, which buffers the ATP stores during exercise (see Moyes and West 1995 for review). Although free adenylate concentrations were not directly measured, the significant decline in creatine charge immediately following exercise by ammocoetes indicates that net dephosphorylation of PCr was likely due to an increase in the free ADP/free ATP ratio (Moyes and West 1995). The absence of a significant drop in ATP, however, was likely because PCr and glycogen reserves were not completely depleted, which would have continued to buffer ATP supplies.

As alluded to previously, the high postexercise acid excretion rates and greater whole-body lactate concentrations indicate the presence of a significant metabolic acidosis in the muscle of ammocoetes, which would also promote PCr dephosphorylation (Schulte et al. 1992) along with changes in the free ADP/free ATP ratio. Despite the absence of a significant decrease in ATP, these collective observations tend to confirm that ammocoetes were using anaerobic metabolism during exercise.

In general, the muscle glycogen stores of fishes are proportional to body size (Ferguson et al. 1993; McDonald et al. 1998). Accordingly, much larger adult sea lampreys would also be predicted to have greater resting glycogen stores than ammocoetes. The glycogen stores of ammocoetes were similar, however, to those reported in adult sea lampreys (Boutilier et al. 1993) and were also comparable to resting levels measured in

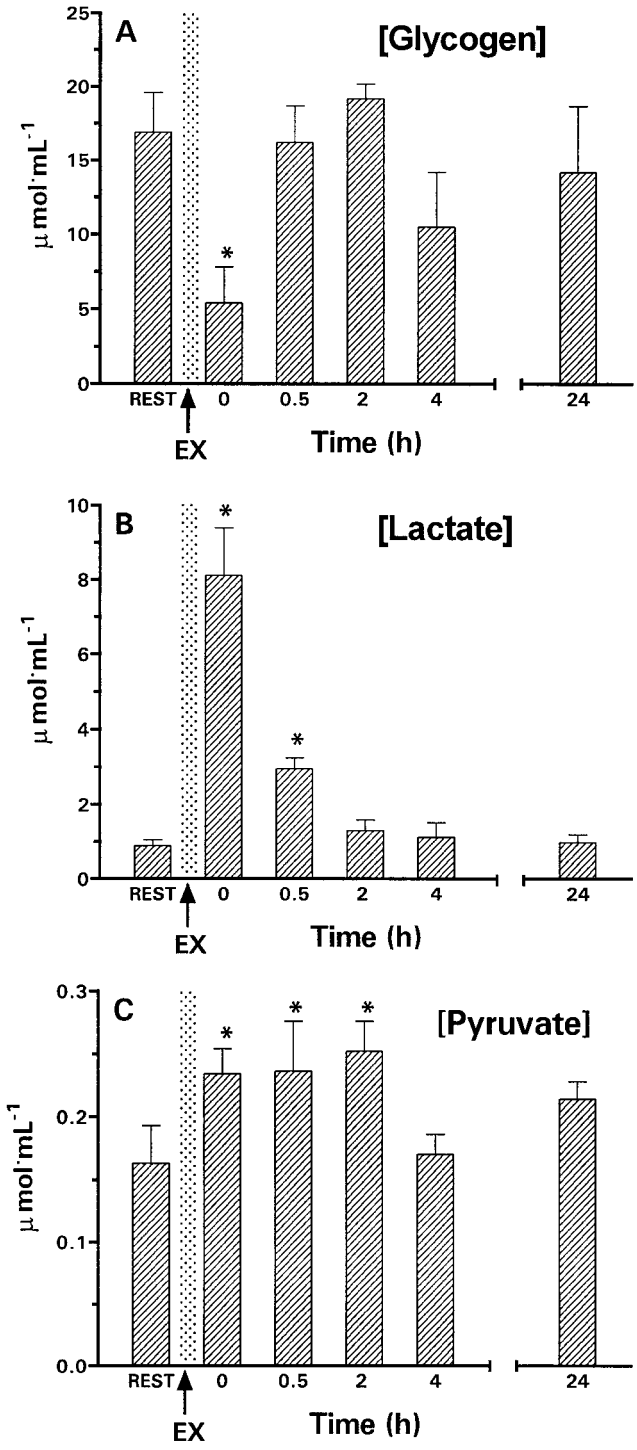

Figure 5. Changes in the whole-body concentrations of (*A*) glycogen, (*B*) lactate, and (*C*) pyruvate of ammocoetes following 5 min of vigorous chasing. The vertical stippled bar represents the period of exercise (*EX*). All data expressed as the mean ± 1 SEM ($N = 6$ at each sample period). Asterisks indicate statistically significant differences between resting and postexercise concentrations ($P < 0.05$).

the white muscle of many salmonids (see Milligan 1996 for review). In salmonids and adult lampreys, high muscle glycogen stores are required to fuel burst swimming. This may be the case in ammocoetes, but high glycogen reserves may also be required to support burrowing activities. Goolish (1991) has argued that muscle glycogen concentrations increase as fish get larger because additional fuel is needed to overcome the greater drag encountered during burst or sprint swimming. Since the resistance provided by stream substrate to a burrowing ammocoete could be considerable, high glycogen stores would give ammocoetes the additional onboard fuel needed to support vigorous burrowing activity. Indeed, burrowing episodes may last several minutes, and Pagget et al. (1998) recently reported that vigorous burrowing by ammocoetes requires muscular contractions that are at least as intensive as those observed during fast swimming.

As expected, exercise-induced declines in glycogen accompanied simultaneous increases in whole-body lactate following exercise by ammocoetes, but this accumulation was about 50% lower than observed in adult sea lampreys, despite comparable reductions in whole-body glycogen (Boutilier et al. 1993). In addition, the ratio of glycogen disappearance versus lactate appearance in ammocoetes was not 1 : 2, as reported in adult lampreys and in many salmonids following anaerobic exercise (e.g., Milligan and Wood 1986; Schulte et al. 1992; Boutilier et al. 1993; Kieffer et al. 1994; Wang et al. 1994*a*; Wilkie et al. 1997). One must keep in mind, however, that the glycolytic intermediates (e.g., glucose-6-phosphate, fructose-6-phosphate, glycerol-3-phosphate, pyruvate) plus lactate, should be considered when attempting to account for postexercise drops in glycogen concentration, as demonstrated by Pearson et al. (1990) in their studies on rainbow trout. Most glycolytic intermediates were not measured in this study, but the sustained elevation of pyruvate suggests that at least some of the apparent lack of agreement between glycogen disappearance and lactate appearance was due to an accumulation of glycolytic intermediates, rather than lactate, in the muscle of ammocoetes.

Another possible explanation for the unexpectedly low levels of whole-body lactate following exercise is that lactate oxidation may have contributed to aerobic energy production during exercise, as suggested for vigorously exercised herring larvae (Franklin et al. 1996). In ammocoetes, some of the lactate generated via anaerobic glycolysis may have been oxidized in the swimming musculature, where there are overlapping layers of glycolytic and oxidative muscle fibres (Peters and Mackay 1961; Meyer 1979).

Estimates of the Ammocoetes' Anaerobic Capacity

Burrow-dwelling ammocoetes are relatively poor swimmers compared with their freely swimming adult counterparts, but they have a surprisingly high anaerobic capacity. Based on changes in ATP, PCr, and lactate immediately following exercise,

Table 1: Changes in whole-body ATP, PCr, and lactate and the estimated AEE of sea lamprey ammocoetes and adults, rainbow trout, and Atlantic salmon fingerlings immediately following brief (5–8-min) periods of exhaustive chasing

	Petromyzon marinus			
	Ammocoetes[a]	Adult[b]	*Oncorhynchus mykiss*[c]	*Salmo salar*[d]
Weight (g)	2.5	300–400	2–3	1.9
ΔATP	−.7	−1.1	−1.2	−2.4
ΔPCr	−11.6	−10.3	−4.4	−5.0
ΔLactate	+6.0	+17.5	+7.9	+9.6
AEE[e]	20.3	37.7	17.5	21.8
LDH activity	351 ± 20	581 ± 61	…	350 ± 32

Note. All data expressed as μmol g^{-1} wet weight, except for LDH activity, which is expressed in μmol product formed min^{-1} g^{-1} wet tissue wt.

[a] This study at 15°C.

[b] Taken from data supplied by Boutilier et al. (1993) at 10°C.

[c] Based on whole-body data taken from Scarabello et al. (1992) at 15°C.

[d] Data from McDonald et al. (1998) at 15°C.

[e] AEE = 1.5(Δlactate) + ΔATP + ΔPCr (see Pearson et al. 1990 and McDonald et al. 1998 for details of calculations).

the whole-body anaerobic energy expenditure (AEE) of ammocoetes was calculated, which would be a reflection of their anaerobic capacity (Pearson et al. 1990; McDonald et al. 1998). Although changes in ATP were not significant in exercised ammocoetes, their AEE was still about 20 μmol g^{-1} wet weight, largely based on the pronounced drop in whole-body PCr and increases in lactate (Table 1). This AEE value was about 46% lower than the AEE calculated for exhaustively exercised spawning lampreys (Boutilier et al. 1993) but very similar to the values determined for similarly sized Atlantic salmon (McDonald et al. 1998) and rainbow trout fingerlings (Scarabello et al. 1992), which may frequently rely on short bursts of anaerobic swimming to maintain position in streams or to avoid predation (Table 1). Further, the maximal activities of muscle LDH (Table 1) greatly exceeded values reported for sluggish, benthic fishes and are actually in the same range measured in the muscle of actively foraging, pelagic fishes with moderate to high anaerobic capacities (Castellini and Somero 1981). Clearly, ammocoetes are not an active pelagic fish, but these estimates of AEE and LDH activity suggest that they have a high anaerobic capacity and a metabolic makeup that is well suited for short bursts of intensive activity, such as fast swimming or burrowing.

Since their burrow-dwelling lifestyle could make them vulnerable to fluctuations in environmental oxygen, a high anaerobic capacity could also benefit ammocoetes during hypoxia. High LDH activity might also be beneficial under hypoxic conditions by promoting pyruvate conversion to lactate, which would yield the NAD^+ needed for continued ATP production via anaerobic glycolysis. Indeed, other aspects of ammocoete physiology, including a very high haemoglobin-oxygen binding affinity (Bird et al. 1976) and a low resting metabolic rate, are thought to be adaptations that allow ammocoetes to withstand drops in environmental oxygen.

Summary

This study demonstrates that ammocoetes were able to increase oxygen consumption by up to sixfold, which may be important when metabolic demands increase during sustained exercise or following brief periods of intensive swimming or burrowing. Further, they are capable of rapid recovery from such vigorous activities, as indicated by their rapid rates of acid excretion, lactate elimination, and glycogen replenishment. The surprisingly high anaerobic capacity of ammocoetes is also indicative of an animal that is well suited for performing vigorous anaerobic activities such as rapid swimming or possibly burrowing and may also help explain their tolerance to hypoxic conditions. In conclusion, the exercise physiology of burrow-dwelling ammocoetes is very similar to that of free-swimming adult lampreys, despite their different habitats, trophic status, body structure, metabolic rate, and respiratory physiology.

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