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THE GILL ARCH OF THE STRIPED BASS, MORONE SAXATILIS. II. MICROVASCULATURE STUDIED WITH VASCULAR CORROSION CASTING AND SCANNING ELECTRON MICROSCOPY

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Abstract

The gill vasculature of euryhaline striped bass, Morone saxatilis , was examined by scanning electron microscopy of corrosion casts prepared by injecting resin (either Mercox/Sevriton or L.R. White) into the ventral aorta. The vasculature of the striped bass gill appears to be similar to that of other euryhaline species. The striped bass gill has three major vascular systems: (1) a respiratory system, (2) an arterio-venous system, and (3) a nutritive system. In the respiratory system, blood from the afferent branchial artery flows to each filament via an afferent filamental artery, and from there to the highly vascularized respiratory lamellae. Lamellar blood is conducted back to the efferent branchial artery via the efferent filamental artery. In the second system arterio-venous anastomoses transport blood from the efferent filamental artery to the central venous sinus. Blood then flows to the branchial vein either directly or via paired afferent companion vessels. No arterio-venous anastomoses connecting the prelamellar vessels with the central venous sinus have been found. Finally, nutritive branches to the arch are provided by the efferent branchial artery and the efferent filamental artery. The striped bass does not have a lamellar bypass system involving the central venous sinus as reported in other species. Intralamellar distribution mechanisms and lamellar recruitment may account for changes in respiratory lamellar perfusion during decreased and increased oxygen demand, respectively. The central venous sinus' role may be partially nutritional since its blood is oxygenated. However, its complex vascular connections may permit a variety of other functions.

KEY WORDS: striped bass, Morone saxatilis, gill, scanning electron microscopy, vascular casting, injection replica, blood vessels, vasculature, microcirculation, microcorrosion casts

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Introduction

Recently, striped bass (Morone saxatilis) have become the subject of increased study because pollution of their native spawning grounds has led to a decline in their numbers (Dawson, 1982), and because this species has become increasingly important to landlocked sport fisheries (Parker and Geiger, 1984). Their euryhalinity (Tagatz, 1961) also makes them an excellent species for osmoregulation studies. Although striped bass gill morphology has been studied (Bauer, 1972; Groman, 1982; Harpole and Hossler, 1984; Hossler et al., 1986b), no detailed study of the branchial and filamental vasculature has been reported. Since osmoregulatory chloride cells (Keys and Wilmer, 1932) in striped bass are abundant on the afferent surface of the filament (Hossler et al., 1986b), a study of the blood flow to that area could be useful in understanding the osmoregulatory role of the chloride cell. Vascular corrosion casting has been used to study the complex gill vasculature of various species including the bowfin (Olson, 1981), perch (Laurent and Dunel, 1976), trout (Laurent and Dunel, 1976; Olson, 1983), ling cod (Farrell, 1980), eel (Laurent and Dunel, 1976; Donald and Ellis, 1983), spiny dogfish shark (Olson and Kent, 1980; DeVries and DeJager, 1984), and skate (Olson and Kent, 1980). In the present study, the microvasculature of the striped bass gill is described using vascular corrosion casting and scanning electron microscopy, a technique which allows the three-dimensional vasculature to be viewed without interference from surrounding tissues.

Materials and Methods

Striped bass (Morone saxatilis) 13-27 cm long were obtained from Eagle Bend Fish Hatchery (Clinton, Tennessee), Morristown State Fish Hatchery (Morristown, Tennessee), and the Southeastern Fish Cultural Laboratory (Marion, Alabama) and transported in styrofoam containers in oxygenated 0.1% salt water (1 g/L NaCl; Parker and Geiger, 1984). Striped bass were maintained in 100L tanks with aerated, hatchery-aged tap water (0.011 Osm) or 3% saltwater (w/v; specific gravity 1.02; 1.01 Osm; Instant Ocean Salts,

Aquarium Systems, Mentor, Ohio), at room temperature (20-24°C) with a cycle of approximately 11 h subdued light and 13 h of dark, and fed trout chow (Silver Cup Feed, Murray Elevators, Murray, Utah) ad libitum for at least one week before experimentation.

For light microscopic studies, striped bass were killed by decapitation, and their gill arches were removed, rinsed in 0.9% NaCl to remove blood, and immersed in freshly prepared 2.5% glutaraldehyde-1.8% paraformaldehyde in 0.1M cacodylate-HCl (pH 7.2) overnight at 4°C or for 2 h at 20-24°C. After fixation, the gill arches were washed in 3 changes of 0.1M cacodylate-HCl (pH 7.2), postfixed in 2% OsO_4 (buffered with 0.1M cacodylate, pH 7.2) for 2 h, and then washed again in several changes of 0.1 M cacodylate-HCl (pH 7.2) overnight. The specimens were dehydrated in a graded ethanol and propylene oxide series, and embedded in eponaraldite (Mollenhauer, 1964). Sections (1-2 μm) were cut with an ultramicrotome (Ultracut, American Optical Instruments, M.O.C. Inc., Valley Cottage, NY), mounted on glass slides, stained with 1% toluidine blue (in 1% Na borate), and viewed and photographed on a Zeiss standard light microscope.

For vascular casting, striped bass were anesthetized with ethyl-p-amino-benzoate (benzocaine; see Ferreira et al., 1979; Olson, 1985). After an intraperitoneal injection of heparin (approximately 30 U/g), the fish were placed ventral side up in a V-shaped trough (Olson, 1985) and a medial, longitudinal slit was made near the pectoral fin to expose the heart. Additional heparin (approximately 15 U/g) was then injected into the heart. The ventral aorta was cannulated and the gills were cleared of blood by flushing with fish Ringer's solution (Lockwood, 1961) at physiological perfusion pressure (30 mm Hg; constant flow, pulsatile pressure; Olson 1983; 1985). Pulsatile was used instead of constant pressure in order to mimic gill blood flow (Farrell et al., 1979; Part and Svangberg, 1981; Davie and Daxboeck, 1982; Daxboeck and Davie, 1982). Resin was then infused through the same cannula until the onset of polymerization (approximately 5 min). Physiological perfusion pressure (30mm Hg) was used for resin injection in most cases to avoid distention of the vessels (Olson, 1983), but occasionally higher pressures (50-60 mm Hg) were used in an effort to obtain filling of the smaller vessels. The resin used was either a combination of Mercox (80%; Ladd Research Industries, Burlington, VT) and Sevriton (20%; Dentsply Limited, Surrey, England), or L.R. White (The London Resin Co., Ltd., Hampshire, England). The fish were immersed in warm water (50°C) for at least 20 min to cure the resin. Tissue was removed with alternating rinses of 20% NaOH and distilled H_2O over a period of several days. Casts were rinsed thoroughly in distilled water, air dried, attached to stubs with silver paste, coated with gold or gold-palladium in a Desk-1 Sputter Coater (Denton Vacuum Corp., Cherry Hill, NJ), and examined in a Hitachi S-430 electron microscope. Approximately 100 arches from 15 fish were studied. Measurements were made from the electron micrographs. Extravasation of resin from vessels was rarely observed.

Results

Striped bass are very sensitive to handling and transport. Disease and mortality are decreased by transporting fish in 0.1% NaCl (Parker and Geiger, 1984; Hossler et al., 1986b).

Different casting media were tested in an effort to obtain well filled striped bass gill vasculature. The Mercox/Sevriton mixture has a viscosity about half that of Mercox alone (usually 10-20 cps; Hossler et al., 1986a), and the viscosity of L.R. White is reported to be 8-10 cps (Sage and Gavin, 1984; F.E. Hossler, unpublished findings). The Mercox/Sevriton mixture usually provided complete gill casts. The L.R. White produced more extensive casts of the whole fish, but the vessels tended to collapse upon digestion. Only Mercox/Sevriton specimens are illustrated in the figures.

No differences in filamental casts from seawater and freshwater specimens have as yet been documented, but subtle differences might be difficult to verify because of individual variations in perfusion and fish. Casts from fish adapted to either salinity are shown.

A typical vascular corrosion cast of striped bass gills is shown (Fig. 1). The four pairs of gill arches are designated I, II, III, and IV, rostral to caudal. No differences among the filaments from the different arches were observed. Striped bass have three major vascular systems: (1) a respiratory system, (2) an arterio-venous system, and (3) a nutritive system (Boland and Olson, 1979). The major components of the vasculature of the gill arch and gill filaments are represented schematically in Fig. 2.

Respiratory system

In the respiratory system, blood from the heart is pumped via the ventral aorta to the four pairs of afferent branchial arteries (ABA; Figs. 2, 3 and 4). As seen in other teleosts (Muir, 1970; Boland and Olson, 1979), each ABA divides into posterior concurrent and anterior recurrent branches allowing all parts of the arch to receive blood from the heart. Blood enters each filament via an afferent filamental (primary) artery (AFA), is distributed to the highly vascularized respiratory (secondary) lamellae (RL, Fig. 5) via afferent lamellar arterioles (ALA), and then passes to the efferent filamental (primary) artery (EFA) via the efferent lamellar arterioles (ELA). Blood from the EFAs is collected in the efferent branchial artery (EBA) and is carried to the dorsal aorta. The EBA is a single vessel centrally, but splits at either end of the holobranch, providing a vessel for each hemibranch (Muir, 1970; Farrell, 1980). It was not unusual to see adjacent AFAs with a common origin from the ABA, or adjacent EFAs which fused before entering the EBA.

In the striped bass the ABA appears to be symmetrically located in the middle of the arch, not aligned next to the cartilage as in perch (Laurent and Dunel, 1976), pike (Dornesco and Miscalenco, 1968), and other perciform species

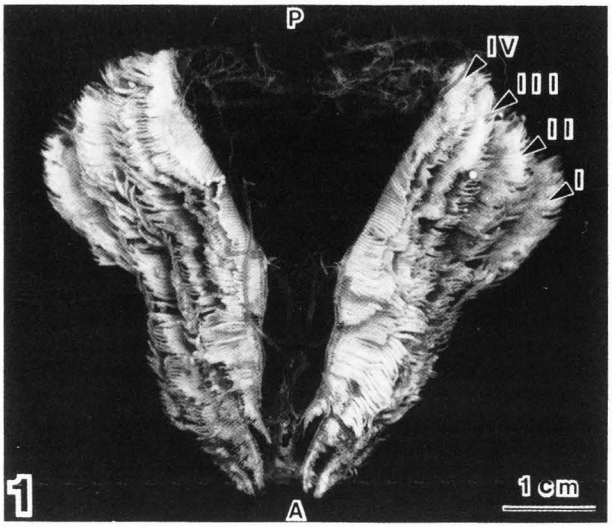


Fig. 1 Ventral view of a vascular cast of all four pairs of gill arches of a 24 cm striped bass. Photographed with a Leicaflex SL 35 mm camera with a 100mm macro lens.

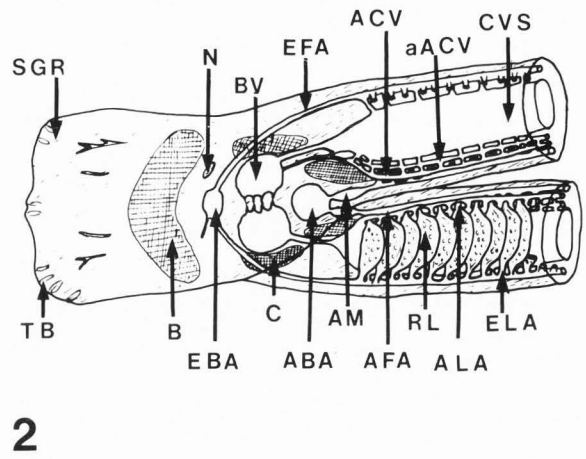


Fig. 2 Schematic cross-section of striped bass gill arch II showing vasculature. The lamellar vasculature has been removed from the upper filament.

The symbols for Figures 1-15 are as follows:

A	-	anterior
aACV	-	"accessory" afferent companion vessel
ABA	-	afferent branchial artery
ACV	-	afferent companion vessel
AFA	-	afferent filamental (primary) artery
ALA	-	afferent lamellar arteriole
AM	-	ampulla of afferent filamental artery
B	-	gill arch bone
BC	-	basal channel of respiratory lamella
BV	-	branchial vein
C	-	cartilage
CVS	-	central venous sinus
EBA	-	efferent branchial artery
EFA	-	efferent filamental (primary) artery
ELA	-	efferent lamellar arteriole
LGR	-	long gill raker
MV	-	marginal vessel of respiratory lamella
N	-	nerve
NU	-	impression of endothelial cell nucleus
P	-	posterior
RL	-	respiratory (secondary) lamella
SGR	-	short gill raker
TB	-	taste bud
I,II,III,IV	-	gill arches, rostral to caudal

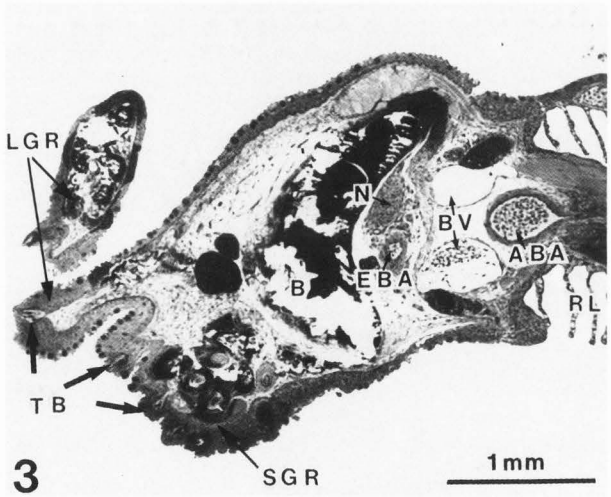


Fig. 3 Light micrograph of a cross-section of gill arch I. Compare with the vascular schematic on Fig. 2.

(Dornesco and Miscalenco, 1967). Only one or two small branches beside the AFA were observed stemming from the ABA.

The AFAs of the two hemibranches of each arch alternate with each other as do the filaments, and their number and size depend on the growth of the fish. The proximal aspect of the AFA has a dilation or ampulla (AM) with approximately 8-10 pairs of ALAs coming from it. No communications were found between the AMs of either the same or opposite hemibranches. The AFA narrows toward the distal end of the filament as the RLs become smaller. No branches other than the ALA were seen stemming from the AFA.

The RLs of the striped bass have prominent marginal vessels (MV) and basal channels (BC), as well as a complex respiratory vascular network

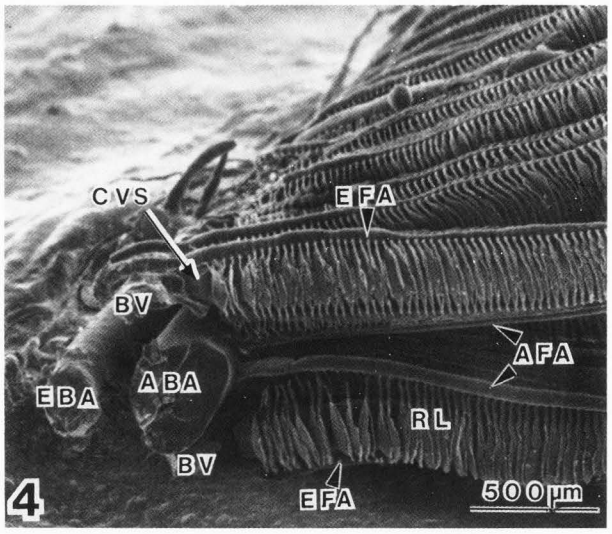


Fig. 4 Scanning electron micrograph of a cross section of a vascular cast of the second gill arch. Compare with the vascular schematic on Fig. 2.

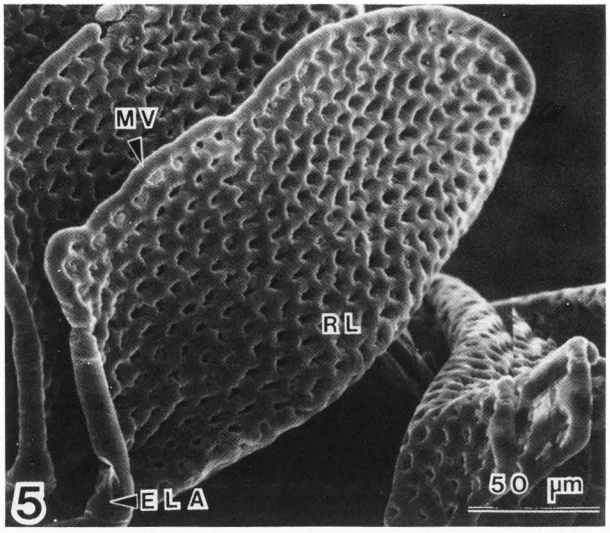


Fig. 5 Vascular network of the respiratory lamellae (RL). Note the marginal vessel (MV). The openings in the capillary network mark the positions of the pillar cells.

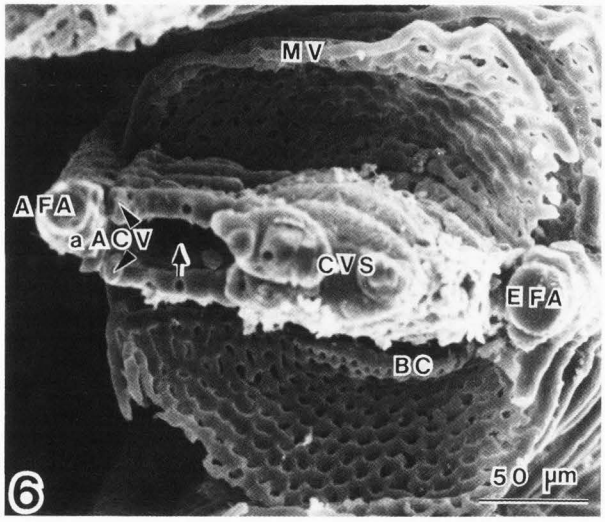


Fig. 6 Cross-section of the vasculature of the gill filament. Note the position of the central venous sinus (CVS) and the accessory afferent companion vessels (aACV). The afferent companion vessels are absent. The arrowhead marks the position where the cartilage would normally be. Note the basal channel (BC) and marginal vessel (MV) of the respiratory lamellae (RL).

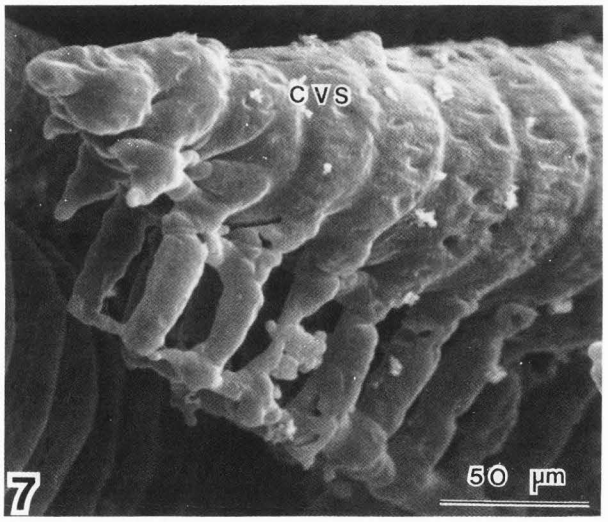
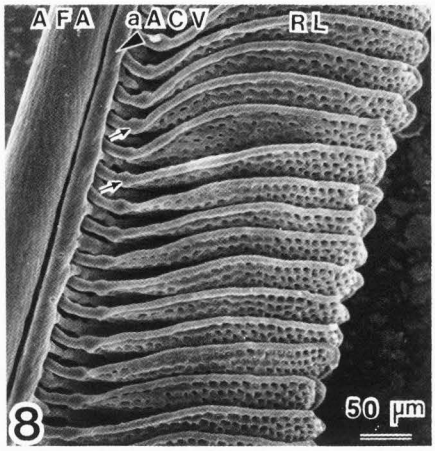


Fig. 7 Cast of central venous sinus (CVS) at apex of filament. The surrounding respiratory lamellae, afferent filamental artery, and the efferent filamental artery were removed. The afferent side of the CVS is at the bottom.

(Fig. 5) interwoven between pillar cells (J.A.C. King, unpublished observation; and see Groman, 1982). In most instances, each RL has one ALA and one ELA. The ALA is longer because it must pass around the filamental cartilage (C) to get to the RL. The ALAs of some of the specimens have enlargements at the junctions of the MV and BC.

Variations on the general respiratory scheme include: (1) common origin of two ALAs on the same or opposite sides of the central venous sinus (CVS); (2) several RLs drained by a single ELA; (3) extra, direct connections between the EFA and the RL or ELA; (4) an area in the middle of the filament with a double EFA with remnants of a RL vascular network on the aberrant EFA; (5) an accessory EFA which then empties into the main EFA; and (6) a filament and its vascula-



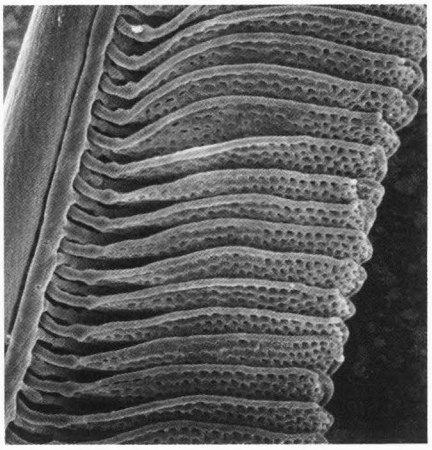


Fig. 8 Stereo pair of the afferent filamental artery (AFA), respiratory lamellae (RL), and the afferent companion vessel (ACV). Arrows: enlargements in the afferent lamellar arterioles (ALA).

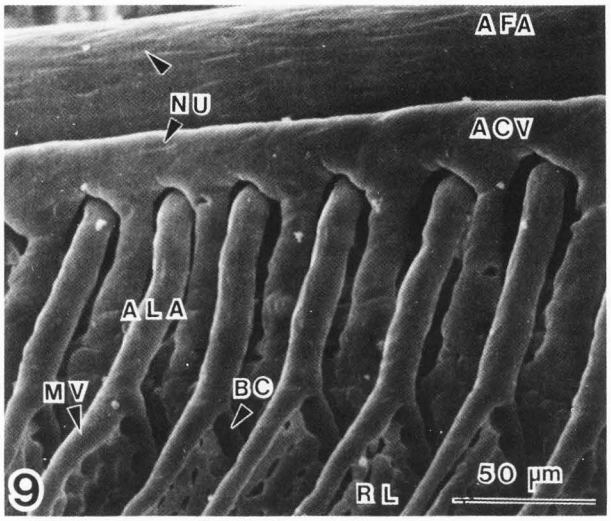


Fig. 9 Cast of blood supply of respiratory lamellae (RL) showing the marginal vessels (MV) and basal channels (BC). Note the afferent companion vessel (ACV) and its connections to the central venous sinus which alternate with the afferent lamellar arterioles (ALA).

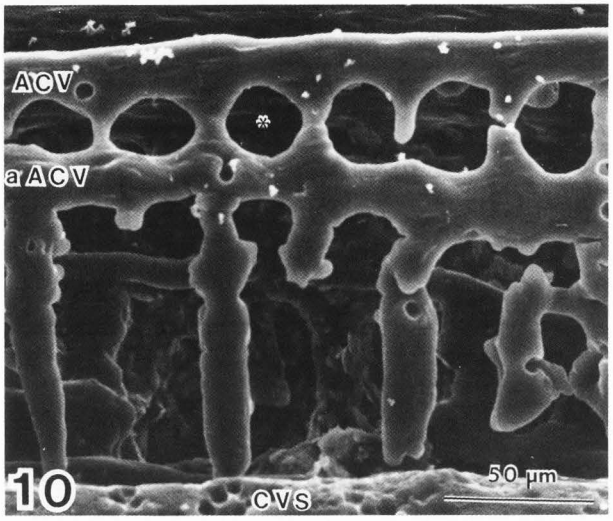


Fig. 10 Cast of "accessory" afferent companion vessel (aACV) and afferent companion vessel (ACV). The asterisk indicates the area where an ALA would exit to attach the RL to the AFA.

ture divided near its distal tip.

Arterio-venous system

Each gill filament contains a CVS located between the filamental cartilage (C) and the EFA (Figs. 2, 6 and 7). Arterio-venous connections occur between the EFA and the CVS, but not between the AFA and CVS. The CVS empties either directly into the branchial vein (BV) or indirectly via small, paired afferent companion vessels (ACV) which lie just medial to the AFA (Figs. 6, 7, 8 and 9). The vessels connecting the CVS and the ACV alternate with the ALA and are regularly spaced, about one for every ALA (Figs. 8 and 9). An "accessory" ACV (aACV) located on either side of the cartilage and paralleling the ACV, allows blood flow between the CVS-ACV connections (Fig. 10). The aACV is separated from the ACV by the ALA. The BV receives blood from the CVS-ACV complex, and apparently from the rest of the filament. Groman (1982) states that blood in the BV empties into the EBA in the striped bass, but in all the casts examined only two small connections were found.

The CVS of striped bass gill filaments is usually a single, sack-like structure which narrows distally (Figs. 7, 11 and 12). Casts of the CVS often exhibit indentations from their overlying RL (Fig. 11). The CVS and EFA have regularly spaced "anastomoses" (see Donald and Ellis, 1983; Laurent, 1984), about one for every two RL. Because of their position and size, however, these anastomoses are difficult to view and count (Laurent and Dunel, 1976). The proximal end of the CVS-ACV complex (Fig. 12) has

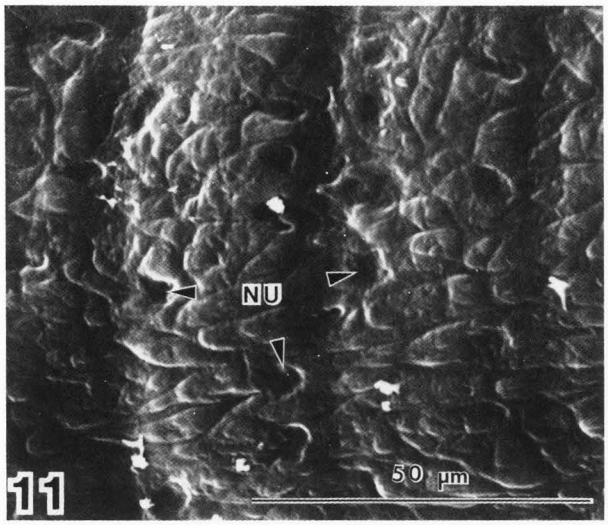


Fig. 11 Cast of the central venous sinus. Note the indentations produced by the respiratory lamellae and the impressions left by the nuclei of endothelial cells (NU).

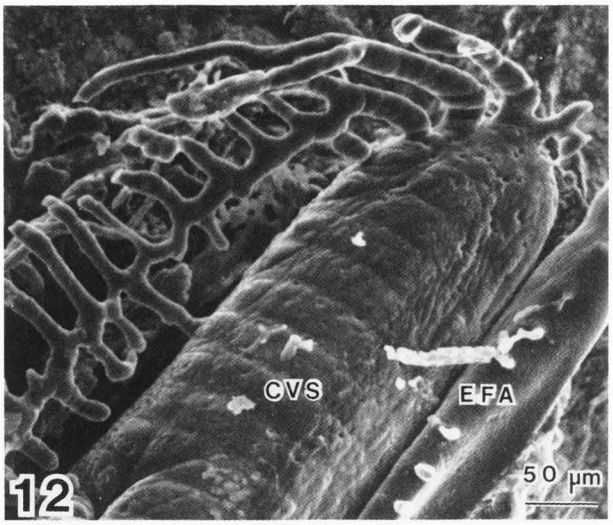


Fig. 12 Vascular cast of the proximal end of the central venous sinus (CVS). Note the CVS-ACV complex at upper left.

various connections to the EBA, adjacent CVSs, and the BV (Fig. 13). No connections between the CVS and the RL or between the ACV and the AFA were observed.

Nutritional system

Nutritive branches to the arch proper are provided by the EBA and the proximal part of the EFA. The EBA gives off large nutritive vessels which parallel it and give off branches to each filament (Fig. 14). Coiled vessels from the EFA proximal to the first RL of the filament anastomose with the nutritional vessels from the EBA (Figs. 14 and 15). Occasionally a nutritional vessel in the proximal part of the EFA will anastomose with a RL rather than the EFA. Vessels providing nourishment to the area around the EFA arise from the vascular network around the base of the EFA or directly from the EFA.

The irregularly shaped BVs in each arch receive blood from the filament proper as well as the CVS-ACV complex described above (Fig. 13). Smaller vessels combine to form larger vessels which parallel the filaments and eventually join the BV on the gill raker side (Fig. 3). The two BVs in each arch communicate by small vascular connections all along the length of the arch. In all the casts studied only two small connections were found between the BV and the EBA.

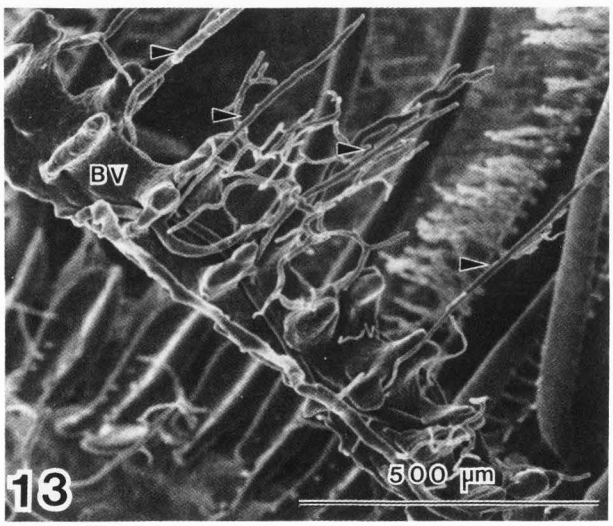


Fig. 13 Vascular cast of the branchial vein (BV) and its connections. Note the vessels from each filament and the tributaries that are interwoven with the BV. Arrowheads indicate small vessels which drain the CVS-ACV complex and the nutrient supply to the filament.

Discussion

As with most other euryhaline species (Laurent, 1984), striped bass gill vasculature consists of 3 major systems (Boland and Olson, 1979): (1) a respiratory system including the afferent filamental artery (AFA), afferent lamellar arteriole (ALA), respiratory lamellae (RL), efferent lamellar arteriole (ELA), and the efferent filamental artery (EFA); (2) an arteriovenous pathway including the EFA, the central venous sinus (CVS), and the branchial veins (BV); and (3) a nutritive system including vessels from the EFA and the efferent branchial artery (EBA). For a general discussion of gill vasculature, the reader is referred to the excellent descriptions by Laurent (1984) and Boland and Olson (1979). Several features of the striped

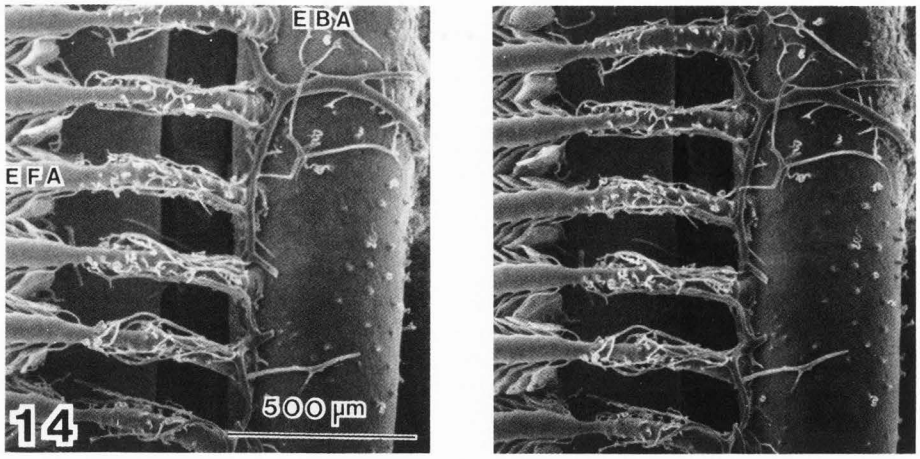


Fig. 14 Stereo pair showing nutrient vessels from the efferent branchial artery (EBA) and the efferent filamental artery (EFA). Note the coiled origins of the nutritional vessels from both the EFA and EBA, and the connections between those vessels.

bass gill vasculature in particular merit mention here.

Respiratory system

Enlargements (called ampulla or "blebs") of the AFA proximal to the "bifurcation of the two hemibranches" observed in striped bass have been reported in other species (Fromm, 1974; Laurent and Dunel, 1976; Olson, 1981). The ampulla may be a damper for the pulsatile blood flow in striped bass as proposed by Fromm (1974). The ALA enlargements may represent sites adjacent to sphincters (Wright, 1973).

The many variations observed in the filamental vasculature are relatively uncommon, probably are due to disease and growth abnormalities (Hughes, 1984), and would not have been of great functional consequence to the fish.

Arterio-venous pathway

The arterio-venous pathway has become the focus of many recent studies. Most of the differences between species studied to date appear in the location of their arterio-venous anastomoses. Prelamellar arterio-venous anastomoses with the central venous sinus are thought to be part of a lamellar bypass for blood when the O_2 demand decreases (Steen and Kruysse, 1964; Richards and Fromm, 1969), and at least some anatomical evidence for lamellar bypass has been reported in: channel catfish (Ictalurus punctatus; Olson et al., 1978; Holbert et al., 1979; Boland and Olson, 1979), eel (Anguilla anguilla; Laurent and Dunel, 1976), short-finned eel (Anguilla australis; Donald and Ellis, 1983), smooth toadfish (Torquigener glober; Cooke and Campbell, 1980), cichlid (Tilapia mossambica; Vogel et al., 1973; 1974), dogfish shark (Squalus acanthias; Olson and Kent, 1980), dogfish (Centrophorus scalpratus; Cooke, 1980), bowfin (Amia calva; Olson, 1981), trout (Salmo gairdneri; Richards and Fromm, 1969), and eel (Anguilla anguilla; Steen and Kruysse, 1964). However, lamellar bypasses have not been found in: trout (Salmo gairdneri; Gannon et al., 1973; Vogel et al., 1976; Laurent and Dunel, 1976), ling cod (Ophiodon elongatus; Farrell, 1980), perch

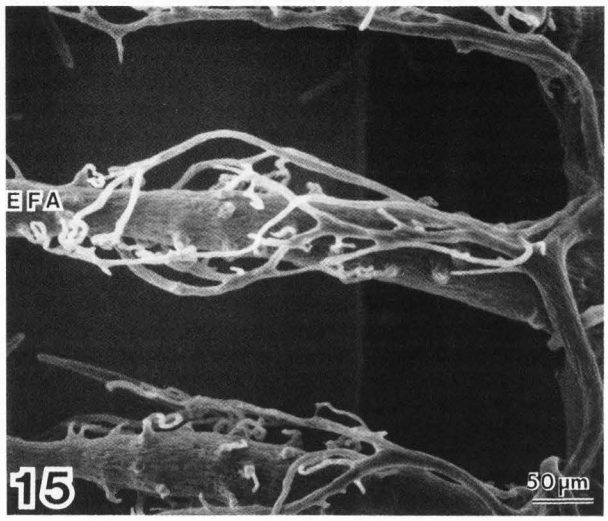


Fig. 15 Details of the vascular network around the proximal aspect of the efferent filamental artery (EFA).

(Perca fluviatus; Laurent and Dunel, 1976), and striped bass (present manuscript). We observed post-lamellar, but not prelamellar, arterio-venous anastomoses. Therefore, the CVS cannot be acting as a shunt mechanism in striped bass. The blood must first be oxygenated before entering the CVS.

Intralamellar distribution mechanisms (Farrell et al., 1980; Soivio and Tuurala, 1981) may be used by the striped bass to regulate O_2 and ion exchange in the gills. Both the marginal vessel (MV) and the basal channel (BC) are prominent in striped bass and are filled before the respiratory capillaries during vascular casting. Hughes (1976) reported that in resting fish blood flows preferentially through the MV, and both the MV (Hughes and Grimstone, 1965; Newstead, 1967; Laurent and Dunel, 1976) and BC (Smith and Johnson, 1977; Part et al., 1984; Tuurala et al., 1984) have been suggested as

possible shunts. Contractile pillar cells (Bettex-Galland and Hughes, 1972) present in striped bass (Groman, 1982; J.A.C.King, unpublished light microscopic and TEM studies) may also help to control intralamellar blood flow (Hughes and Grimstone, 1965; Newstead, 1967; Morgan and Tovell, 1973).

"Lamellar recruitment" (Hughes, 1972; Hughes and Morgan, 1973; Cameron, 1974; Booth, 1978, 1979; Farrell et al., 1979; Holbert et al., 1979; Jackson and Fromm, 1981) may occur in striped bass during increased oxygen demand. Randall (1970), Hughes (1972), and Hughes and Morgan (1973) found that the number of RL receiving blood at a given time changes with O_2 demand and this may be controlled by ALA sphincters (Wright, 1973). Incomplete casting of some RL in the present study could be the result of such selective RL recruitment or of perfusion differences.

The CVS has been described in some species as a sack-like structure (Laurent and Dunel, 1976), but recent studies (Olson, 1983) have shown that the CVS may be composed of several vessels which appear as a single structure when distended by excessive perfusion pressure. Although physiological perfusion pressures were used here, the normally distinct CVS-ACV connections were meshed together in some filaments. Since endothelial nuclear impressions were evident, the size and shape of the CVS could not have been the result of resin extravasation, but could have been affected by distension.

The CVS may provide support to the filament (Wright, 1973), act as a reserve for oxygenated blood (Laurent and Dunel, 1976), or supply nutrition to the tissues (Groman, 1982). However, in our view no one function can satisfactorily explain the complex CVS-ACV network observed in the striped bass gill filament.

Nutritional system

Nutritional vascular networks around the proximal aspect of the EFA have been reported previously (Laurent and Dunel, 1976; Boland and Olson, 1979), and are probably providing nourishment to the abductor muscle bundles of the filaments (Groman, 1982). Nutritional vessels from the EBA probably supply the rest of the arch including the gill rakers and taste buds.

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Discussion with Reviewers

K.R. Olson: A variety of resins were used in your methods. Are these all methacrylate resins? Why did you combine the Mercox and the Sevriton? Would you please give a brief description of these resins.
Authors: L.R. White (London Resin Co., Ltd., Hampshire, England) is an acrylic resin used for embedding. Its viscosity is about 8-10 cps at 30°C (Sage and Gavin, 1984; F.E. Hossler, unpublished findings), its polymerization is exothermic, and it has a setting time of 5-15 min Mercox (Ladd Research Industries, Burlington, VT) is an acrylic resin. Its viscosity is reported as 20-30 cps by the manufacturer, its polymerization is exothermic, and it has a setting time of 5-10 min.
Sevriton (De Trey Division, Dentsply Ltd., Surrey, England) is an acrylic resin used as a dental sealant. We did not measure the viscosity of Sevriton alone, but when mixed with Mercox (1:4) it reduces the viscosity of Mercox by half without interfering with polymerization. Sevriton has previously been combined with Batson's medium (Nopanitaya et al., Scanning Electron Microsc. 1979, II, 751-755). We observed that the viscosity of Mercox varied from one shipment to the next, and that we could lower its viscosity by adding Sevriton and thus obtain better vessel filling.

K.R. Olson: Did the L.R. White collapse due to the digestion procedure or the inherent fragility of the resin?
Authors: The L.R. White casts collapsed during the digestion procedure probably due to lack of rigidity of the plastic.

K.R. Olson: Did you notice any special vascular arrangements between the central sinus vessels and the chloride cells?
Authors: As with other fish, chloride cells in striped bass are located on the afferent filamental surface and between the respiratory lamellae (Hossler et al., 1986b). Chloride cells have been functionally linked to the arterio-venous system in teleosts (Payan and Girard; In: Fish Physiol. XB, pp. 39-63, 1984) as well as to the slower blood flow in the basal channel (BC) of the respiratory lamellae (Hughes, 1984). The location of chloride cells in striped bass may allow them to be affected by both vessel systems. No other vascular arrangements were observed.

D.E. Hinton: It seems as if the first heparinization was done before surgical removal of the heart. If so, how was this performed? Why?
Authors: The initial heparinization was done with an i.p. injection so that anticoagulation would be initiated before and during the approximately 10 min surgery time. As indicated, the additional heparin was added to the heart before cannulation.

D. Schraufnagel: The three-dimensional relationships you show in the different vascular systems would facilitate counter-current ion exchange. Could you elaborate on this?
Authors: The function of the central venous sinus-accessory companion vessel (CVS-ACV) network is not known. It is thought that the blood flow in the adjacent ACV and afferent filamental artery (AFA) are in opposite directions, possibly providing a potential site for counter-current ion exchange. This exchange, if present, would be in the area of chloride cells, which are thought to be responsible for osmoregulation.

K.R. Olson: There has been considerable speculation about the ability of the basal channel to act as a thoroughfare channel because in many species this pathway is not enlarged all the way across the lamellae. From your Fig. 6 it appears that this channel is also reduced toward the efferent end of the lamella. Do you think it can act as a "shunt" or "preferential" channel?
Authors: During casting we observed that both the marginal vessel and the basal channel filled before the respiratory network. Both are continuous channels, but as you correctly observed, the basal channel occasionally narrows on the efferent side. This could indicate that the marginal channel which does not appear to contain such narrowings is the "preferential" channel.

D. Schraufnagel: How does the gill vasculature of the striped bass compare to fish which tolerate more and less salinity?
Authors: To date we are not aware of any differences between the vasculature of freshwater and seawater adapted fish. The differences seem to be species specific and not related to salinity. We are however, continuing to look at the gill vasculature of striped bass adapted to different salinities (see discussion).

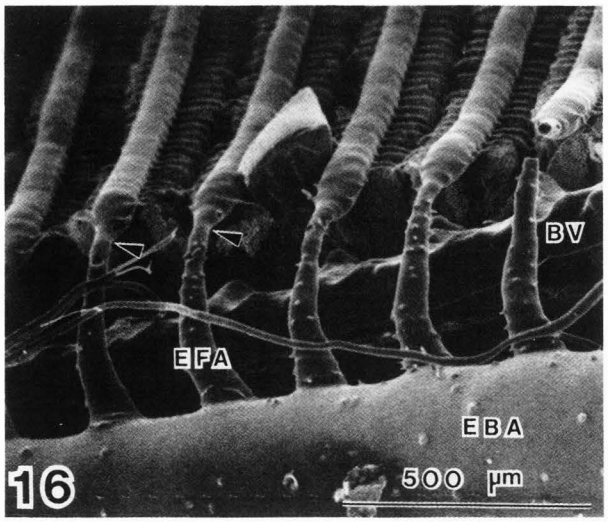

Fig. 16 Cast of the efferent filamental artery (EFA) and the efferent branchial artery (EBA). Arrowheads, possible sites of sphincters.

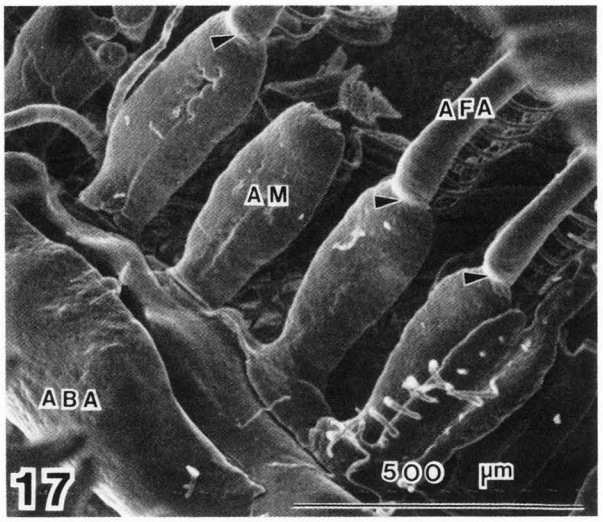

Fig. 17 Cast of the ampulla (AM) of the afferent filamental artery (AFA). Note the narrow junctions of the AMs with the afferent branchial artery (ABA), and the constrictions (arrowheads) in the AFAs.

K.R. Olson: The osmoregulatory "work" that many fish must perform while in freshwater depends on the concentration of calcium in the water. Did you measure ambient calcium? Is it possible that this could account for the lack of any differences between the freshwater and saltwater adapted fish?

Authors: Ion concentrations in the laboratory tap water as reported by Culligan of the Tri-Cities, Inc. (Blountville, Tennessee) vary from day to day but on the average are as follows: calcium 70 mg/L; magnesium 27 mg/L; sodium 62 mg/L; sulphate 68 mg/L; bicarbonate alkalinity 68 mg/L; silica 9.1 mg/L; iron 0.04 mg/L; manganese 0.01 mg/L; copper 0.03 mg/L; zinc 0.27 mg/L; pH 7.3. The calcium level fluctuates somewhat due to the water source. The 3% saltwater was prepared by using Instant Ocean Salts (w/v; Aquarium Systems, Mentor, Ohio). The calcium hardness of the hatchery water at the Southeastern Fish Cultural Laboratory (Marion, Alabama) was 81.1 p.p.m.. Yes, it is possible that the ambient calcium could account for the lack of any differences between the freshwater and saltwater adapted fish, but that has yet to be determined.

K.R. Olson: Most of the nutrient circulation in the medial "afferent" border of the filament goes to the adductor muscles. These are found near the EFA but the muscles attach to the contralateral hemibranch. The abductor muscles usually are quite small. Could the vessels shown in Fig. 13 be the capillary-venous vessels of the adductors?

Authors: Groman (1982) states that the abductor muscles are located "along the outer lateral gill arch between the base of the gill ray and the bone of the gill arch in bony fish like striped bass. The paired adductor muscles,

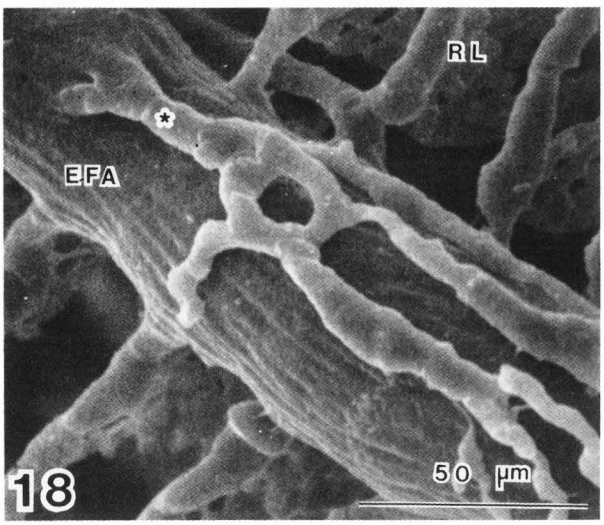

Fig. 18 Cast of the efferent filamental artery (EFA). Note nutritional vessels (asterisk) arising from the EFA and anastomosing with each other.

however, are located between hemibranches and cross over each other." Hughes (1984) seems to support this view. The nutritional vessels described in striped bass connecting the EFA and the EBA are located only around the proximal part of the filament on the lateral (EFA) aspect and do not extend to the area between the hemibranches.

P. Laurent: You do not give any interpretation concerning the enlargements visible on ALA (Fig.

8). Have you seen any peculiarity on the EFA's close to their junction with the EBA (efferent filamental artery sphincters)?
Authors: The apparent ALA enlargements (Fig. 8) at the bifurcation of the marginal vessel and basal channel may result from sphincters just proximal to them. Similarly, constrictions are present in the casts of the efferent filamental arteries just proximal to the first respiratory lamellae (Fig. 16), and in the casts of the afferent filamental arteries just distal to their ampullae (Fig. 17). These constrictions could represent the sites of sphincters, as you suggest, which regulate the flow of blood to and from the filaments.

K.R. Olson : In some fish the nutritional vessels that arise from the efferent filamental arteries (Figs. 14, 15) anastomose and one or two branches re-enter the filament to form the arterial supply for the filamental nutrient vessels. Did you observe this in any of your presentations ?

Authors: Yes, nutritional vessels from the efferent filamental artery (EFA) anastomose with each other and with efferent lamellar arterioles and supply adjacent regions of the filament (Fig. 18). In addition, nutritional vessels from the proximal end of the EFA joined with nutritional vessels from the efferent branchial artery. Most of the nutrient supply to the filament seems to come from these anastomoses (Fig. 14).