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(Leptocottus armatus)

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John A. Ruben

Long-term subaerial survival has been anecdotally noted
in the staghorn sculpin, Leptocottus armatus. In this study,
I quantified this phenomenon physiologically by examining the
following parameters: standard metabolic rate and indications
of reduced standard metabolic rate during subaerial exposure;
capacity for and mechanism(s) of oxygen consumption in air;
capacity for anaerobic production of lactate and/or ethanol;
and blood pH. The staghorn sculpin appears well-suited for
the subaerial exposure it experiences in nature. Mean
subaerial survival time was 6 hours which would enable fish to
survive intertidal stranding. Staghorns breathe air through
well-supported gills and supplement depressed rates of
subaerial aerobiosis by anaerobic production of lactic acid.
Most remarkably, the staghorn sculpin may also substantially
reduce minimal energy expenditure requirements during periods
of hypoxia by reducing resting metabolic rate.

Response to Hypoxia
in the Staghorn Sculpin, (<u>Leptocottus</u> <u>armatus</u>)

by

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(_Leptocottus armatus_)

INTRODUCTION

In fish, respiratory stress and death may result from exposure to low water oxygen concentrations. Consequently, many have developed means to deal with hypoxia, including structural adaptations for air-breathing as well as enhanced physiological mechanisms for expanded anaerobic metabolic capacity. Air breathing occurs most widely in tropical freshwater teleosts which often must endure seasonal hypoxia related to a local abundance of decaying ambient organic matter. Air-breathers are also common in other regions where seasonal drought may persist for weeks or months at a time.

A variety of accessory respiratory organs occur in freshwater teleosts. These include vascularization of cutaneous, buccal or pharyngeal surfaces (Johansen et al., 1968) and pharyngeal or opercular lung-like outpocketings (Hughes and Munshi, 1973). In some species, gills are structurally modified to prevent collapse in air and the opercular chamber itself is highly vascularized (Johansen, 1966). Others swallow inhaled air and utilize gastric or intestinal gas exchange (Carter, 1935). The South American catfish, _Plecostomous plecostomus_, uses intestinal surfaces

for gas exchange during voluntary forays onto land (Carter, 1935).

A number of species utilize the lung/swimbladder as a combination respiratory/buoyancy organ. Indeed, most lungfish are obligate air-breathers and will drown if denied access to air.

Due to the action of tides, currents and winds, most oceanic environments are rarely hypoxic. Nevertheless, some marine habitats are regularly oxygen depleted. These include some estuaries, mangrove lagoons, tidepools and intertidal zones where fish become stranded between tides. Typically, fish in these environments rely on cutaneous or pharyngeal specializations to breathe air during periods of aquatic hypoxia (Graham, 1976).

Many species rely on the anaerobic metabolism to endure hypoxia. Most produce lactate in response to acute hypoxia (Jorgensen and Mustafa, 1980; Scholander et al., 1962) but the goldfish (Carassius auratus) has demonstrated remarkable tolerance to anoxia by systemic production of ethanol (Shoubridge and Hochachka, 1980).

Surprisingly, mechanisms of subaerial survival remain unknown in a number of familiar species. Particularly remarkable tolerance to hypoxia in the Pacific staghorn sculpin, Cottidae: Leptocottus armatus, has been anecdotally noted only (John Ruben, pers. comm.). This sculpin is commonly

found in bays, estuaries and rarely along rocky shorelines of the Pacific coast of North America (Miller and Lea, 1972; Hart, 1973). Its name is derived from its preopercular spines which have three, upward facing, recurved hooks. Color varies with the substrate but usually ranges from an olive-gray or green to a light or dark brown. The largest recorded specimen was 46cm in length (Hart, 1973), although most individuals are less than 30 cm. The diet of the staghorn sculpin consists of various marine arthropods, mollusks, annelids, insect larvae, and small fish including members of its own species (Tasto, 1974). Spawning occurs from mid-December through mid-March with a very high rate of post-spawning mortality (Tasto, 1974). Thus, populations consist mostly of fish less than one year old (Tasto, 1974).

In its natural habitat, the staghorn sculpin often experiences extended periods of intertidal stranding in mudflats (and occasionally tidepools) where individuals have been observed to survive subaerially for intervals to several hours (John Ruben, pers. comm.; Tasto, 1974). Under such conditions, the fish are deprived of sufficient water from which to extract oxygen and would seem forced to rely on air-breathing and/or anaerobic metabolism.

The staghorn sculpin's proficiency for subaerial survival seems absent among sympatric fishes. Only the buffalo sculpin, Cottidae: Enophrys bison, can, apparently, tolerate

relatively limited periods of hypoxia but does not appear to possess the same tolerance as the staghorn sculpin (John Nichols, pers. comm.). Thus, the staghorn sculpin appears to be unusually adapted to subaerial survival among bay and estuarine fishes of the Pacific Coast of N. America. The present study is an attempt to determine mechanisms of subaerial survival in the staghorn sculpin.

In addition to more precise quantification of the capacity to survive subaerial exposure in the staghorn sculpin and E. bison, the following physiological/anatomical parameters were examined in the staghorn sculpin: standard metabolic rate under standard conditions; capacity for, and mechanism(s) of, oxygen consumption in air; capacity for anaerobic production of lactate and/or ethanol; and blood buffering capacity.

METHODS AND MATERIALS

Adult Pacific staghorn sculpins (Cottidae: Leptocottus armatus, mean wt. 73.7 grams; range: 29.6 - 136.1 grams) were taken from Yaquina Bay in Newport, Oregon. These fish were acclimated for two weeks before use in experiments in a seawater holding tank under conditions of constant temperature (11 ± 2°C) and a natural photoperiod. Fish were fed a diet of squid and pollock. All were fasted for 96 hours before experimental observations.

Resting oxygen consumption in seawater

Six staghorn sculpins (mean wt. = 57.2 grams; range: 33.8 - 85.3) were used to determine resting oxygen consumption in seawater. For each fish, a 1 liter glass tank was filled with seawater from the holding tank. The PO_2 of the seawater was determined with a Radiometer Copenhagen E 5021 Microelectrode Unit connected to a Radiometer Copenhagen PHM 71 Mk2 Acid-Base analyzer. The PO_2 electrode was regulated at 11°C. Individual fish were placed in the tank which was then sealed with an air-tight glass lid allowing no air/water interface. The tank was placed in a darkened, temperature controlled room maintained at 11°C. After one hour, a sample of the water was drawn from each aquarium and the PO_2 was determined for a second time. The fish were removed and the seawater from each aquarium was collected and the volume of that water was determined. Oxygen consumption was determined

using the following equations :

$$[O_2] \text{ in } H_2O = a_{g \cdot c} \times (PO_2 / 760)(0.8),$$

where $a_{g \cdot c}$ = the solubility coefficient of O_2 in H_2O at 11°C

which is 38.78ccO_2/liter H_2O (Hainsworth, p. 21). Actual ccO_2

consumed = ($\blacktriangle$ [O_2] in H_2O)(total volume H_2O). The µMATP

produced = (ccO_2 consumed) (0.29 µMATP produced/ccO_2 consumed)

(Bennett and Licht, 1972).

Survival in hypoxic water

Survival time in hypoxic seawater was determined for 5

staghorn sculpins (mean wt. = 69.2 grams; range: 44.2 -

87.7). For each fish, a one liter tank (25x20x15 cm) was

filled with seawater from the holding tank. The tank was

sealed with a lid that allowed no air/water interface and

kept in a temperature-controlled room at 11°C. Water was

deoxygenated by bubbling with nitrogen for approximately one

hour. PO_2 was determined as described above and was

approximately 15mmHg. The fish was quickly placed in the

deoxygenated seawater and the system resealed. Individuals

were then observed in the aquarium until death occurred at

which point survival time was noted. Criteria for death were

cessation of coordinated respiratory and locomotory movements.

Subaerial survival and oxygen consumption

Survival time and oxygen consumption in air were measured

in 14 staghorn sculpins (mean wt. 75.5 grams; range: 29.6 -

126.9). Oxygen consumption was measured in 11 individuals

(mean wt. 77.3 grams; range: 29.6-126.9). Fish were placed in a large glass petri dish (1 liter) with a thin film of water (< 0.25 cm deep) covering the bottom of the dish. Individual animals in petri dishes were placed in air-tight, transparent Lucite chamber, measuring 52x30x1cm in a darkened, temperature controlled room maintained at 11°C. Room air was metered through the chamber at 200cc/min. Air was subsequently passed through columns of Drierite (anhydrous $CaSO_4$) and Ascarite (Na-hydrate asbestos) and then metered through a Beckman paramagnetic oxygen analyzer.

Oxygen consumption determinations were taken at 30 minute intervals over a 6 hour period or until the death of the fish. At the end of 6 hours, or upon death of the fish, total oxygen consumption and length of survival time were recorded.

Subaerial survival time was measured for 4 buffalo sculpins (mean wt. 280.0g; range: 85.1 - 396.9) from Yaquina Bay and obtained from the Hatfield Marine Science Center in Newport, Oregon. Each fish was placed in a large glass Petri dish (1 liter) with a thin film of water (< 0.25cm deep) covering the bottom of the dish. All fish were kept in a darkened, temperature controlled room maintained at 11°C. Individuals were then observed at 30 minute intervals until death occurred at which point survival time was noted. Criteria for death were cessation of coordinated respiratory and locomotory movements.

Subaerial cutaneous oxygen consumption

Capacity for subaerial cutaneous exchange of gases was also determined. The mouths of 6 staghorn sculpins (mean wt.= 53.1 grams; range: 38.6 - 68.3) were wired closed with copper thread and the opercula were held closed with rubber bands. It appeared that this arrangement prevented air from entering the buccal and opercular cavities. Fish did not resist this constraint and remained docile throughout the course of the experiment.

Individuals in Petri dishes (1 liter) were placed in an air-tight, transparent Lucite chamber, measuring 52x30x15 cm, in a darkened, temperature controlled room maintained at 11°C. Oxygen consumption was measured by the method previously described for subaerial survival time and oxygen consumption. Oxygen consumption readings were taken every 15 minutes for 1 hr.

Whole body lactate

Whole body lactate determinations were made for 6 resting and 6 subaerially exposed staghorn sculpins (mean wt. 80 grams; range: 44.1 - 136.1 grams). Fish were exposed subaerially for 6 hours in a darkened, temperature controlled room at 11°C. Resting, normoxic fish were taken directly from a tank of previously undisturbed fish. For each sample, a fish was quickly collected and stunned with a blow to the head. The fish was then homogenized in 5x its weight in

cold, 0.6N perchloric acid. A sample of the homogenate (approx. 5 mls) was collected and centrifuged for ten minutes at 3600g. The supernatant solution was filtered and centrifuged for ten minutes at 12000g and this clarified fraction then analyzed for lactate using a Sigma Diagnostics kit (Sigma Diagnostics, St. Louis, Missouri) for lactate. Absorbance was measured using a Beckman 24 Spectrophotometer set at 340 nm.

Blood pH and ethanol levels

Blood pH and ethanol concentrations were measured in 6 resting and 6 subaerially exposed staghorn sculpins (mean wt. 79.9 grams; range: 29.4 - 157.6). Subaerial exposure of fish in was for 5.5 hours in a darkened, temperature controlled room maintained at 11°C. Resting fish were taken from a tank of previously undisturbed fish.

A 0.3 ml sample of blood was drawn from experimental individuals via cardiac puncture with a heparinized syringe. Struggling was minimal and the entire procedure took less than 30 seconds. Part of the sample (0.1 ml) was then immediately analyzed for Ph using a Radiometer BMS 3 MK Blood Micro System connected to a PHM 73/ Blood Gas Monitor. The electrode was regulated at 11°C. The remaining 0.2 ml of the blood sample was analyzed for ethanol content using a Sigma Diagnostics kit as described for lactate determination.

Orthoscopic visualization and dissection of the buccal cavity, esophagus and stomach

Six adult staghorn sculpins were left exposed to air at 20°C for one hour. An endoscopic system consisting of a telescope, light source and fiber optic cable (catalogue No. 7200B, Karl Stortz Endoscopy-America, Inc. Culver City, CA) and a miniature endovideo (Circon, Santa Barbara, CA) (designed for use in arthroscopic surgery) was used for endoscopic visualization of the buccal cavity, esophagus and stomach of each fish. These areas were observed for increased vascularization through which gas exchange might occur during subaerial exposure.

Determination of gill surface area

Four live specimens each of adult staghorn sculpins (mean wt. 62.4g ; range: 42.4 - 85.1) and buffalo sculpins (mean wt. 280.0g; range: 85.1 - 396.9) from Yaquina Bay obtained from the Hatfield Marine Science Center in Newport, Oregon were used to estimate gill surface area during subaerial exposure. The right second and third gill arches of each fish were removed and total length of these arches was measured to the nearest 0.5mm. A 1mm cross section was taken from the center of these arches. This 1mm section contained about 2 gill filaments. Given that the gill filaments collapsed upon aerial exposure, I measured the exposed surface area of the filaments as an index of the area of respiratory surface

available for aerial respiration. Subsequently, an estimate of the total surface area available for aerial respiration was calculated by multiplying the length of the gill arch by the surface area of the 1mm section. For each specimen, gill surface area per unit of body weight was calculated for interspecific comparison as follows: [Surface area of 2nd gill arch + surface area of 3rd gill arch]/body weight.

Calculation of ATP contribution
from aerobic and anaerobic sources

It is possible to estimate the percentage contribution of aerobic and anaerobic energy sources to the total energy utilized from oxygen consumption and lactate production. The amount of ATP generated from these sources are (Bennett and Licht, 1972):

$$1.0 \text{ mg lactate formed} = 0.0167 \text{ }\mu\text{mATP}$$
$$1.0 \text{ cc } O_2 \text{ consumed} = 0.290 \text{ }\mu\text{mATP}$$

RESULTS

Resting oxygen consumption in sea water

The mean (± S.E.) rate of oxygen consumption for 11 staghorn sculpins at rest in seawater was 0.14 ccO_2/gxhr ± 0.03 (Figure 1).

Subaerial survival time and oxygen consumption

Eighty-six percent of 14 staghorn sculpins tested survived a minimum of six hours of subaerial exposure. Subaerially exposed fish occasionally opened their mouths, trapping a small volume of air which was then held for a short time before apparently being forced out through the opercular slits. This activity was repeated approximately every two or three minutes and was similar to that described for Clinocottus recalvus by Wright and Raymond (1978).

Mean (± S.E.) oxygen consumption for 11 the staghorn sculpin in air was 0.05 ccO_2/gxhr ± 0.03. Oxygen consumption in subaerially exposed staghorn sculpins examined here provided about 88% of total ATP production (Figure 1).

None of the buffalo sculpins survived longer than six hours of subaerial exposure.

Survival time in hypoxic water

None of the 5 fish tested survived longer than 38 minutes in hypoxic water. Mean (± S.E.) survival time was 20 minutes ± 7.5.

Subaerial cutaneous oxygen consumption

None of the six fish which had their mouths and opercula sealed survived longer than one hour. No measurable oxygen consumption was recorded for any of the fish tested. While in the metabolic chamber, the fish remained calm and did not struggle against restraints placed upon them. One individual survived for an exceptionally long period of time but close examination revealed that the wires holding the mouth shut had worked loose. Bubbles were apparent around the opercula from air passing through the buccal cavity. The datum for this individual was not included in the reported results.

Whole body lactate, blood pH and ethanol levels

Following subaerial exposure, lactate values were 50% greater than those of resting fish ($p < 0.05$, t-test; Table 1). Hypoxic fish suffered a significant drop in blood pH of an average of 0.95 pH units from the resting level ($p < 0.002$, t-test, table 1). Ethanol was absent from the blood of all fish tested.

Orthoscopic visualization and dissection of the buccal cavity, esophagus and stomach

No evidence of internal accessory respiratory organs, including vascularization of the surface buccal cavity, esophagus or stomach was noted through endoscopic observation or dissection of the animals. Few morphological differences

were visible by dissection of the staghorn sculpin and the buffalo sculpin.

The gills consist of four arches, the fourth being reduced and fused to the rear of the opercular chamber. Dissections revealed the staghorn sculpin to have slender gill arches and filaments with spaces between the individual arches. This arrangement allows the filaments to remain separated in air with only the tips of the filaments touching the filaments of adjacent arches. The gill filaments of the buffalo sculpin were closely packed under the opercula and did not remain separated in air. Pseudobranchs were present on both the buffalo sculpin and the staghorn sculpin.

Comparison of gill surface area

Mean (± S.E.) gill surface area in the staghorn sculpin ($23.2mm^2/g$ ± $1.4mm^2/g$) was 2.4 times greater than that in the buffalo sculpin ($9.4mm^2/g$ ± $3.1mm^2/g$). This difference was statistically significant ($p<0.05$, Mann-Whitney U-test).

DISCUSSION

Staghorn sculpins inhabiting mudflats, bays and estuaries customarily experience subaerial tidal stranding (Tasto, 1974). During ordinary tidal series, individuals may experience six to eight hours of stranding. Air breathing in these fish, and perhaps many Cottidae, appears to be a major means of tolerating subaerial exposure. Confamilial _Clinocottus_ _recalvus_ are also particularly adept air-breathers (Wright and Raymond, 1978).

Air-breathing fish often possess a variety of specialized, highly vascularized surfaces to facilitate subaerial O_2 exchange. These range from vascularized cutaneous surfaces to specialized internal organs that function primarily for aerial gas exchange. Of possible regions of arterial gas exchange in the staghorn sculpin, a cutaneous site seems least likely. Oxygen uptake ceased in subaerially exposed experimental individuals when mouths and opercula were sealed.

The presence of accessory, non-pharyngeal internal respiratory organs specialized for O_2 consumption in air seems unlikely. Arthroscopic inspection and dissection revealed no apparent sites of enhanced vascularization in either the buccal, esophageal or gastric regions.

Aerial gas exchange can also take place at the gills. The marine fish _Mnierpies_ _macrocephalus_ uses gills for air-

breathing (Graham, 1973). Its gill filaments are long, thick and well supported out of water. Significantly, the buffalo sculpin possesses gills with little interbranchial spacing and exhibits far less tolerance to subaerial stranding than the staghorn sculpin. The gills of the staghorn sculpin have more relative surface area in contact with air when these fish are subaerially exposed. This probably allows for more oxygen uptake in subaerially exposed staghorn sculpins, and would account for subaerial oxygen consumption rates of the staghorn sculpin.

While out of water, both in the field and in the laboratory, the staghorn sculpin remained in an upright position supported by its broad pectoral fins. This posture is similar to that observed in the air-breathing bald sculpin, Clinocottus recalvus (Wright and Raymond, 1978) and probably prevented the weight of the fish from collapsing the gill filaments.

It is well documented that vertebrates utilize anaerobiosis to generate ATP during periods of intense exercise (Ruben and Bennett, 1980) and hypoxia (Bennett, 1978; Scholander, et al., 1962; Caillouet, 1968). Although staghorn sculpins apparently do not anaerobically generate ethanol as a fuel for ATP production, production of lactic acid helps extend hypoxia tolerance in subaerially exposed staghorn sculpins. Staghorns investigated here relied on glycolysis

and lactate formation to provide almost 12% of ATP generated during experimental exposure (Figure 1). This is broadly comparable to levels of lactate-related ATP generation in hypoxic Periopthalmus (mudskippers) and Platichthyes (flatfish) which also increased significantly during periods of extended subaerial exposure (Bandurski et al., 1968; Leivestad et al., 1957).

Reliance on anaerobic generation of lactate during subaerial hypoxia in the staghorn sculpin exacts a toll: although total lactate content following extended exposure increased by only 120%, blood pH dropped almost a complete pH unit. This is significantly greater than previously reported rates of blood pH reduction in Torpedo (electric ray) (-0.45 pH units following 11 hours of hypoxia [Hughes and Johnston, 1978]). Severe depression of pH is associated with depression of blood oxygen capacity, decrease of blood bicarbonate and the general disruption of circulation (Bennett, 1978).

The possible inability of experimentally exposed staghorn sculpins to rid themselves of CO_2 may also have contributed to the marked observed pH depression. A number of air-breathing fish including Electrophorous (Johansen, et al., 1968) and Protopterus (Lenfant and Johansen, 1968) excrete CO_2 in hypoxic water via the skin or by aquatic ventilation of the gills. Experimental conditions utilized here (complete subaerial exposure) may have prevented cutaneous or branchial

excretion of CO_2. However, exposed sculpins in the field may be periodically sprayed or soaked with sea water and individuals in tidal flats may partially bury themselves in mud. This might allow removal of CO_2 and lactate through the skin and/or gills.

While aquatic resting metabolic rate in sculpins examined here was broadly comparable to that of most similar sized teleosts, subaerially exposed individuals exhibited almost a 58% reduction in total resting metabolic rate (anaerobic + aerobic ATP production). In the field, such a response might reasonably be expected to extend the duration of intertidal stranding that can be tolerated by these fish.

Depressed resting metabolic rate during hypoxia in other fish is relatively uncommon. <u>Acipenser</u> <u>transmontanus</u> (sturgeon) reduce their total energy expenditure during hypoxia to less than 10% of normal without accrual of oxygen debt (Burggren and Randall, 1978) but there is no previous record of such a reduction in any marine fish. Thus, reduced resting metabolic rate in staghorn sculpins may represent a relatively unique response to extended periods of subaerial intertidal exposure and its attendant hypoxia.

In conclusion, the staghorn sculpin appears well-suited for the subaerial exposure it experiences in nature. It is able to breathe air through well-supported gills and supplements necessarily depressed rates of subaerial

aerobiosis by anaerobic production of lactic acid. Most
remarkably, it may also substantially reduce minimal energy
expenditure requirements during periods of hypoxia. These
adaptations probably greatly enhance the range of intertidal
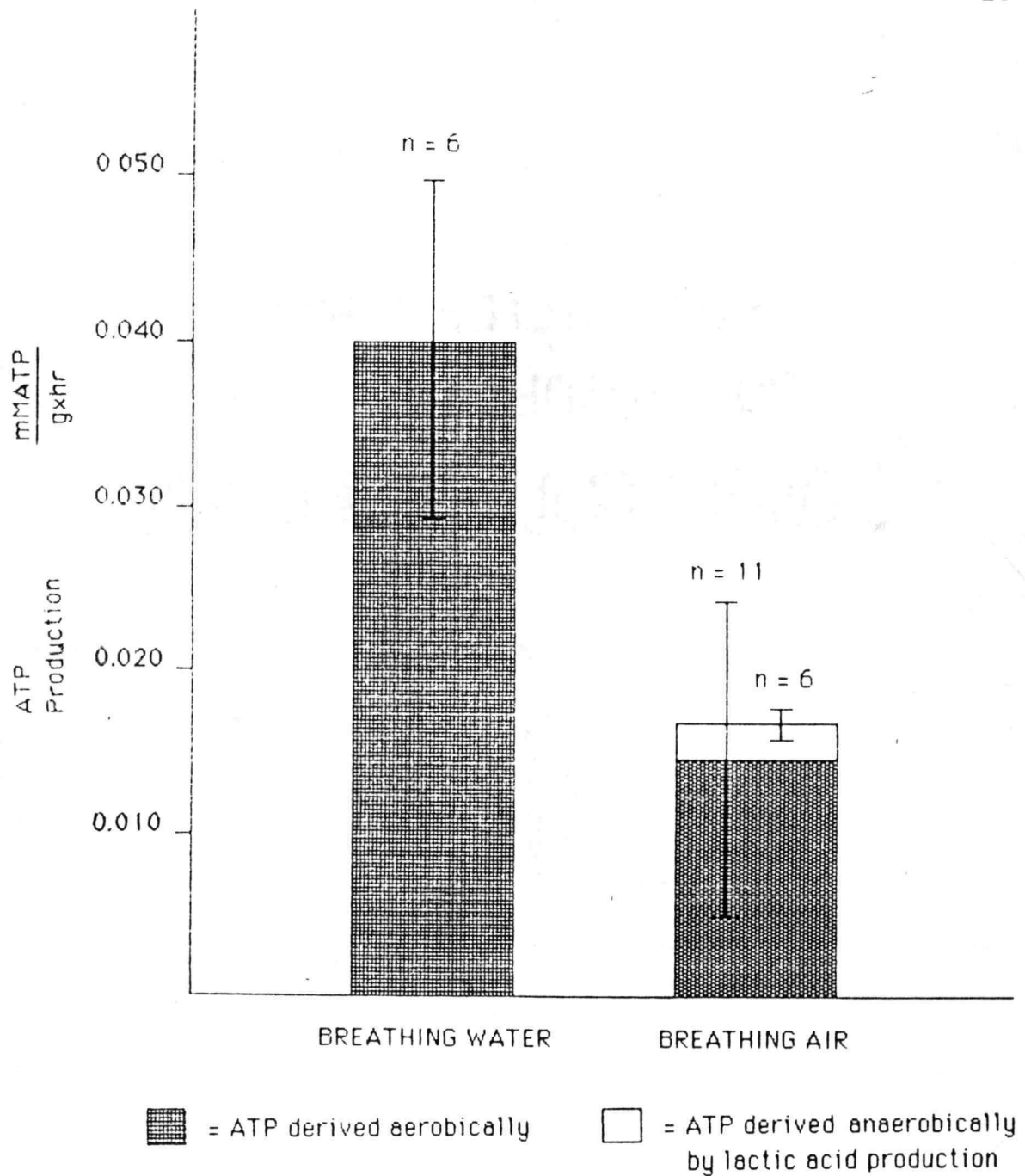environments in which the staghorn sculpin can survive.

Figure 1. Mean (± S.E.) rates and sources of ATP production during water and air breathing in the staghorn sculpin. Sample sizes are indicated above each histogram.

Table 1. Mean (± S.E.) whole body lactate concentrations and blood pH values at rest and following subaerial exposure for 6 staghorn sculpins. Means separated by * and ** are significantly different at p<0.05 and p<0.002, respectively (t-test).

	Whole body lactate (mg/g)	Blood pH
At rest	0.343 (± 0.171)	7.50 (±0.28)
	*	**
Following subaerial exposure	0.742 (± 0.273)	6.55 (± 0.22)

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