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Adaptation of Striped Bass to Sea Water Following Direct Transfer from Freshwater: Morphological, Biochemical, and Physiological Parameters

Judy A. King
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ADAPTATION OF STRIPED BASS TO SEA WATER FOLLOWING DIRECT TRANSFER FROM FRESHWATER: MORPHOLOGICAL, BIOCHEMICAL, AND PHYSIOLOGICAL PARAMETERS

A Dissertation Presented to the Faculty and Department of Anatomy

Quillen-Dishner College of Medicine

East Tennessee State University

In Partial Fulfillment

of the Requirements for the Degree of

Doctor of Philosophy

Judy Ann Curtis King

May, 1987

APPROVAL

This is to certify that the Graduate Committee of

JUDY ANN CURTIS KING

met on the

Fifth day of December, 1986.

The Committee read and examined her dissertation, supervised her defense of it in an oral examination, and decided to recommend that her study be submitted to the Graduate Council and the Associate Vice President for Research and the Dean of the Graduate School in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biomedical Science.

Fred E. Hossler
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Signed on behalf of
the Graduate Council

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Associate Vice President for
Research and Dean of the
Graduate School

ABSTRACT

ADAPTATION OF STRIPED BASS TO SEA WATER FOLLOWING DIRECT TRANSFER FROM FRESHWATER: MORPHOLOGICAL, BIOCHEMICAL, AND PHYSIOLOGICAL PARAMETERS

by

Judy Ann Curtis King

There has been heightened interest in the biology of striped bass (Morone saxatilis) because of increased pollution in their native spawning grounds and because of their extensive use in landlocked sport fisheries. Their euryhalinity makes them an excellent species for osmoregulation studies. The objective of this research was to study the rate of adaptation of striped bass gills to sea water (3% salt) after direct transfer from freshwater using biochemical (ion transport enzyme levels), physiological (chloride efflux), and ultrastructural methods.

Striped bass have specialized osmoregulatory cells located on the interlamellar and afferent surfaces of their gill filaments as shown by light microscopy (LM), transmission electron microscopy (TEM), and scanning electron microscopy (SEM). SEM studies show that apical pit (opening of one or more chloride cells) morphology changes during sea water adaptation, and the number of apical pits increases by 32.5% after two weeks in sea water. Chloride cell size and number, extent of basolateral tubular system, and number of mitochondria per chloride cell appear to increase upon adaptation to sea water. Autoradiographic studies using tritiated thymidine indicate that the nondifferentiated basal epithelial cells may be precursors for chloride cells.

Sodium-potassium adenosine triphosphatase (Na,K-ATPase) activity is maximal on day 3 after transfer to sea water. However, measurements of the binding of ouabain, an inhibitor that combines 1:1 with the Na,K-ATPase complex, could not demonstrate that the number of Na,K-ATPase complexes changed during the first 24 h after transfer to sea water. Studies suggest that cortisol may act as a hormonal mediator for long term adaptation to sea water. Salt extrusion measured by ^{36}Cl efflux begins to increase 3-6 h after transfer and increases gradually to become 26 times the freshwater level on day 7. Serum osmolality increases within 3-6 h after transfer and remains elevated throughout the first week.

The general morphology of both freshwater and sea water adapted fish gills were studied using LM, SEM, and TEM. Sonication was used to break away the epithelia of the gill arches and filaments in order to expose the taste buds and basement membranes. Preliminary studies indicate that the osmium-dimethylsulfoxide-osmium method can be used to investigate intracellular structural changes in striped bass gills using SEM.

Since the chloride cells are associated with the afferent surface of the filament, the blood supply to that area is also of great interest in osmoregulation studies. Studies of the gill vasculature using corrosion casting (i.e. filling blood vessels with plastic resins) and SEM indicate that the blood vessel distribution in the striped bass gill is similar to that of other euryhaline species with arterio-arterial, arterio-venous, and nutritive pathways. Blood flow may be controlled at a variety of places by sphincters, shunts and cellular contraction. Correlation of these biochemical, physiological and anatomical measurements will aid in the understanding of the process of adaptation to sea water.

To Jimmy, Roy and Naoma

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I would like to thank Dr. Fred E. Hossler for his advice and guidance.

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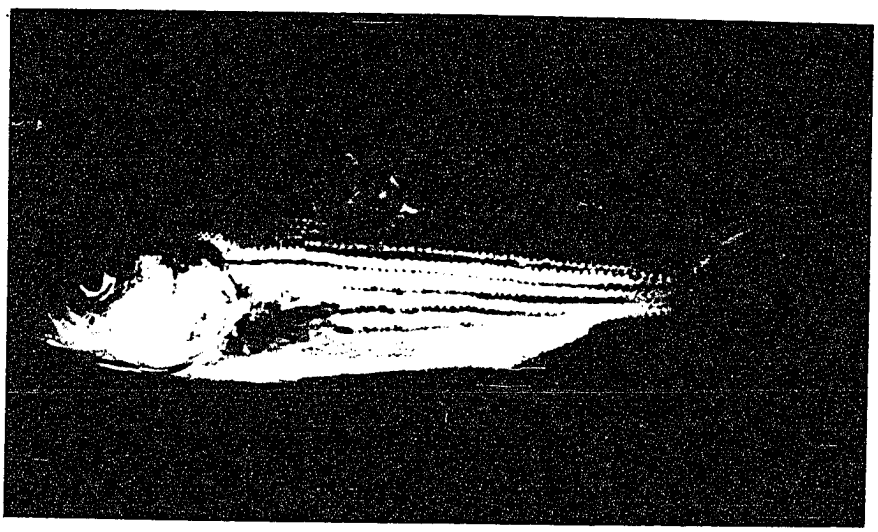
TABLE OF CONTENTS

LIST OF FIGURES



GENERAL INTRODUCTION

There has been increasing interest in the biology of the striped bass, Morone saxatilis, in recent years because of the value and extent of the sports fishery of this species in both marine (St. Laurence Seaway to Florida; Gulf of Mexico; and California coast) and freshwater environments (Raney, 1952; Tagatz, 1961; Nichols, 1966; Pfuderer et al., 1975; see Coutant 1986), and because of the recent decline in the numbers of this species as a result of pollution of these environments (Chittenden, 1971; Hazel et al., 1971; Pfuderer et al., 1975; Dawson, 1982; Hall et al., 1982, 1984; Mehrle, 1982; Wright et al., 1985). Reproducing populations have been established in the Kerr Reservoir (North Carolina), Millerton Lake (California), Santee-Cooper Reservoir (South Carolina) and probably in the Kentucky Lake (Kentucky-Tennessee; Nichols, 1966).

Because striped bass are an anadromous species and are known to tolerate abrupt changes in salinity (Tagatz, 1961; Otwell and Merriner, 1975; King, Hossler, and Harpole, unpublished observations) they provide a useful model system for the study of the rate of osmotic adaptation and the mechanism of osmoregulation.

Teleosts, or bony fish, can be divided into two groups - those that can survive only a small change in external salinity (the stenohaline teleosts), and those that can tolerate drastic changes in salinity (the euryhaline teleosts). No matter whether teleosts live in freshwater or sea water, they have severe osmotic problems. Water enters the gills of freshwater fish osmotically. To compensate, the kidneys increase salt absorption and excrete a dilute urine. The uptake of ions to replace those lost during excretion is thought to be the

responsibility of the gills. In sea water, fish drink to replace water lost osmotically by the gills. As a result salt intake is increased (see Smith, 1930; Black, 1957; Maetz, 1968, 1976; Berridge and Oschman, 1972; Prosser, 1973; Evans, 1984; Karnaky, 1986). Bivalent ions absorbed by the gut are excreted by the kidneys, while the monovalent ions (Na^+, Cl^-) are excreted primarily by the gills (Motais and Maetz, 1965; Prosser, 1973; Maetz, 1976; Girard and Payan, 1980). This extrarenal osmoregulatory mechanism is necessary since teleost kidneys cannot produce a concentrated urine (Threadgold and Houston, 1964; Foskett and Scheffey, 1982).

In 1930, Homer Smith found that sodium chloride is excreted by an extrarenal site in sea water fish. Keys (1931a,b) used a heart-gill perfusion preparation and discovered that chloride ions are excreted in the region of the gills. In 1932, Keys and Willmer examined the gills of sea water teleosts and found a cell resembling the acid-secreting cells in the stomach which they named the "chloride-secreting" cell. Since that time the "chloride cell" and its proposed osmoregulatory mechanisms have been the focus of many investigations (see reviews by Conte, 1969; Maetz, 1969, 1971; Evans, 1979; Karnaky, 1980; Lahlou, 1980; Degnan and Zadunaisky, 1982; Evans et al., 1982; Foskett et al., 1983; Degnan, 1984; Karnaky, 1986).

The gill epithelium of teleosts consists of four major cell types: (1) pavement ("respiratory"), (2) mucous, (3) non-differentiated, and (4) chloride (Karnaky and Kinter, 1977). Pavement cells (Copeland, 1948) are flat squamous cells which cover the entire surface of the gill except for openings for mucous and chloride cells. The nondifferentiated cells are basal epithelial cells which do not extend to the surface. A chloride cell can be easily identified as an eosinophilic columnar cell with a single nucleus, extensive basolateral plasma

membrane tubular system, numerous mitochondria, and an opening to the surface called an apical crypt. An apical crypt of a freshwater teleost exhibits cellular extensions visible between the pavement cells (Hossler et al., 1979b, 1985; Hossler, 1980). Crypts from freshwater species are generally considered to be the openings of single chloride cells since multicellular groups do not exist (Sardet, 1980; Sardet et al., 1980). Chloride cells have also been reported in opercular (Karnaky and Kinter, 1977; Karnaky et al., 1984) and buccal epithelia (Karnaky et al., 1984). Cells referred to as "accessory" or "adjacent" cells have been reported in the gill epithelium (Hootman and Philpott, 1979, 1980; Sardet et al., 1979; Dunel-Erb and Laurent, 1980; Laurent and Dunel, 1980). They resemble chloride cells, but differ in electron density and size (Hootman and Philpott, 1980; Laurent and Dunel, 1980), and in the lack of sodium-potassium adenosine triphosphatase (Na,K-ATPase) on their tubular system (Hootman and Philpott, 1980). Accessory cells are generally observed only in sea water adapted species (Hootman and Philpott, 1980; Laurent and Dunel 1980). Some authors feel that accessory cells are young chloride cells (Sardet et al., 1979; Hootman and Philpott, 1980), while others feel that they are a totally different cell type (Laurent and Dunel, 1980; Chretien and Pisam, 1986).

Extensive studies of chloride cell ultrastructure have been completed (Doyle and Gorecki, 1961; Kessel and Beams, 1962; Philpott and Copeland, 1963; Straus, 1963; Threadgold and Houston, 1964; Philpott, 1965; Oberg, 1967; Shirai and Utida, 1970; Olson and Fromm, 1973; Laurent and Dunel, 1980). Reported changes in chloride cell ultrastructure during adaptation to higher salinities include increased numbers of mitochondria (Maetz and Bornancin, 1975; Karnaky et al., 1976a; and see reviews on chloride cells listed above) and an elaboration of the basolateral plasma membrane tubular system (Shirai and Utida, 1970; Doyle and Epstein, 1972; Karnaky et al., 1976a,b; Philpott, 1980; Pisam, 1981).

As adaptation occurs, the apical crypt takes on a deeper "pit" structure that has few if any visible cellular extensions and often is shared by several chloride cells (Bierther, 1970; Maetz and Bornancin, 1975; Hossler et al., 1979b, 1985; Hossler, 1980). Increased numbers of chloride cells (Jozuka, 1966; Conte and Lin, 1967; Newstead, 1967; Olivereau, 1970; Shirai and Utida, 1970; Utida et al., 1971; Doyle and Epstein, 1972; Sargent et al., 1975; Karnaky et al., 1976b; Thomson and Sargent, 1977; Hootman and Philpott, 1980), and chloride cell hypertrophy (Liu, 1942; Utida et al., 1971; Karnaky et al., 1976 a,b; Hootman and Philpott, 1979, 1980; Foskett et al., 1981) have also been reported during acclimation to sea water. The increase in chloride cell numbers, the elaboration of the basolateral tubular system where transport could occur, the increased number of mitochondria which supply the energy for transport, and the localization of chloride in the chloride cell apical pit region (Copeland, 1948; Philpott, 1965; Petrik, 1968; Bierther, 1970; Masoni and Garcia Romeu, 1973) support the hypothesis that the chloride cell is responsible for sodium chloride excretion.

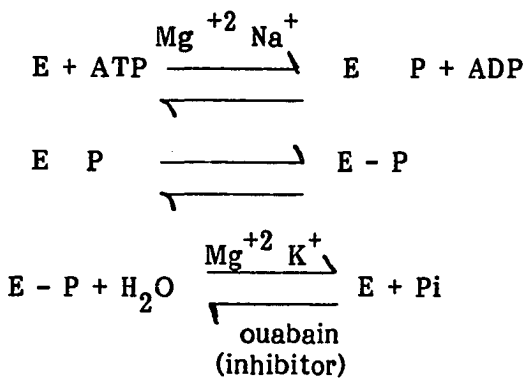
Ion transport models for chloride cells have been proposed by Maetz (1971) and Silva et al. (1977). According to Maetz's model, chloride and sodium from the plasma are exchanged for bicarbonate and hydrogen ions, respectively, from the interior of the chloride cell. Chloride diffuses passively across the apex of the chloride cell. A sodium-potassium exchange system accounts for the efflux of sodium. According to the model proposed by Silva et al. (1977), the primary transport is accomplished by the Na,K-ATPase which creates a sodium gradient in the tubular system. It is known that there are higher concentrations of sodium in the tubular lumen than in the chloride cell cytoplasm (Frizzel et al., 1979). The secondary transport involves the movement of chloride into the cell via a sodium chloride cotransport driven by

the sodium gradient. Chloride then travels to the apex of the cell where it enters the crypt lumen by electrical forces. Sodium enters the crypt lumen by way of the junctions between the chloride cells, driven by the gradient (Epstein et al., 1980). Sardet et al., (1979) suggest that when fish move from freshwater to sea water, the junctions between chloride cells change from tight to leaky to allow passive sodium extrusion. Ion flux studies under short circuit current conditions imply that chloride transport is active while sodium transport is not (Degnan et al., 1977; Karnaky et al., 1977; Marshall and Bern, 1980; Foskett et al., 1981; Foskett and Scheffey, 1982). Using x-ray emission and analysis, Potts and Oates (1983) found that the intracellular concentrations of sodium, potassium, chloride, and phosphate ions in the chloride cells of Fundulus heteroclitus are consistent with Silva's model (Silva et al., 1977).

Chloride cell-pavement cell and accessory cell-pavement cell junctions are deep elaborate "tight" junctions composed of five or more strands. Chloride cell-chloride cell and chloride cell-accessory cell junctions are shallow and are composed of one to two strands (Philpott and Copeland, 1963; Bierther, 1970; Shirai and Utida, 1970; Morgan and Tovell, 1973; Karnaky and Kinter, 1977; Ernst et al., 1978; 1980; Sardet et al., 1979; Dunel-Erb and Laurent, 1980; Hootman and Philpott, 1980; Laurent and Dunel, 1980; Sardet, 1980). The only junctions which appear to undergo modifications with salinity changes are the junctions between adjacent chloride cells or between chloride cells and accessory cells (see Karnaky, 1986).

Na,K-ATPase and its inhibition by ouabain, a cardiac glycoside, were discovered by Skou (1957, 1960) while studying crab nerves. Na,K-ATPase is thought to be present in most cells (see reviews by Schwartz et al., 1975; Skou and Norby, 1979) and is responsible for maintaining the high potassium, low

sodium content of cells by transporting three sodium ions out of the cell for every two potassium ions it transports in (Sen and Post, 1964). The proposed mechanism of ion transport is as follows (Bonting, 1970; see Schwartz et al., 1975):



Ouabain inhibits from the serosal (blood) side of chloride cells (Epstein et al., 1980) by non-competitive inhibition with potassium (Bonting, 1970; Hansen, 1984). In the presence of potassium and magnesium, Na,K-ATPase can also hydrolyze p-nitrophenylphosphate (PNPP) but at about 1/6 to 1/10 the rate of ATP (Skou, 1975). Magnesium adenosine triphosphatase (Mg-ATPase) and other ATPases are not inhibited by ouabain (Bonting, 1970).

In order to find out which cell type in the gill contained Na,K-ATPase, Kamiya (1972b) dissociated the cells of eel gill filaments with elastase and separated the cells with dextran density gradient centrifugation. The chloride cell fraction was the one rich in Na,K-ATPase. Mizuhira et al. (1970), Sargent et al. (1975), Hootman and Philpott (1978), and Naon and Mayer-Gostan (1983) have verified that Na,K-ATPase activity is located in chloride cells. Karnaky et al. (1976a) used autoradiography (^{3}H-ouabain) to show that the chloride cells

of killifish are the primary site of Na,K-ATPase. Mizuhira et al. (1970), Shirai (1972), Karnaky et al. (1976b), Silva et al. (1977), and Hootman and Philpott (1979) showed that the Na,K-ATPase sites were located on the basolateral cell surface, including the tubular system. No Na,K-ATPase sites were found on the apical crypt. Ouabain, an inhibitor of Na,K-ATPase, binds to the large subunit of Na,K-ATPase on a 1:1 basis (Jorgensen, 1974), and thus is often used in Na,K-ATPase quantitation studies (Hossler, 1980). By using ouabain binding, Karnaky et al. (1976b) determined that there are 1.5×10^8 Na,K-ATPase sites on each chloride cell. The ouabain binding capacity of sea water fish is 3.2 to 6 times higher than that of freshwater animals (Sargent and Thomson, 1974; Hossler, 1980). Utida et al. (1971) showed a correlation beween Na,K-ATPase activity and the increased number of chloride cells upon adaptation of eels to sea water. Na,K-ATPase activity in gill homogenate is calculated as the difference between the amount of inorganic phosphate released from ATP in the presence of Na^+,K^+, and Mg^{+2} (experimental assays) and that released in the presence of Mg^{+2} plus ouabain (control assays). Many studies have shown that gill Na,K-ATPase activity increases as much as two to six fold when fish are exposed to various concentrations of sea water (Utida et al., 1966, 1971; Epstein et al., 1967; Jampol and Epstein, 1968, 1970; Kamiya and Utida, 1968, 1969; Shirai and Utida, 1970; Zaugg and McLain, 1970; Pfeiler and Kirschner, 1972; Evans et al., 1973; Forrest et al., 1973a; Greenwald et al., 1974; Kirschner et al., 1974; McCormick and Naiman, 1984; Sargent and Thomson, 1974; Evans and Mallery, 1975; Sargent et al., 1975; Karnaky et al.,

1976a,b; Thomson and Sargent, 1977; Hootman and Philpott, 1979; Hossler et al., 1979b; Epstein et al., 1980; Abo Hegab and Hanke, 1986; see reviews by Kirschner, 1969, 1980), but exceptions where the enzyme does not increase have been recorded (Lasserre, 1971; Gallis and Bourdichon, 1976; Gallis et al., 1979; Doneen, 1981).

Most studies of striped bass to date have dealt with culture conditions, growth rates, and distribution (see bibliography by Pfuderer et al., 1975; and reviews by Kerby et al., 1983, and Geiger and Parker, 1985), but very few reports have dealt with the physiology, biochemistry, and anatomy (Groman, 1982) of this species. The purpose of this research was to study the rate of adaptation of striped bass gills to sea water following direct transfer from freshwater using biochemical, physiological, and ultrastructural methods. Although adaptation studies have been reported using various percentages of sea water compared to freshwater, very few studies of adaptation rates have been conducted (Bornancin and De Renzis, 1972; Forrest et al., 1973a,b; Evans and Mallery, 1975; Boeuf et al., 1978; Jacob and Taylor, 1983) and none of these have dealt with striped bass. The results of the sea water adaptation studies are presented in the following chapters which include descriptions of gill morphology, chloride cell ultrastructure, and gill vasculature; and measurements of changes in Na,K-ATPase content and activity, plasma osmolality, chloride efflux, plasma osmolality, plasma chloride, and plasma corticosteroids.

CHAPTER 1

STRIPED BASS GILL ULTRASTRUCTURE: RATE OF RESPONSE TO SALINITY CHANGE

Introduction

Because striped bass are euryhaline teleosts, they must have a mechanism for osmoregulation to survive changes in salinity. It is thought that the sodium and chloride taken in by sea water teleosts are excreted by the gills (Prosser, 1973), and that the chloride cell is responsible for the excretion of these ions (Keys and Willmer, 1932; Motais and Garcia Romeu, 1972; Maetz and Bornancin, 1975).

Conte (1965) found that when cell division is inhibited by X-irradiation, fish are unable to osmoregulate in sea water. Increased salinity is known to cause an increase in DNA synthesis (Conte and Lin, 1967; Tondeur and Sargent, 1979) and thus cell turnover and movements (Conte and Newstead, 1965). The number and size of chloride cells, the number of mitochondria, and the volume of the basolateral plasma membrane tubular system increase during acclimation of euryhaline teleosts to sea water (Maetz and Bornancin, 1975; see General Introduction for a review of literature). Chloride cell apical crypts also undergo alteration during acclimation to sea water (Bierther, 1970; Maetz and Bornancin, 1975; Hossler et al., 1979b,d, 1985; Hossler, 1980; and see General Introduction).

As part of the overall study of the mechanism of osmoregulation by striped bass gills the following chapter includes (1) a description of the surface ultrastructure of the gill arches of the striped bass, and (2) details of the morphological changes of chloride cells and their apical crypts during acclimation to sea water. Gill arch morphology and chloride cell ultrastructure are examined with light microscopy (LM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). The results of preliminary work on striped bass gills with a technique known as the osmium-DMSO-osmium method (Tanaka, 1981) are described. This relatively new procedure has been used successfully in other tissues (Tanaka, 1981; Tanaka and Naguro, 1981; Osatake et al., 1985; Fukudome and Tanaka, 1986) to reveal intracellular structures in three-dimensional form with SEM instead of the traditional two-dimensional TEM sections. Preliminary studies of epithelial cell renewal in striped bass gill filaments using ^{3}H-thymidine are also reported.

Partial accounts of this study have been published (Hossler et al., 1986b; see Appendix).

Materials and Methods

Striped bass, Morone saxatilis, 3-25 cm in length were donated by the 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 100 L tanks with aerated, hatchery-aged tap water (0.011 Osm; "freshwater") or sea water (3% salt water, w/v; Instant Ocean Salts, Aquarium Systems,

Mentor, Ohio; specific gravity 1.02; 1.01 Osm) at room temperature (20-24°C) with a cycle of approximately 11 h subdued light and 13 h dark. The fish were fed trout chow (Silver Cup Feed, Murray Elevators, Murray, Utah) ad libitum for at least one week before experimentation. For acclimation studies, fish were transferred directly from freshwater (FW) to 3% salt water and sacrificed at designated times (45 min, 3 h, 6 h, 12 h, 24 h, 3 days, 7 days, or 14 days).

Scanning Electron Microscopy

Fish were killed by decapitation, the opercula were spread laterally, and each gill arch was carefully removed, rinsed free of blood with 0.9% NaCl and placed in freshly prepared fixative for 2 h at 20-24°C or overnight at 4°C. Fixative consisted of 2.5% glutaraldehyde and 1.8% paraformaldehyde in 0.1 M cacodylate-HCl buffer (pH 7.2). After fixation gill arches were rinsed for 1 h with 3 changes of excess buffer (0.1 M cacodylate-HCl, pH 7.2), then post-fixed for 2 h at 4°C in 2% OsO_4 in 0.1 M cacodylate-HCl (pH 7.2). Gill arches were rinsed in buffer as before, then dehydrated in a graded ethanol series during a period of 1-2 h. Gill arches were placed in a critical point drying apparatus (Model E3000, Polaron Instruments, Inc., Hatfield, PA) in 100% ethanol, dried with liquid CO_2, affixed to specimen stubs with silver paste, coated with a thin layer of gold or gold-palladium in a sputter coating apparatus (Model Desk 1, Denton Vacuum Inc., Cherry Hill, NJ), and observed in a scanning electron microscope (Model S430, Hitachi Scientific Instruments, Mountview, CA; Model JSM-35C, JEOL (U.S.A.) Inc., Peabody, MA). Measurements were made from electron micrographs.

Light Microscopy

For light microscopy, gills were fixed as described previously, dehydrated first in a graded ethanol series, followed by propylene oxide, and embedded in epon-araldite (Mollenhauer, 1964). Sections (2 µm) were cut with an ultramicrotome (Ultracut, American Optical Instruments, M.O.C. Inc., Valley Cottage, N.Y.), mounted on glass slides, stained with toluidine blue (1% in 1% Na-borate), and viewed and photographed with a Zeiss standard light microscope.

Transmission Electron Microscopy

For transmission electron microscopy, gills were processed and embedded in epon-araldite as described for light microscopic specimens. Thin sections were cut with glass knives, stained with uranyl acetate and lead citrate, and viewed in a Hitachi H-500 transmission electron microscope (Hitachi Scientific Instruments, Mountain View, CA). Measurements were made from electron micrographs.

Osmium-DMSO-Osmium

This procedure has been reported previously by Tanaka and Naguro (1981). The gills were rinsed in excess 67 mM phosphate buffer (pH 7.4) and fixed at 4°C for 1-2 h in 1% OsO_4 buffered with phosphate (67 mM, pH 7.4). After rinsing with the buffer solution, the tissue was immersed in 15, 30, and 50% DMSO (dimethylsulfoxide) for 30 min each. The specimens were frozen on a SEM stub chilled with liquid nitrogen and cracked with a razor blade. The specimens were placed in 50% DMSO to allow thawing, and then rinsed again in buffer solution (3 changes). The tissue was placed in 0.1% OsO_4 buffered with 67 mM phosphate buffer (pH 7.4) at 4°C for 24-72 h. After washing in the

buffer solution, the specimens were fixed in the buffered OsO_4 for 1 h, dehydrated in a graded ethanol series, dried with liquid CO_2, affixed to stubs with silver paste, coated with a thin layer of gold or gold-palladium in a sputter coater and observed in a scanning electron microscope. Measurements were made from electron micrographs.

Autoradiography

This autoradiographic procedure was adapted from Rasch et al. (1982). After sacrifice, the gills and gut were removed, rinsed in a wash solution containing 0.65% NaCl with antibiotic-antimycotic (Penicillin, 10,000 units/ml.; Fungizone, 25 mcg/ml; Streptomycin, 10,000 mcg/ml; Gibco Laboratories, Grand Island Biological Company, Grand Island, NY), and placed in the wash solution enriched with 5 µCi/ml ^{3}H-thymidine (New England Nuclear) for 5 h at room temperature. The specimens were rinsed again in the wash solution, fixed in ethanol-acetic acid (3:1) overnight, dehydrated, and embedded in paraffin. Sections were cut (5 µm thick) and mounted on glass slides. Following paraffin removal, the slides were coated with Ilford K-5 emulsion (diluted 1:4, w/v with water), held for 6 weeks at 4°C, and then developed at 15°C for 3½ min in Kodak D-19 and cleared for 5 min in Kodak fixer. Using replicate slides of gill and gut sections as controls for non-specific isotope incorporation, deoxyribonucleic acid (DNA) was extracted in 5% trichloroacetic acid for 15 min at 90°C prior to coating the slide with emulsion. The slides were stained with hematoxylin and eosin and photographed with a Zeiss standard light microscope.

Counts of Chloride Cell Apical Pits

Apical pits of chloride cells were counted on filaments in the middle third of gill arch II on the right side of the fish. In each case an area on the afferent surface next to the respiratory lamellae (not including the interlamellar regions) was chosen. One 400 μm^2 area was counted on each of 10 filaments, five from each hemibranch, and the 10 values were then averaged. The data was evaluated by analysis of variance ($p < 0.05$) for overall significance. Comparisons between experimental groups and control groups [freshwater (FW) or 0 h in sea water] were evaluated by Dunnett's test ($p < 0.025$; Winer, 1971).

Chloride Cell Counts

Chloride cells were counted on 20 filament cross-sections, 10 from each hemibranch of both a freshwater (0 h) fish and a 7-day sea water fish. The numbers were then averaged. Each cross-section contained at least three pairs of respiratory lamellae.

Results

Striped bass are especially subject to shock during transport and handling. The mortality rate is usually reduced by covering tanks and adding up to 0.1% sea salts to the water during transport (Parker and Geiger, 1984), and by maintaining fish in tanks partially covered with translucent shields or cardboard and located in low-traffic, low-light areas of the laboratory (Hossler et al., 1986b; King and Hossler, 1986).

General Morphology

Gill arch structure is similar to that of other teleosts (see Hughes, 1984; Laurent, 1984). Two rows of filaments (anterior and posterior hemibranches) extend posterolaterally and two rows of rakers extend anteromedially from each of the four arches, designated I, II, III and IV, rostral to caudal (Figs. I-1, I-2, and I-3). Both rows of filaments curve slightly posteriorly in vivo, and are free for most of their length (i.e., are perciform type teleost gills; Dornesco and Miscalenco, 1968a). Filament length varies with different gill arches and with different locations on a given arch. Filaments of the posterior hemibranch are longer than those of the anterior hemibranch on the dorsal aspects of arches I and II. Filaments of the anterior hemibranch are longer than those of the posterior hemibranch on the ventral aspects of arches II, III and IV. Elsewhere the filaments of each hemibranch are similar in length.

Filaments in the two hemibranchs of the same arch are not paired but rather alternate and interdigitate somewhat basally. Distally, the tips of filaments of one hemibranch lie close to those of the adjacent hemibranch on the next arch. Similarly, the pharyngeal surfaces of adjacent arches are closely aligned with each other such that the rakers of one arch could mesh with those of an adjacent arch to form a seal between the pharyngeal and gill cavities. Both the length and number of filaments and rakers per arch increase with the growth of the fish (see Hossler et al., 1986b).

Each filament supports two rows of triangular, leaflike, respiratory lamellae, one row on each side of the filament (Figs. I-3 and I-4a,b). In the

Fig. I-1 Lateral view of the right gill arches of a 20 cm striped bass. Arches are designated I, II, III and IV, rostral to caudal. The gills were photographed with a Leicaflex SL 35 mm camera with a 100 mm macro lens. R: rakers, F: filaments.

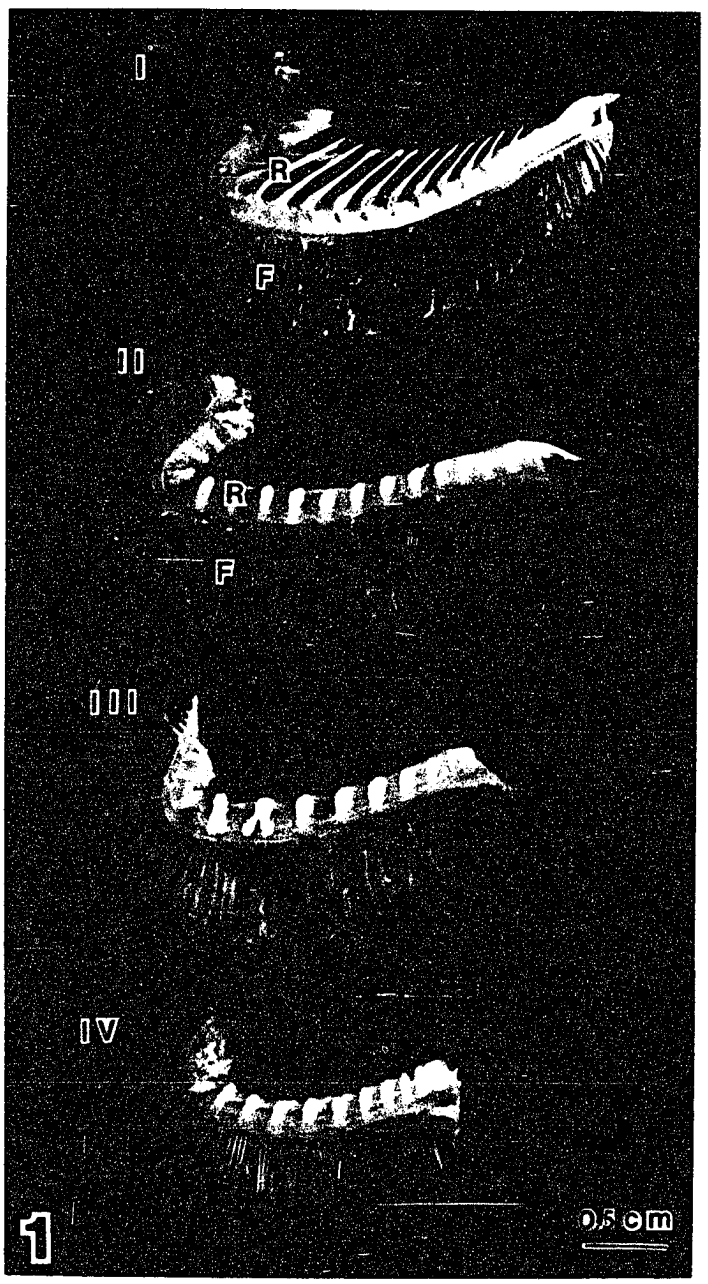
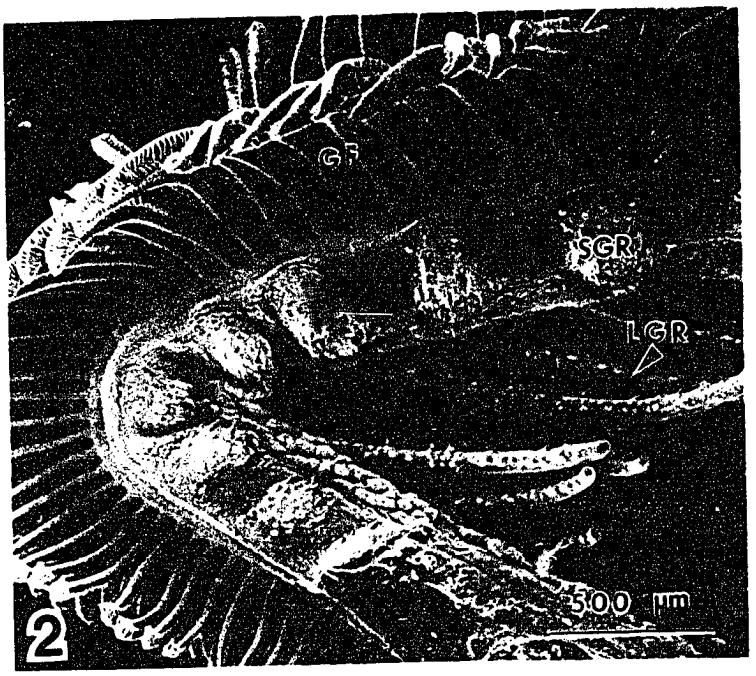
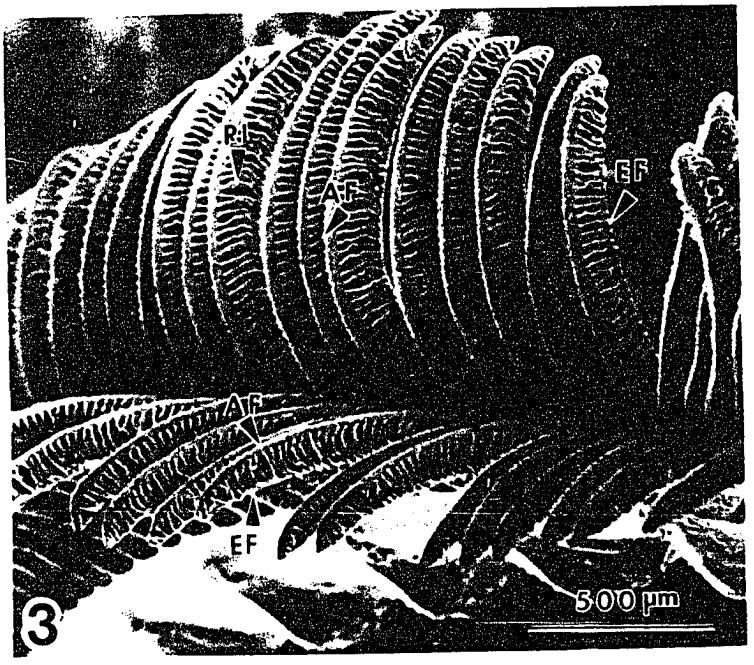


Fig. I-2 Medial view of gill arch I. SGR: short gill raker; LGR: long gill raker; GF: gill filament.

Fig. I-3 Structure of a holobranch. AF: afferent filament surface; EF: efferent filament surface; RL: respiratory lamellae.





center of gill arch I on a typical 10 cm fish, there are about 40-50 lamellae per mm on each side of a filament. In cross-section, filaments are rounded on their afferent surfaces but more flattened on their efferent surfaces. The respiratory lamellae conform to the shape of the filament, are present from its base to its apex, and greatly increase its surface area. Each of the triangular-shaped lamellae has its leading, free edge aligned with the efferent surface of the filament. Cross-sections of the respiratory lamellae reveal pillar cells within the complex vascular network (Fig. I-5a,b).

With few exceptions all surfaces of each gill arch are covered by a mosaic of flattened, polygonal pavement cells (Copeland, 1948) which exhibit concentrically-arranged microplicae (Andrews, 1975) on their surfaces (Figs. I-6 and I-7). Cell borders can usually be discerned by changes in the surface pattern or by prominent intercellular ridges. These cells measure 7.5 ± 2.7 μm in width and the surface microplicae measure 0.2 ± 0.3 μm in width. On the interlamellar surfaces, the surface microplicae are often discontinuous or replaced by short surface projections (Fig. I-6). On the respiratory lamellae, however, the pavement cells lack microplicae and have distinct cellular borders outlined by rows of short surface projections (Figs. I-4 a,b and I-6).

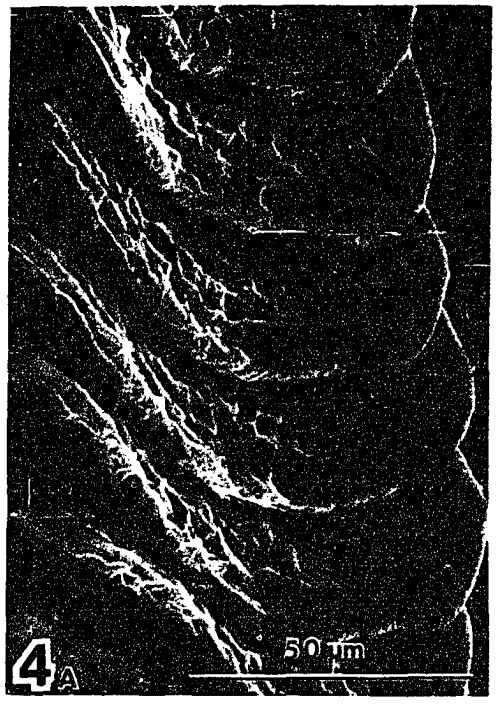
On the afferent surfaces of filaments, on the interlamellar surfaces, and around the bases of respiratory lamellae the pavement cell epithelium is interrupted by the apical crypts of chloride cells (Figs. I-6 and I-7). These apices open along the borders of adjacent pavement cells, measure 3.16 ± 1.05 μm in diameter, and consist of tufts of short, cellular projections (Fig. I-7). Chloride cell apices are not observed on the efferent filament surface, on the raker surfaces (Figs. I-8 to I-11), or on any other surface of the gill arch.

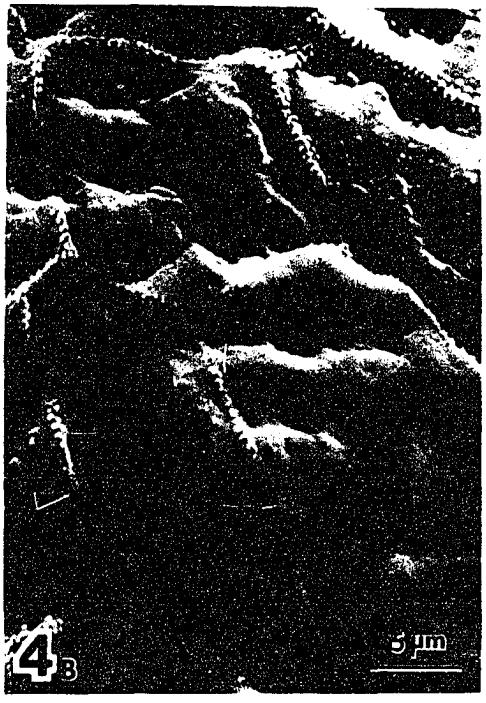
Fig. I-4a Scanning electron micrograph of respiratory lamellae.

Fig. I-4b Polygonal, smooth-surfaced pavement cells on respiratory lamella. Note the absence of microplicae, but the presence of short surface projections especially along intercellular borders (arrow).

Fig. I-5a Pillar cell and vascular network of respiratory lamella of specimen treated with osmium-DMSO-osmium. N: nucleus of pillar cell; V: vascular network of respiratory lamellae; PC: pavement cells have been pulled away at this site.

Fig. I-5b Transmission electron micrograph of pillar cell of respiratory lamella pillar cell. N: nucleus; PC: pavement cell; V: vascular network of respiratory lamella.





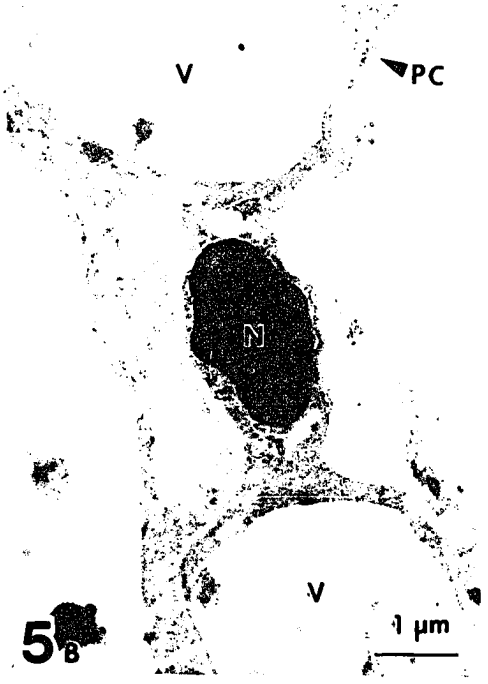
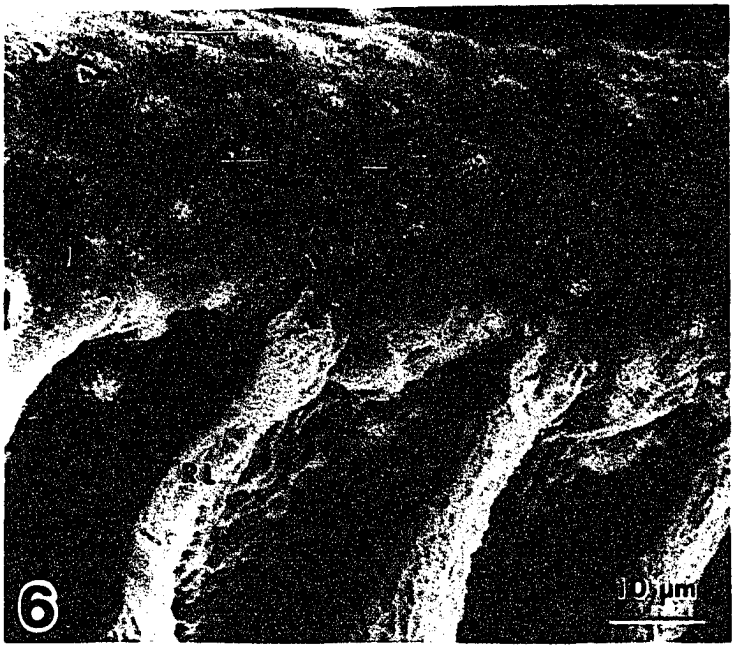
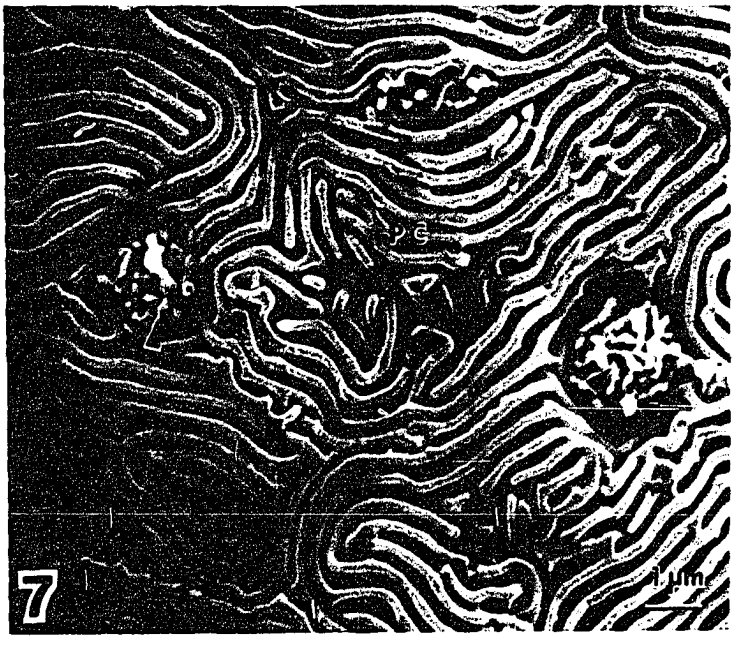


Fig I-6 Afferent and interlamellar surfaces of filament from a freshwater fish. CC: apical crypts of chloride cells; RL: respiratory lamella; PC: pavement cell.

Fig. I-7 Apical crypt of chloride cell from a freshwater fish. PC: pavement cell microplicae; arrow: apical extensions of the chloride cell.





The anterior row of rakers on gill arch I consists of long, finger-like appendages which span the slit between gill arch I and the operculum, and thus separate the pharyngeal cavity from the opercular cavity (Figs. I-2 and I-8). The anterior surface of the rakers is convex, but the posterior (pharyngeal) surface (Fig. I-10) is flattened and contains a single row of spines along each edge. The posterior row of rakers on gill arch I (Fig. I-8) and both rows of rakers on the remaining gill arches (Fig. I-9) are reduced to short, raised areas generously studded with spines (Fig. I-12).

Taste buds are observed in a single row along the center of the pharyngeal surface of each gill arch (Figs. I-8, I-9 and I-11), along the center of each of the long rakers (Fig. I-10), and among the spines of each of the short rakers (Figs. I-11 and I-13). Taste buds are located on raised areas of the epithelium (Figs. I-11 and I-13) and consist of tufts of large and small microvilli which project above the epithelial surface along the lateral borders of adjacent pavement cells. Taste buds usually contain about 9 4 large microvilli and 163 56 small microvilli, and measure about 3.06 1.1 μm in diameter.

Response to Salinity Change

Apical crypts of chloride cells of striped bass are observed only on the afferent and interlamellar surfaces of the filaments (Figs. I-14 to I-20). In FW (0 h in sea water) adapted fish, the chloride cell apical crypts have numerous short cellular extensions of similar length in their interiors (Figs. I-6, I-7, I-21, and I-22). At 3 h after direct transfer from freshwater to sea water, the cellular extensions become longer and more prominent (Figs. I-16 and I-17). The 6 h, 12 h, and 24 h specimens show progressively more variation in the length of the cellular extensions within the pits (Figs. I-18 and I-19). For example, one pit might exhibit some very long cellular extensions with bulbous tips resembling

Fig. I-8 Pharyngeal surface of gill arch I. Note the single row of taste buds along the center of the pharyngeal surface of the arch (arrows). SGR: short gill raker; LGR: long gill raker; GF: gill filament.

Fig. I-9 Pharyngeal surface of gill arch II. SGR: short gill rakers. Note the single row of taste buds along the center of the pharyngeal surface of the arch.

Fig. I-10 Pharyngeal surface of long raker of gill arch I. S: spine; arrows: raised areas of epithelium containing taste buds.

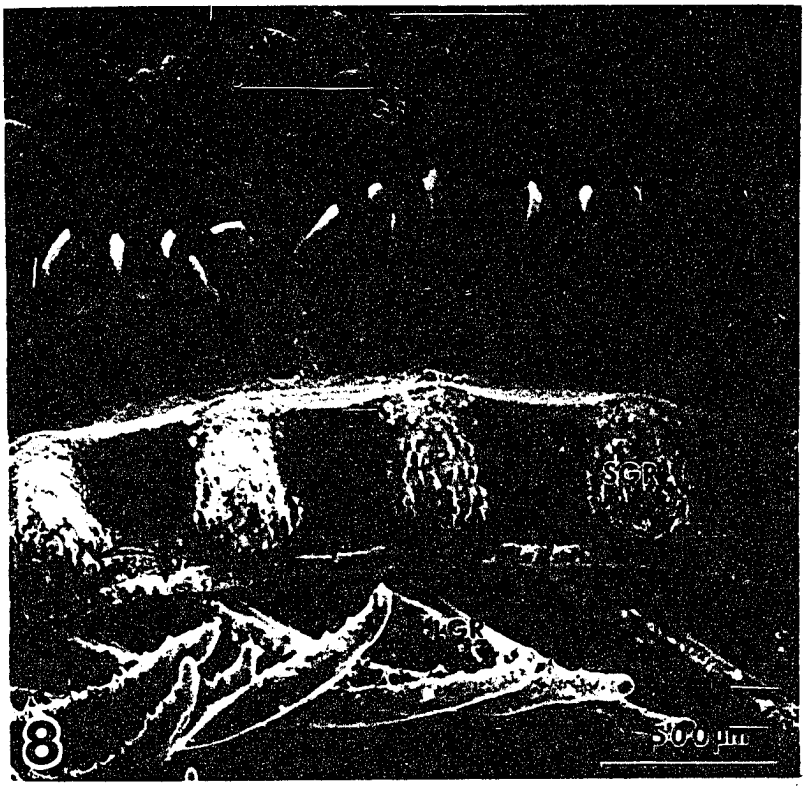


Fig. I-11 Short gill raker. Note raised areas of epithelium containing taste buds (TB) alternating with spined (S) epithelial projections.

Fig. I-12 Spine of short gill raker (S) with pavement cells (PC).

Fig. I-13 Light micrograph of a taste bud. A taste bud (TB) is characterized by dark and light cells on a raised area of the epithelium. PC: pavement cells; MC: mucous cells; CT: connective tissue core of raker.

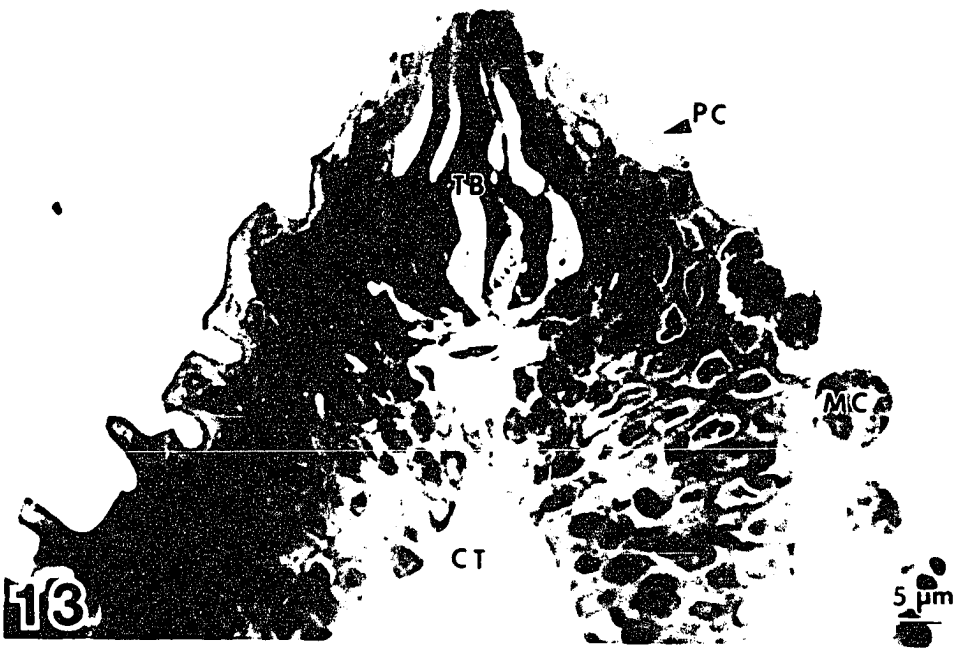


Fig. I-14 Cross-section of a gill filament from a freshwater-adapted fish. Note presence of clear, columnar chloride cells (arrows) on afferent (A) and interlamellar, but not efferent (E) filament surfaces. RL: respiratory lamellae.

Fig. I-15 Cross-section of the afferent surface of a gill filament from a fish acclimated to sea water for 7 days. Note the abundant chloride cells (arrows); RL: respiratory lamellae; MC: mucous cells.

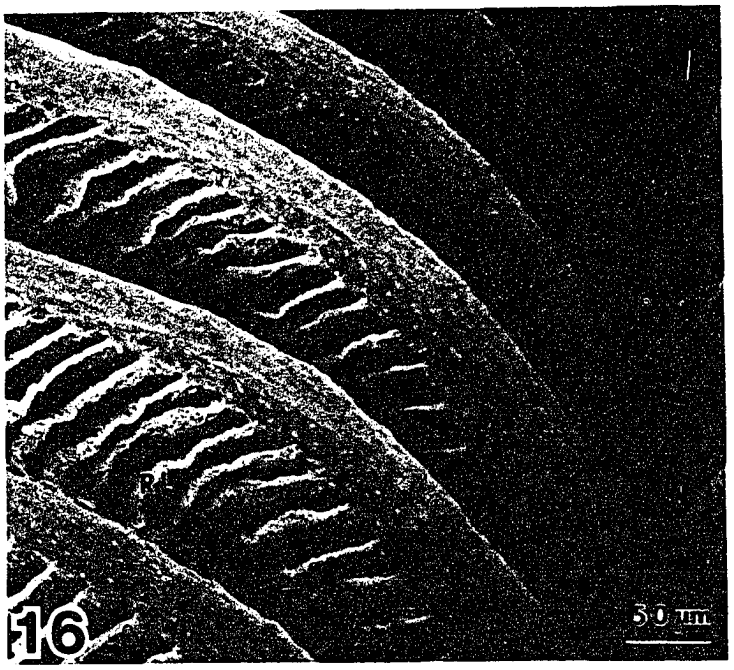
14

50 µm

15

Fig. I-16 Afferent surfaces of filaments from one hemibranch of a fish acclimated to sea water for 3 h. Arrows: apical crypts of chloride cells; RL: respiratory lamellae.

Fig. I-17 Afferent and interlamellar filamental surfaces of a fish acclimated to sea water for 3 h. Note that the cellular extensions of the chloride cell apical crypts are more obvious than those from freshwater fish (compare with Fig. I-6). RL: respiratory lamellae; CC: apical crypts of chloride cells.



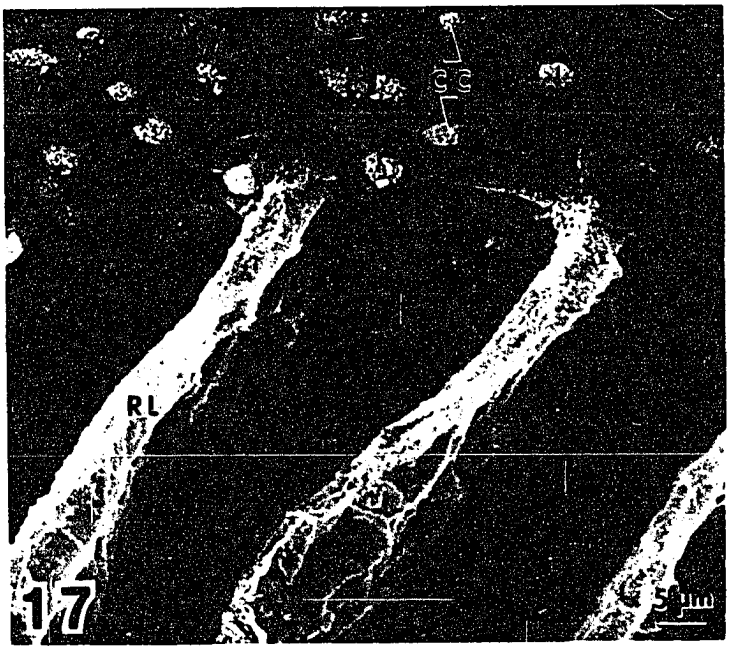
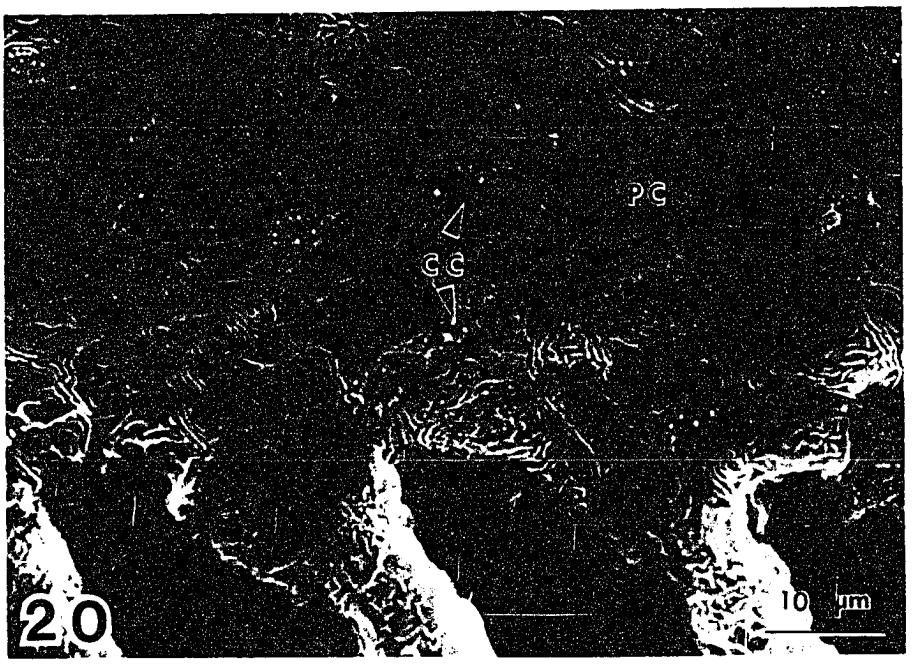


Fig. I-18 Apical crypt of chloride cell of a fish acclimated to sea water for 6 h. CC: apical crypts of chloride cell; PC: pavement cell.

Fig. I-19 Apical crypt of chloride cell of a fish acclimated to sea water for 12 h. Note the different lengths of the chloride cell (CC) extensions. PC: pavement cell.

Fig. I-20 Afferent and interlamellar filamental surfaces of a fish acclimated to sea water for 7 days. Note that some of the chloride cell apical crypts have visible extensions while others exhibit a "pit" structure with few or no visible extensions. PC: pavement cell; CC: apical crypts of chloride cells.



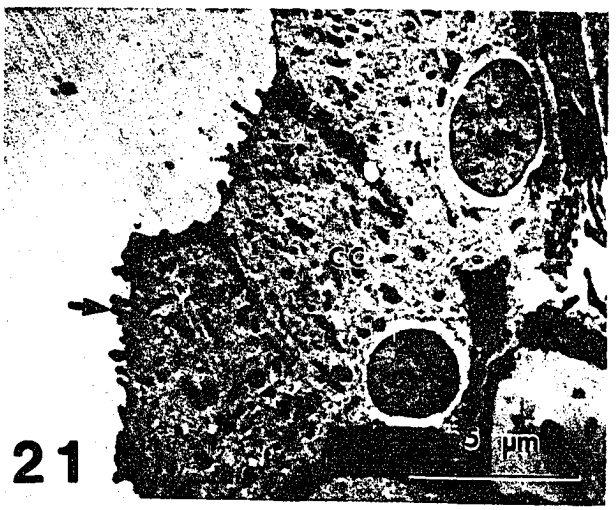
those of the 3 h specimens adjacent to short nub-like extensions resembling those of FW crypts (Fig. I-20). After 7 days in sea water, the crypts show a full range of morphological characteristics including the two extremes: shallow crypts with cellular extensions covering all of their interior, and deep pits with few or no cellular extensions visable (Figs. I-20, and I-23). The 14-day specimens resemble those of the 7-day fish. No change in the number of apical crypts is seen until 7 days of sea water adaptation and a significant increase in crypt number (32.5%) was not recorded until 14 days of adaptation (Fig. I-24). Chloride cells increase in number by 16.1% during the first 7 days in sea water. (The average number of chloride cells per cross-section of the freshwater fish was 14.6 as compared to 16.95 for the 7-day sea water fish).

Chloride cells of fish adapted to freshwater usually occur singly, do not share crypts, and have relatively few mitochondria (Figs. I-21 and I-22). Chloride cells of fish adapted to sea water for 7 days occur in groups, have more mitochondria, have a more elaborate basolateral plasma membrane tubular system, and often share apical crypts or pits (Fig. I-23). Cells resembling chloride cells but with increased cellular density are observed next to chloride cells, both in freshwater and in sea water, and are probably those that other authors have termed "accessory cells", or "adjacent cells" (Hootman and Philpott, 1980; Laurent and Dunel, 1980). Tight junctions are present at apical regions of pavement cell-chloride cell and chloride cell-chloride cell junctions (Figs. I-25 and I-26). The extent of the tight junctions could not be determined. Chloride cells thought to be in a stage of degeneration, with enlarged tubular system lumens and decreased numbers of mitochondria, are observed in fish acclimated to sea water for 7 days (Fig. I-27; Copeland, 1948). No changes in pavement or mucous cells were noted during acclimation to sea water.

Fig. I-21 Transmission electron micrograph of chloride cells (CC) from a freshwater-adapted fish. The chloride cells (CC) are clustered although each has its own crypt. Note the presence of cellular extensions in the apical crypt (arrow).

Fig I-22 Details of a chloride cell from a freshwater-adapted fish. Note the relatively few mitochondria (M).

Fig. I-23 Transmission electron micrograph of chloride cells from a fish acclimated to sea water for 7 days. Note the increased number of mitochondria as compared to FW specimens (Figs. I-21 and I-22). Arrows indicate apical pits shared by several chloride cells. Compare the "pit" structure shown in Fig. I-23 with the crypt structure of a FW fish shown in Fig. I-21.



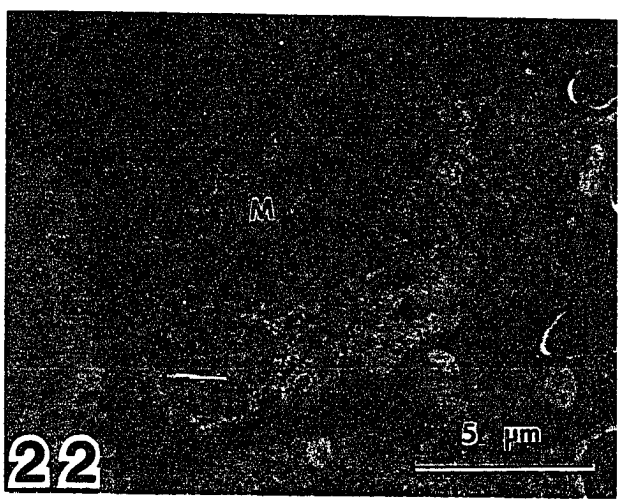


Fig. I-24 Changes in the number of chloride cell apical crypts of striped bass following transfer of freshwater-acclimated fish to sea water. Values represent the mean (M) ± SE. The 0-hour sample was taken prior to transfer.

The actual values are as follows:

FW (0 h)	4.86	±	0.44 (7)
3 h	4.71	±	0.28 (9)
6 h	4.53	±	0.41 (6)
12 h	4.75	±	1.35 (2)
24 h	4.70	±	0.27 (7)
7 days	5.62	±	0.37 (8)
14 days	6.44	±	0.15 (7)*

The numbers in parentheses are the number of fish sampled. The * represents a significant difference between the group indicated and the FW (0 h) group as determined by Dunnett's test ($p < 0.025$; Winer, 1971).

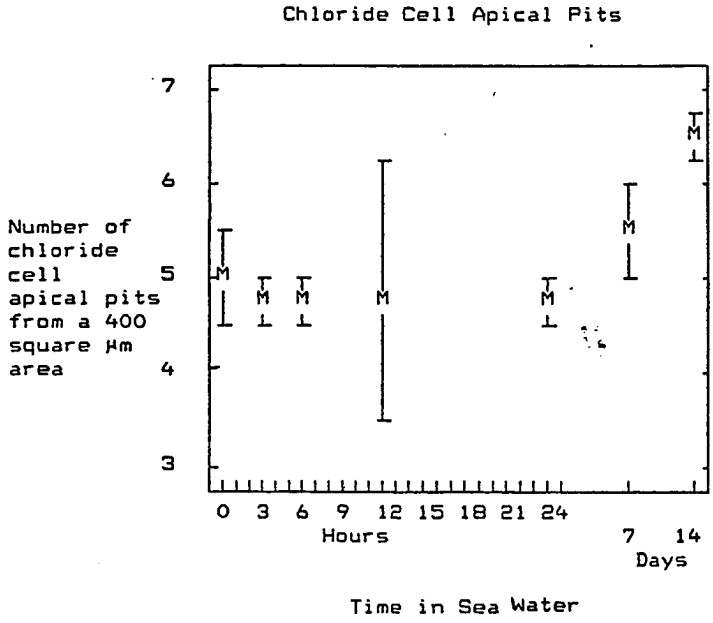
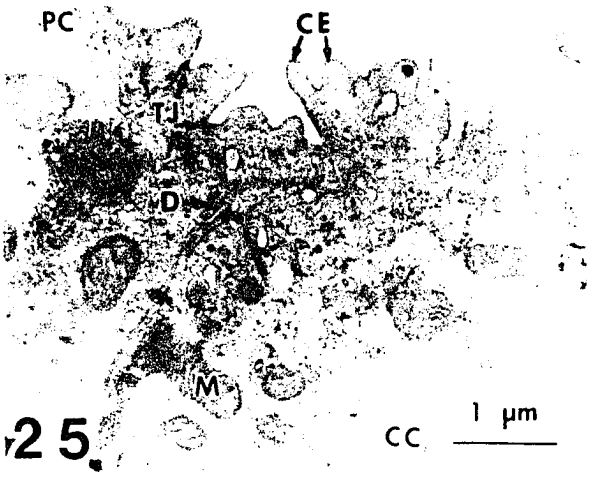


Fig. I-25 Apical region of chloride cell from a fish acclimated to sea water for 45 min. M: mitochondria; TJ: tight junction; D: desmosome; CE: cellular extensions of chloride cell; PC: pavement cell; CC: chloride cell.

Fig. I-26. Apical region of chloride cell from a freshwater fish. Note tight junction (TJ) between chloride cell (CC) and pavement cell (PC). Note tubular system (arrows) and mitochondria (M) and lack of the tubular system in the cellular extensions (CE).

Fig. I-27. A degenerative chloride cell from a fish acclimated to sea water for 7 days. Note relatively few mitochondria (M) compared to normal chloride cells for that stage of acclimation (compare to Fig. I-23), as well as enlarged tubular system (arrows).



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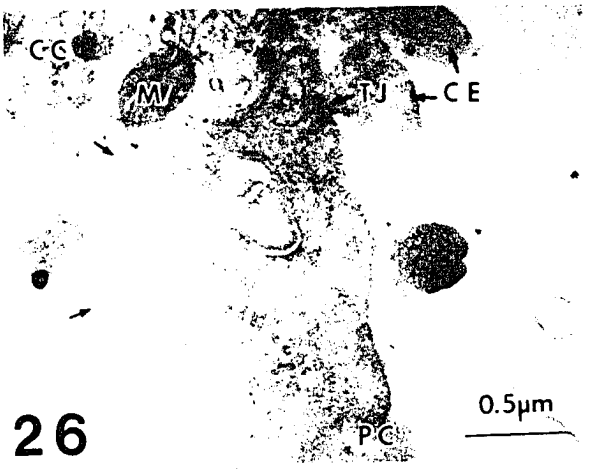

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Osmium-DMSO-Osmium

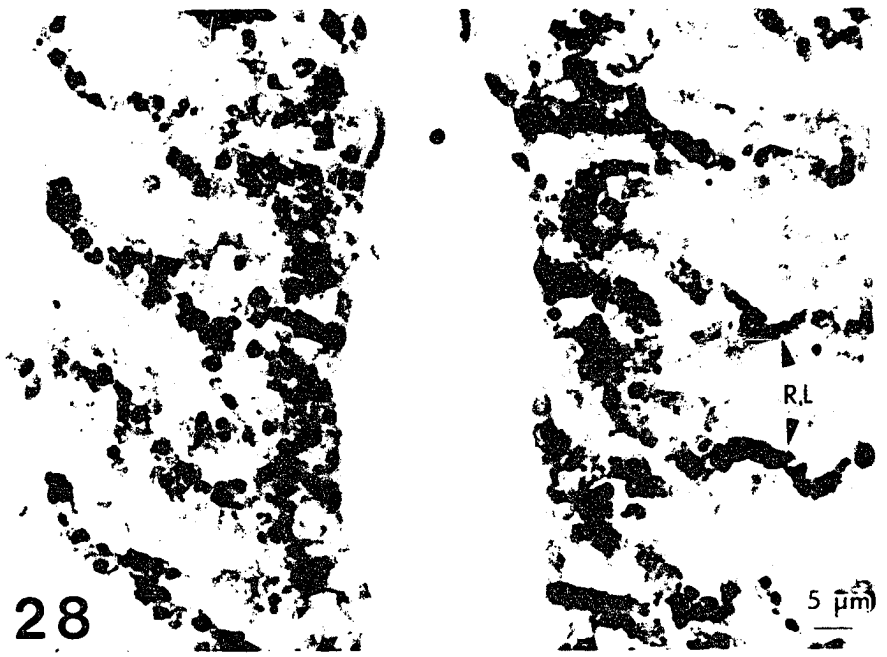
Preliminary work with this procedure on striped bass gills indicates that it can be used for revealing intracellular structures for observation with SEM. Rodlet (Fig. I-5a) and pavement cells are easily cleaved to reveal intracellular structures. This procedure allows the arch to be cross-sectioned evenly while preserving the normal morphological details of bone, cartilage, nerve, and vasculature. Sectioned chloride cells were not observed, but few specimens were examined. Future work with gill tissues will require modifications in the osmolality and/or length of exposure to DMSO and osmium because the present procedure causes the removal of pavement cells in sheets (Fig. I-5a).

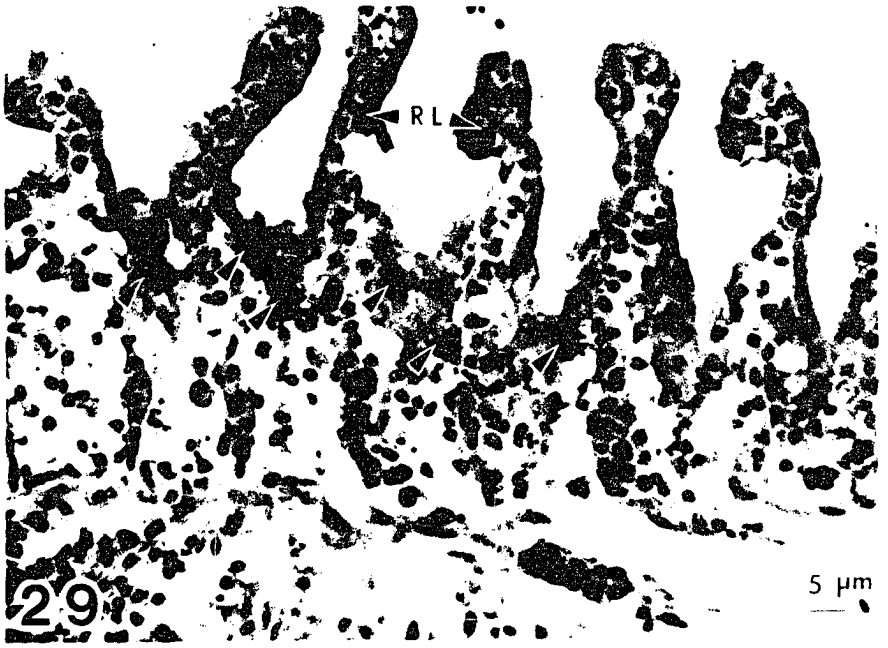
Autoradiography

Freshwater specimens incubated with ^{3}H-thymidine incorporated very little isotope (Fig. I-28), and this incorporation is only observed in the interlamellar region at the base of the filaments. After three days in sea water label is found in the interlamellar region along the length of the filaments. After 7 days in sea water, label is observed in the interlamellar region at the base and near the tip of the filaments (Fig. I-29). At no time did the lamellar pavement cells incorporate thymidine. Label in the interlamellar epithelium can be seen from the basal layers to the surface. A few eosinophilic cells with what appear to be apical pits are labeled, and it is thought that these represent chloride cells. Most of the label in the interlamellar epithelium appears in the hematoxylin-stained sub-surface cells that may be non-differentiated progenitor cells. Mucous cells appear as rounded cells with basal, unlabeled nuclei.

Fig. I-28. Light micrograph of ^{3}H-thymidine incorporation in the gill filament of a freshwater fish. Note the lack of labelled nuclei. RL: respiratory lamellae.

Fig. I-29. Light micrograph of ^{3}H-thymidine incorporation in the gill filament of a fish acclimated to sea water for 7 days. Note labelled nuclei (arrowheads). RL: respiratory lamellae.





Discussion

General Morphology

The gill arches of the striped bass, Morone saxatilis, are typical of teleosts of intermediate activity according to Gray (1954) and as discussed by Hughes (1984). All four gill arches are highly developed, exhibiting relatively long filaments the full length of each arch. The respiratory lamellae are numerous and of average size when compared with other fish of intermediate activity (e.g., mullet: Hossler et al., 1979a; and killifish: Hossler et al., 1985). However, the number of lamellae per millimeter (40-50) on each side of the filament is high compared to the average value (18-25) reported by Hughes (1984) for fishes of intermediate activity. This could indicate that striped bass should be classified near the upper range of activity of this group. However, these high values could be characteristic of the immature fish used in this study.

With the exception of its importance in feeding mechanisms (see discussion by Lauder, 1983), gill raker anatomy has received little attention in the literature. As is the case with some other teleosts (e.g., killifish, Hossler et al., 1985), only the anterior row of rakers on the first gill arch of striped bass is highly developed. These rakers bridge the gill slit between the operculum and the first gill arch, and likely prevent food or ingested debris from entering the opercular cavity and causing possible damage to the respiratory lamellae. This first gill slit would seem to be potentially the largest opening in the gill and could function in expelling water during feeding — hence the importance of this first row of rakers and their well-developed pharyngeally directed spines. All of the remaining rows of rakers in the striped bass gill,

although heavily laden with spines, are reduced to short, raised areas along the pharyngeal surface of each arch. Their positioning, however, allows juxtaposed rakers on adjacent arches to interdigitate when the arches are approximated, thus forming a tight seal between the pharyngeal and gill cavities. Lauder (1983) demonstrated very elegantly in bluegill sunfish that such a seal is essential for the formation of negative pressure in the pharyngeal cavity during suction feeding.

During respiration, however, the slits between gill arches are open, and the primary source of resistance to waterflow is provided by the close approximation of filaments (especially at their tips) and their attached respiratory lamellae (see discussion in Hughes, 1984). This arrangement insures that water entering the gills will flow across the lamellar surface. The respiratory function would also be enhanced by orienting the triangular lamellae such that one broad surface of the triangle would face the incoming water on the efferent surfaces of the filaments as observed here. The reduction in surface sculpturing on the pavement cells of the respiratory lamellae seen here has been observed in other species (trout, Hughes, 1979; mullet, Hossler et al., 1979a; Tilapia, Fishelson, 1980; killifish, Hossler et al., 1985). Although it is possible that pavement cell surface ridges at this site are masked by a covering of mucus, we feel the ridges are truly absent for two reasons. First, the cell-to-cell junctions with their microvilli remain distinct on the lamellae. And second, preliminary transmission electron micrographs of lamellae of striped bass (unpublished) have failed to reveal the surface ridges. Because surface sculpturing is believed to act as an anchoring site for mucus (Hughes and Wright, 1970; Sperry and Wassersug, 1976; Hughes, 1979), its absence in this area might serve to reduce the thickness of the blood-water barrier.

Of special interest in this study was the location and anatomy of the apical crypts of chloride cells, because our intent is to use striped bass as a model-system for studies on the mechanism of osmoregulation. As with most other euryhaline teleosts (see discussion in Laurent, 1984), chloride cells in striped bass are limited to the epithelium of afferent and interlamellar surfaces of filaments. The diameter of the apical crypts (3.16 µm) is similar to that of other euryhaline teleosts adapted to freshwater (e.g., mullet, 4 µm, Hossler, 1980; and killifish, 2.03 µm, Hossler et al., 1985). As observed in other fish, the crypts appear as slight depressions between adjacent pavement cells through which project numerous chloride cell surface extensions.

Response to Salinity Change

Although crypt structure changes during sea water adaptation, the 7-and 14-day sea water-acclimated fish do not exhibit the uniform, deep apical pits as observed in mullet (Hossler, 1980). It has been proposed that deepening of the apical pit increases surface area for ion transport by increasing chloride cell access to the external environment (Hossler et al., 1979b). Since only some of the crypts in striped bass take on the deep pit ultrastructure, only that fraction of the chloride cells may be activated for ion transport. Apical crypt morphology begins to change within 3 to 12 h after transfer to sea water. Copeland (1948) found that in Fundulus cytological changes occur as early as 3 h after transfer to sea water and are completed by 18-24 h.

The internal ultrastructure of a chloride cell is altered during the first week after transfer to sea water yielding a hypertrophied cell with an increased number of mitochondria and a more extensive basolateral tubular network similar to that observed in other species (see General Introduction). This is thought to occur because of the increased need for energy (mitochondria) and enzymes (basolateral plasma membrane tubular system) for ion transport.

Accessory cells are present in freshwater- and sea water-adapted striped bass. Hootman and Philpott (1980) found that freshwater pinfish do not have accessory cells, while pinfish adapted to 33% (isosmotic) and 100% sea water (hyperosmotic) have this cell type. Laurent and Dunel (1980) also reported that accessory cells are present only in sea water fish and that only one accessory cell is associated with each chloride cell. It appears that striped bass may have one to two accessory cells associated with a chloride cell. If accessory cells represent an immature form of chloride cells as proposed by Sardet et al. (1979) and Hootman and Philpott (1980), striped bass may have a more extensive precursor population than do most species, which possibly allows for faster adaptation.

Autoradiography

The greater amount of label observed in 7-day sea water-adapted striped bass, as compared to freshwater bass, is in agreement with the results reported in other species (Conte and Lin, 1967; Chretien and Pisam, 1986). The site of incorporation in striped bass (interlamellar region) is also in good agreement with the results of Conte and Lin (1967) with Oncorhynchus. Although the labeled cells can not be positively identified because of poor tissue preservation, many of the labeled cells are located in the basal and intermediate regions of the epithelium. The cells stain with hematoxylin, suggesting that they are non-differentiated cells (Conte, 1965; Laurent and Dunel, 1980; Chretien and Pisam, 1986) which have numerous ribosomes and rough endoplasmic reticulum. The lack of label in pavement cells is probably because cellular movement did not have time to occur during the incubation. Pavement cells are thought to originate from stem cells in the filamental

epithelium (Laurent and Dunel, 1980), and cellular movement takes days (Conte and Lin, 1967).

Some apparent differences in labeling did occur along the length of the striped bass filament at the different time periods, as reported in other species (MacKinnon and Enesco, 1980). Chretien and Pisam (1986), however, found no difference in labelling of the proximal and distal parts of the filaments in the guppy. Further studies with better specimen fixation are necessary to determine if differences really exist.

These studies of striped bass gill morphology and the changes observed in that morphology as a result of sea water adaptation form a basis for the investigations described in the next three chapters.

CHAPTER 2

MICROVASCULATURE OF THE STRIPED BASS GILL ARCH STUDIED WITH VASCULAR CORROSION CASTING AND SCANNING ELECTRON MICROSCOPY

Introduction

Although striped bass gill morphology has been studied (Bauer, 1972; Groman, 1982; Harpole and Hossler, 1984; Hossler et al., 1986b; see Chapter 1), no detailed examination of the branchial and filamental vasculature has been reported. Since osmoregulatory chloride cells (Keys and Willmer, 1932) in striped bass are abundant on the interlamellar and afferent surface of the filament (Chapter 1; 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; De Vries and De Jager, 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. An account of this study has been published (King and Hossler, 1986; see Appendix).

Materials and Methods

Striped bass (Morone saxatilis) 13-27 cm long were obtained and maintained, and light microscopic sections were prepared as described in Chapter 1 and by King and Hossler (1986).

For vascular casting, striped bass were anesthetized with ethyl-p-amino-benzoate (benzocaine; see Ferreira et al., 1979; Olson, 1985). After an intra-peritoneal 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 Ringers solution (Lockwood, 1961) at physiological perfusion pressure (30 mm Hg; constant flow, pulsatile pressure; Olson, 1983; 1985). Pulsatile pressure 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 (30 mm Hg) was used for resin injection in most casts 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 (100%; 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 electron micrographs. Extravasation of resin from vessels was rarely observed.

Results

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 during tissue removal. Only Mercox/Sevriton perfused specimens are illustrated in the figures.

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

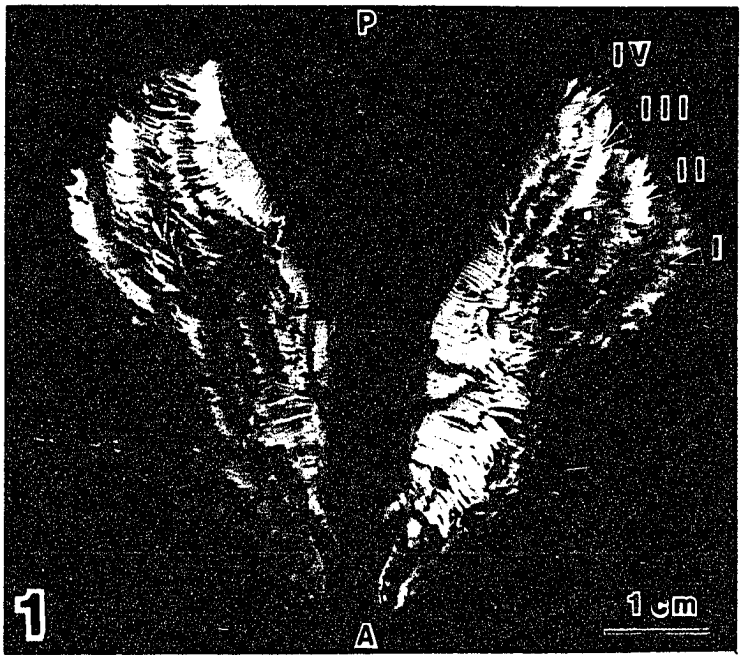
A typical vascular corrosion cast of striped bass gills is shown in Figure II-1. The four pairs of gill arches are designated I, II, III, and IV, rostral to caudal. No major differences among the filaments from the different arches were observed. Striped bass have three major vascular systems: (1) a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

Fig. II-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 35mm camera with a 100mm macro lens.

The symbols for Figures II-1 to II-15 are as follows:

Abbreviations

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



(Boland and Olson, 1979). The major components of the vasculature of the gill arch and gill filaments are represented schematically in Figure II-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. II-2, II-3 and II-4). As 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. II-4) via afferent lamellar arterioles (ALA), and then passes to the efferent filamental (primary) artery (EFA) via the efferent lamellar arterioles (ELA;; see Fig. II-5). 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. Constrictions were observed in some EFA casts just proximal to the first RL (see Appendix).

In the striped bass the ABA appears to be symmetrically located in the middle of the arch (Fig. II-3), not aligned next to the cartilage as in perch (Laurent and Dunel, 1976), pike (Dornesco and Miscalenco, 1968b), and other perciform species (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 with approximately 8-10 pairs of ALAs coming from it. Constrictions were observed in some AFA casts just distal to the ampullae (see Appendix). No communications were found between the ampullae 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.

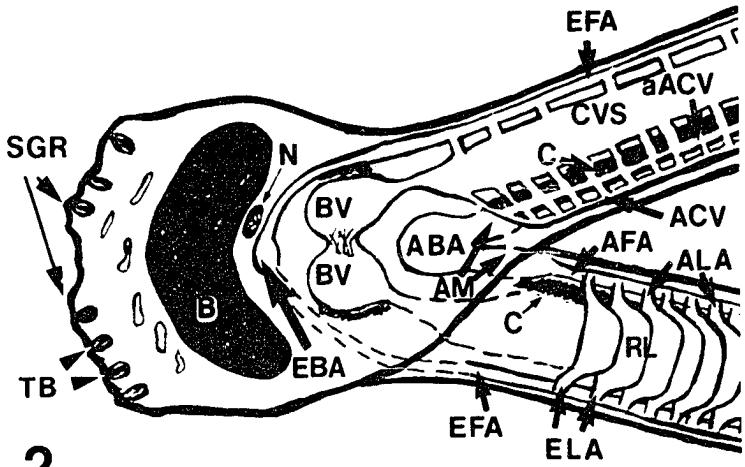
The respiratory lamellae of striped bass have prominent marginal vessels and basal channels (BC) as well as a complex respiratory vascular network (Figs. II-5 and II-6) interwoven between pillar cells (see Chapter 1 and Groman, 1982). In most instances, each RL has one ALA and one ELA. The ALA is longer because it must pass around the filamental cartilage (Fig. II-2) to get to the RL. The ALAs of some of the specimens have enlargements at the junction of the MV and BC.

Variations in 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 vasculature or ELA; (4) an area in the middle of the filament with a double EFA and 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 with the vasculature divided near the distal tip.

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

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

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



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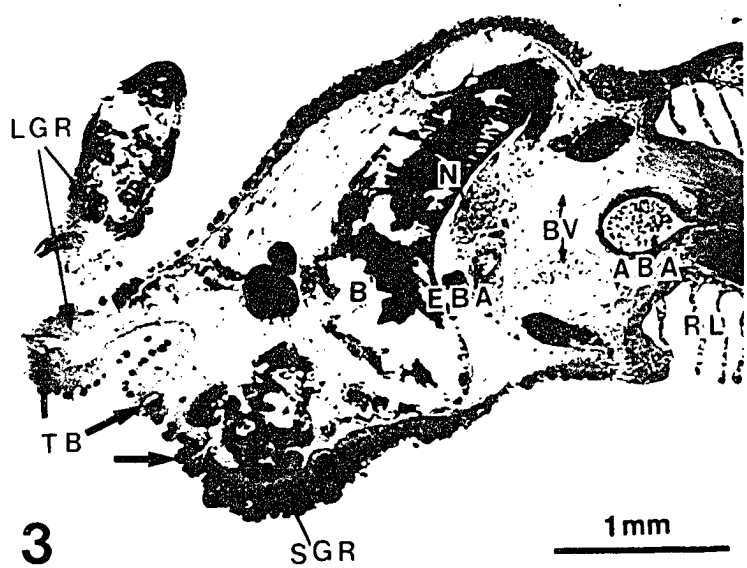

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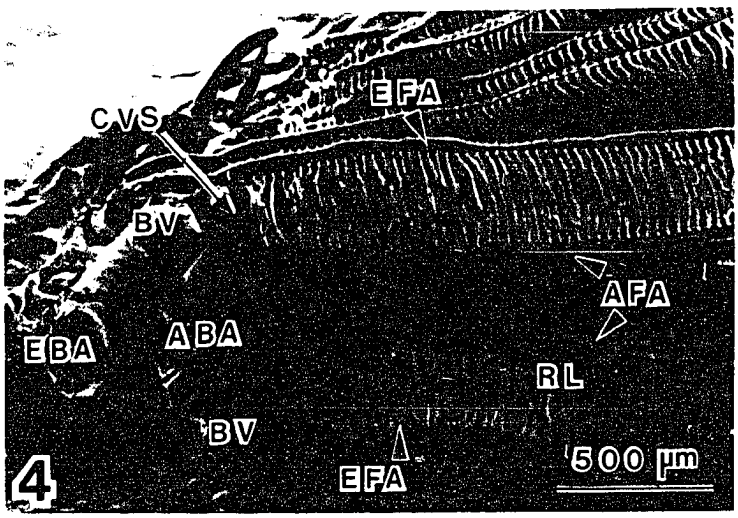


4

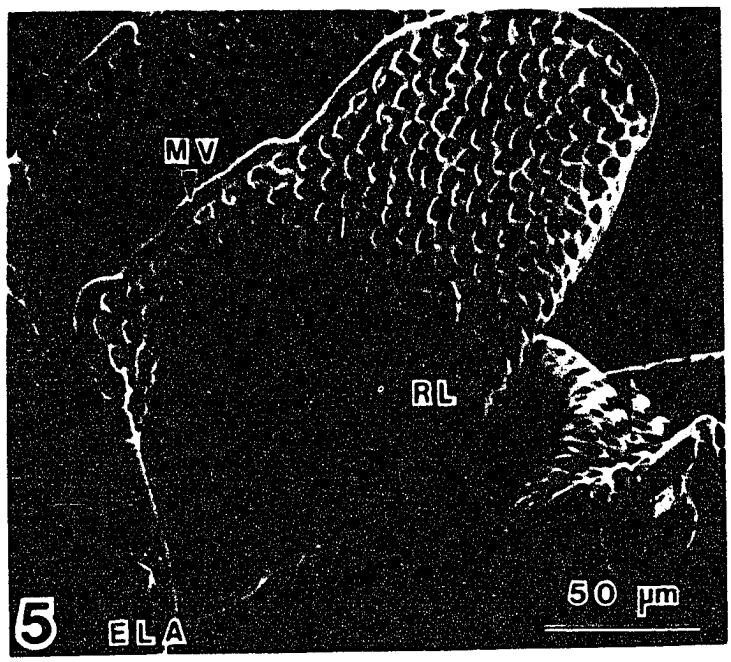
Arterio-venous system

Each gill filament contains a CVS located between the filamental cartilage and the EFA (Figs. II-2, II-6 and II-7). Arterio-venous connections occur between the EFA and the CVS, but not between the AFA and the CVS. The CVS empties either directly into the branchial vein or indirectly via small, paired afferent companion vessels (ACV) which lie on either side just medial to the AFA (Figs. II-6, II-7, II-8 and II-9). The vessels connecting the CVS and the ACV alternate with the ALA and are regularly spaced, about one for every ALA (Figs. II-8 and II-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. II-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. II-7, II-11 and II-12). Casts of the CVS often exhibit indentations from their overlying RL (Fig. II-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. II-12) has various connections to the BV (Fig. II-13), EBA, and adjacent CVSs. No connections between the CVS and the RL or between the ACV and the AFA were observed.

Fig. II-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. II-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).



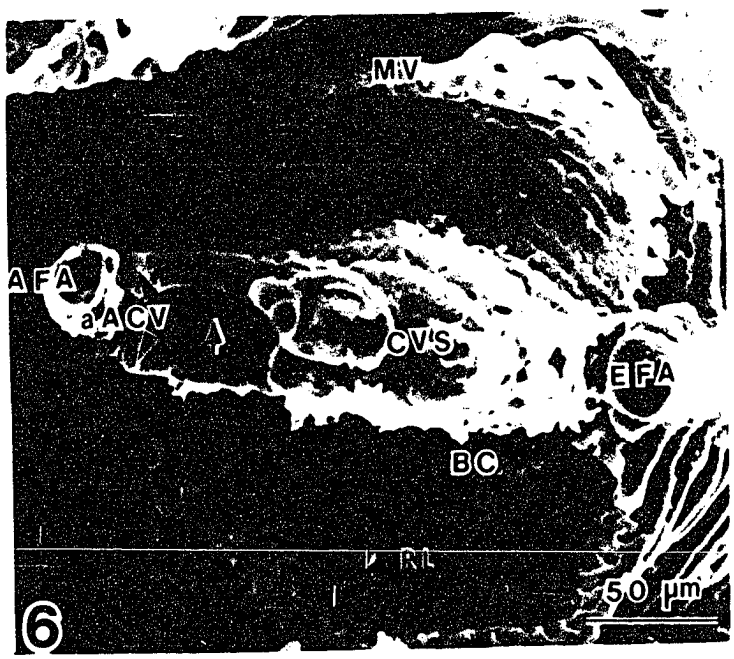
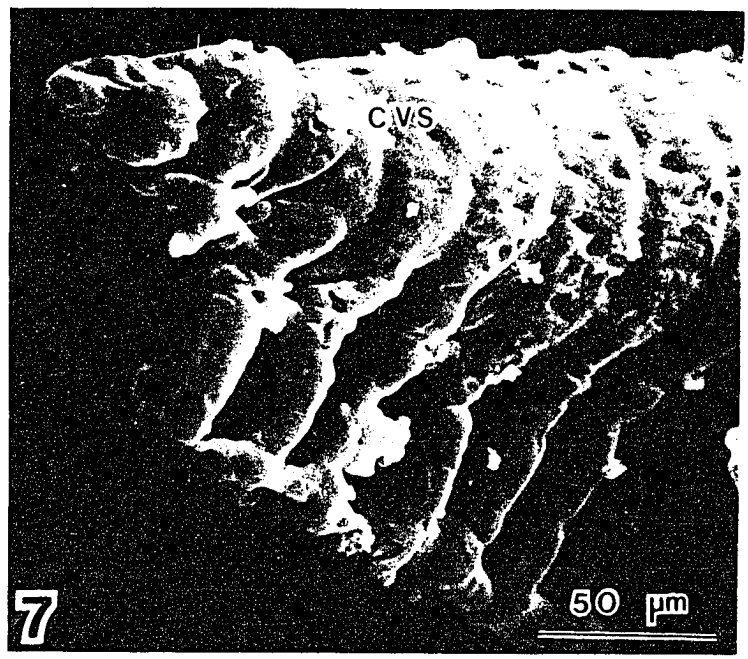
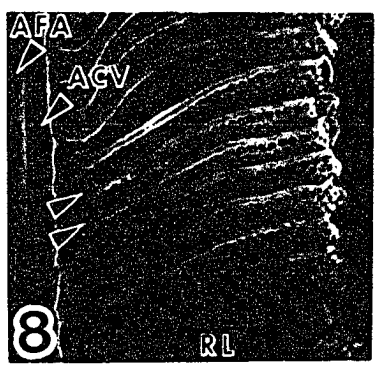
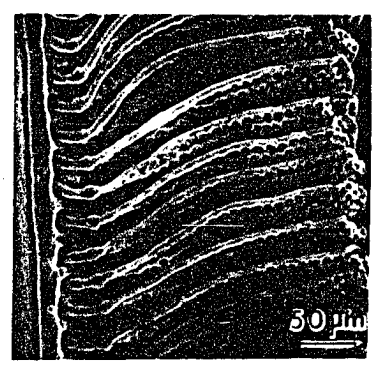


Fig. II-7 Cast of central venous sinus at apex of filament. Note the remnants of connections to the ACV. The surrounding respiratory lamellae, afferent filamental artery, and the efferent filamental artery were removed. The afferent side of the central venous system (CVS) is at the bottom.

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







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. II-14). Coiled vessels from the EFA proximal to the first RL of the filament anastomose with the nutritional vessels from the EBA (Figs. II-14 and II-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 from the CVS-ACV complex described above (Fig. II-13). Smaller vessels combine to form larger vessels which parallel the filaments and eventually join the BV on the gill raker side (Fig. II-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.

Discussion

As with most other euryhaline species (Laurent, 1984), striped bass gill vasculature consists of three 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 arterio-venous pathway

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

Fig. II-10 Cast of "accessory" afferent companion vessel (aACV) and afferent companion vessel (ACV). The asterisk indicates the area where an afferent lamellar arteriole (ALA) would exit to attach the respiratory lamellae (RL) to the afferent filamental artery (AFA).

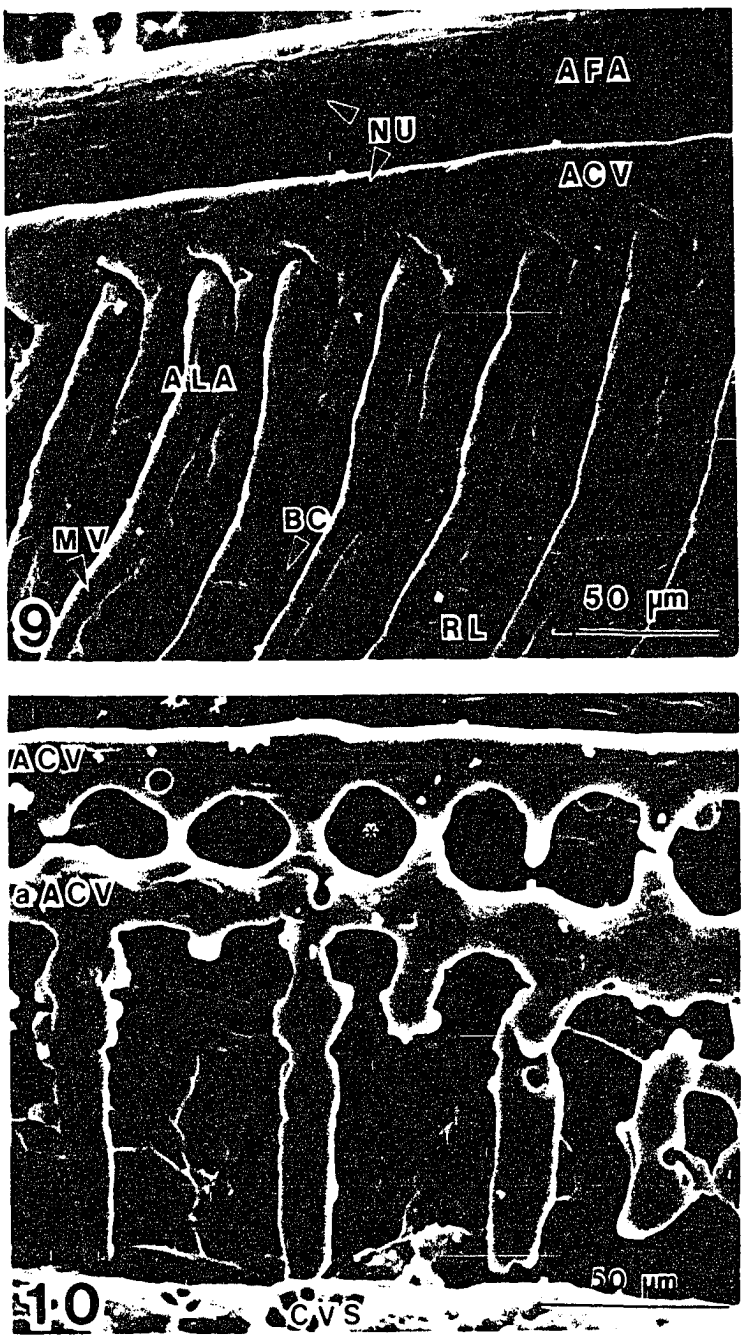
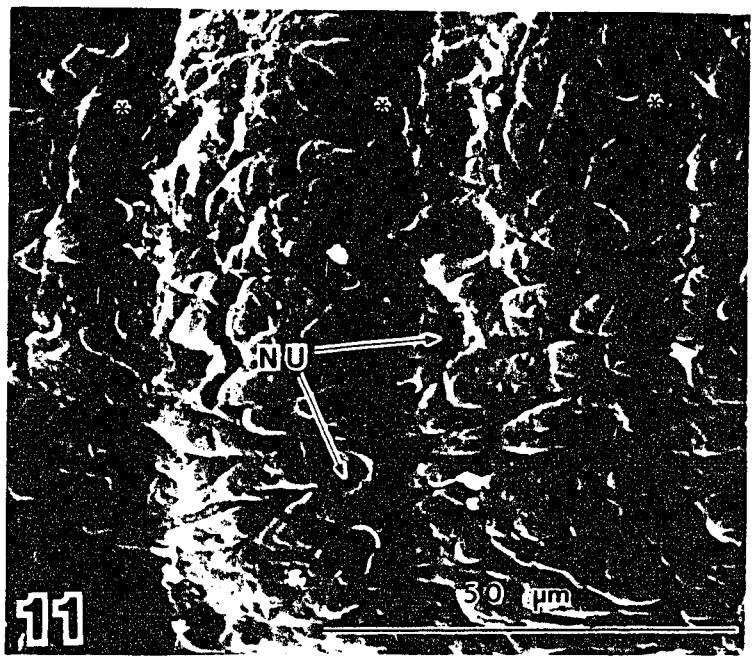


Fig. II-11 Cast of the central venous sinus. Note the indentations produced by the respiratory lamellae (asterisks) and the impressions left by the nuclei of endothelial cells (NU).

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



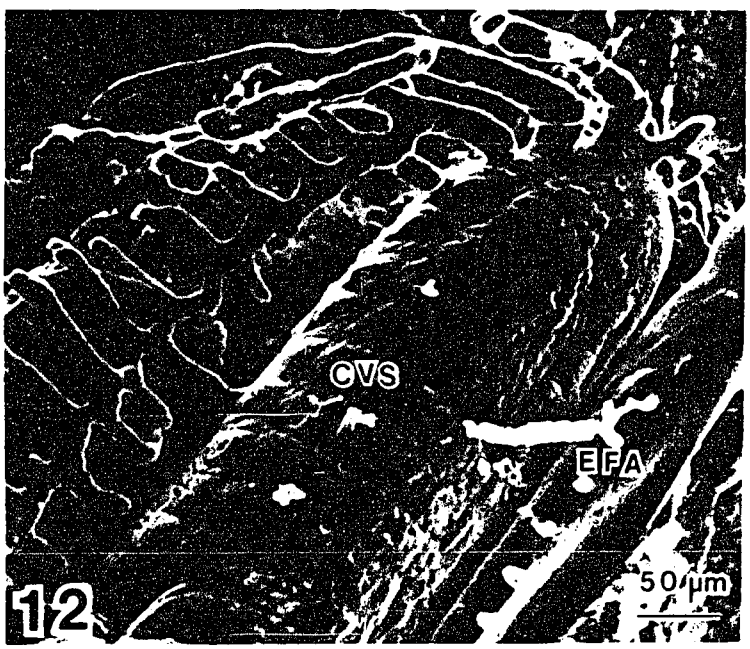


Fig. II-13 Vascular cast of the branchial vein (BV) and its connections. Note the vessels from each filament and the tributaries that are interwoven with the BV. Arrowheads indicate small vessels which drain the CVS-ACV complexes and the nutrient supply of the filaments.

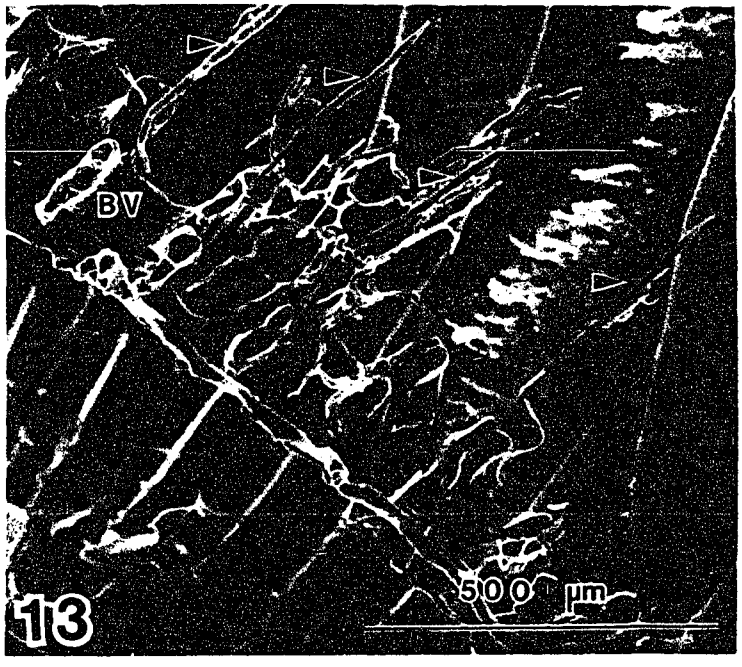
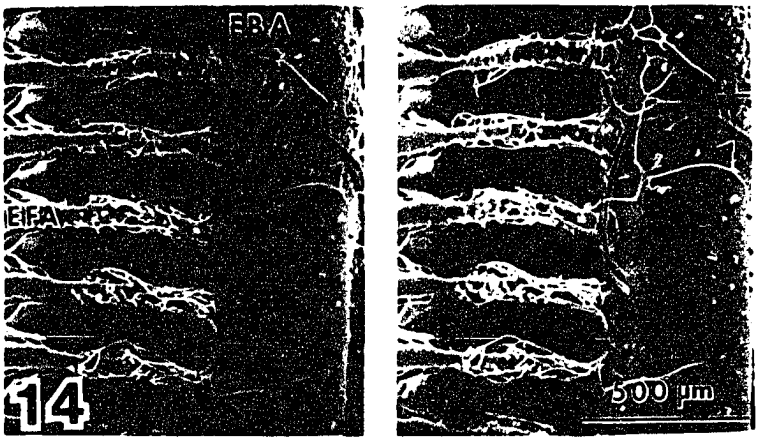
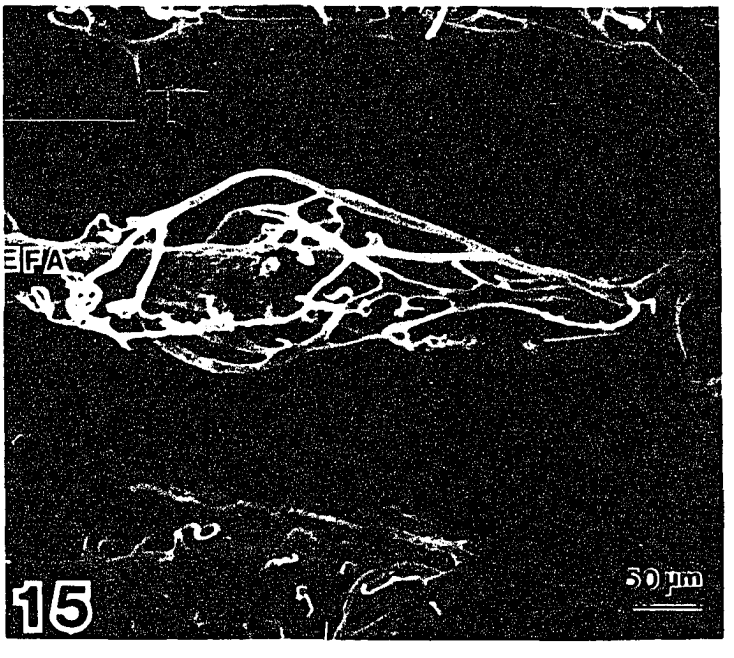


Fig. II-14 Stereo pair of nutrient vessels from the efferent branchial artery (EBA) and the efferent filamental artery (EFA). Note the coiled origins of the nutritional vessels from both the EFA and EBA, and the connections between those vessels.

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





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 bass gill vasculature, in particular, merit mention here.

Respiratory system

Enlargements (called ampullae or "blebs") of the AFA proximal to the "bifurcation of the two hemibranches" observed in striped bass have been reported in some 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 AFA constrictions observed in some casts just distal to the ampullae may represent sphincters which could control blood flow to the filaments. The ALA enlargements may represent sites adjacent to sphincters (Wright, 1973).

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

Arterio-venous system

The arterio-venous system has become the focus of many recent studies. Most of the differences between species studied to date appear in the location of the 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 a 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; Steen and Kruysse, 1964; 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), and trout (Salmo gairdneri; Richards and Fromm, 1969). However, lamellar bypasses have not been reported in trout (Salmo gairdneri; Gannon et al., 1973; Vogel et al., 1976; Laurent and Dunel, 1976), ling cod (Ophiodon elongatus; Farrell, 1980), perch (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 Tuurula, 1981) may be used by the striped bass to regulate O_2 and ion exchange in the gills. Both the MV and the 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. 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 (Smith and Johnson, 1977; Part et al., 1984; Tuurala et al., 1984). Contractile pillar cells (Bettex-Galland and Hughes, 1973) present in striped bass (Chapter 1; Groman, 1982) may also help to control intralamellar blood (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 recruitment 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 affected by resin extravasation but could have been affected by distension. The EFA constrictions may represent the sites of sphincters (Dunel-Erb and Laurent, 1980). Contraction of the EFA sphincter may force blood into the CVS via the arterio-venous anastomoses.

The CVS of the striped bass gill filament 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).

Chloride cells have been linked functionally to the arterio-venous system in teleosts (Payan and Girard, 1984), as well as to the slower blood flow in the basal channel of the RL (Hughes, 1984). The chloride cells of striped bass are located on the interlamellar and afferent surface of the filament (Chapter 1;

Hossler et al., 1986b). This location may allow them to be affected by both vessel systems. It is thought that blood flow in adjacent ACV and AFA are in opposite directions, possibly providing a potential site for counter-current exchange in the area of the chloride cells. However, no single function satisfactorily explains 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 probably provide 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.

CHAPTER 3

MICRODISSECTION OF THE STRIPED BASS GILL ARCH BY ULTRASONICATION

Introduction

Selective dissociation of epithelia by aldehyde fixation, prolonged osmication, and/or boric acid treatments combined with ultrasonic vibration (sonication) has been used successfully with mammalian tissues to permit scanning electron microscopic viewing of subsurface details of lung, choroid plexus, uterus (Highison and Low, 1982), gastrointestinal tract (Low and McClugage, 1984; McClugage and Low, 1984), thebesian veins (Rosinia and Low, 1986), cerebellum (Arnett and Low, 1985) and placenta (Highison and Tibbitts, 1986).

Teleosts have specialized chloride cells located in their gill epithelium that are thought to be osmoregulatory (see Zadunaisky, 1984). The apical pits and internal ultrastructure of chloride cells change in response to altered salinity (see Chapter 1). To permit examination of possible changes in the basolateral plasma membrane of chloride cells during sea water adaptation, and to view the anatomy of the basement membrane of the filament by scanning electron microscopy, microdissection by sonication techniques were used to selectively remove some of the epithelial cells of the gill. This required modification of the methods used with mammalian tissues.

Materials and Methods

Striped bass (Morone saxatilis) 4-18 cm long were obtained and maintained, and light microscopic sections were prepared as described in Chapter 1 and by King and Hossler (1986). For dissection by sonication, striped bass were killed by decapitation. The gill arches were removed, rinsed in 0.9% NaCl to remove the blood, and then sonicated by one of the procedures described below (and as outlined in Table III-1) to achieve removal of all or part of the gill epithelium.

Procedure 1

The gill arches were immersed in 2% OsO_4 buffered with 0.1 M cacodylate-HCl (pH 7.2) for 48-72 h. The arches were then rinsed in 3 changes of 0.1 M cacodylate-HCl (pH 7.2), dehydrated in a graded acetone series, and sonicated in the ice-cooled water bath of a Bransonic 220 ultrasonic cleaner (Branson Sonic Power Co., Danbury, CT) at 50/60 Hz for 0-20 min. The specimens were placed in a model E3000 critical point dryer (Polaron Instruments, Inc., Hatfield, PA) in 100% acetone, dried with liquid CO_2, affixed to specimen stubs with silver paste, coated with gold or gold-palladium in a sputter coating apparatus (Model Desk 1, Denton Vacuum, Inc., Cherry Hill, NJ), and observed in a model S430 scanning electron microscope (SEM, Hitachi Scientific Instruments, Mountain View, CA; Model JSM-35C, JEOL (U.S.A.) Inc., Peabody, MA). Measurements were made from electron micrographs.

Procedure 2

The gills were immersed in aqueous 1% boric acid (H_3BO_3) for 5 h, rinsed in 3 changes of 0.1 M cacodylate-HCl (pH 7.2), osmicated (2% OsO_4 buffered with 0.1 M cacodylate-HCl, pH 7.2) for 48 h, dehydrated in a graded acetone series, sonicated at 50/60 Hz for 0-10 min, critical point dried, and prepared for SEM examination.

Procedure 3

The gills were immersed in 1% H_3BO_3 for 5 h, rinsed in 3 changes of 0.1 M cacodylate-HCl (pH 7.2), dehydrated in a graded acetone series, sonicated at 50/60 Hz for 5-20 min, critical point dried, and prepared for SEM examination.

Procedure 4

The gills were immersed in freshly prepared aldehyde fixative for 2 h at 20-24°C or overnight at 4°C. Fixative consisted of 2.5% glutaraldehyde and 1.8% paraformaldehyde in 0.1 M cacodylate-HCl buffer (pH 7.2). After fixation the specimens were rinsed in excess buffer (0.1 M cacodylate-HCl, pH 7.2), postfixed for 24-48 h at 4°C in 2% OsO_4 buffered with 0.1 M cacodylate-HCl (pH 7.2), rinsed in buffer as before, dehydrated in a graded ethanol or acetone series, sonicated at 50/60 Hz for 5-25 min, critical point dried, and prepared for SEM examination.

Procedure 5

The gills were immersed in 2% OsO_4 buffered with 0.1 M cacodylate (pH 7.2) with 0.1% Triton X-100 for 2-4 h at 20-24°C, rinsed in 3 changes of 0.1 M cacodylate-HCl (pH 7.2), dehydrated in a graded ethanol or acetone series, sonicated for 0-10 min, critical point dried, and prepared for SEM observation.

Results

Morphology Revealed by Sonication

Striped bass have four pairs of gill arches designated I, II, III, and IV, rostral to caudal. Each arch has two rows (hemibranches) of filaments as well as anterior and posterior rows of gill rakers (Fig. III-1). The anterior row of gill rakers of gill arch I, are long, finger-like appendages (long gill rakers). The posterior row of gill rakers of gill arch I and the rakers of the other 3 arches are short, raised areas referred to as short gill rakers. Each filament supports two rows of respiratory lamellae, one row on each side of the filament. (see Chapter 1).

Removal of epithelium from the short gill rakers (Figs. III-1, III-2, and III-3) reveals pharyngeal spines (Figs. III-2 and III-3) and the basal lamina underlying the randomly distributed taste buds (Figs. III-3, III-4, and III-5). Removal of the epithelium covering the long gill rakers reveals that the basal lamina of taste buds is in the form of columns (Figs. III-6 and III-7).

The basal laminae of respiratory lamellae closely conform to the contours of the overlying pavement cells and to the vasculature beneath (Fig. III-8). Occasionally, sonication removed all of the respiratory lamellae, exposing the collagen layer that is continuous with the filamental basal lamina (Fig. III-9; see Hughes 1980, 1984; Laurent and Dunel, 1980).

Selective breakage of the filamental epithelium along the afferent and interlamellar surfaces and at the base of the respiratory lamellae reveals chloride cells (Fig. III-10) which are identified by the short apical projections. The details of the basolateral plasma membrane of chloride cells were not easily

observed due to fragments remaining after the removal of adjacent cells. The lateral borders of rounded mucous cells identified by the mucigen droplets on the surfaces are also observed in some specimens (Fig. III-11).

Sonication Procedures

The five procedures used in this study (Table III-I) provide a range of epithelial removal. The osmicated specimens of procedures 1 and 5 have areas on the proximal parts of the filament and the arch proper which are denuded of epithelium or where the epithelium is cracked revealing lateral cellular borders. With 48 h osmication and 20 min sonication, the respiratory lamellae of the distal filamental regions are removed revealing the basal lamina and collagen as observed in Fig. III-9. Most of the epithelium of the rakers remains attached, but the basal lamina is exposed in certain regions. In other sites the pavement cells are removed to reveal the basal epithelial cells.

Both procedures 2 and 3 include boric acid treatment. With procedure 2 there is a range in the extent of epithelial removal. Without sonication, very few cells are dissociated. After two min of sonication lateral cellular borders are exposed. At two min of sonication the basal lamina is exposed (Fig. III-2). Procedure 3 produces total epithelial removal, from both the gill arch proper (Figs. III-1 and III-3) and the filaments (Fig. III-8).

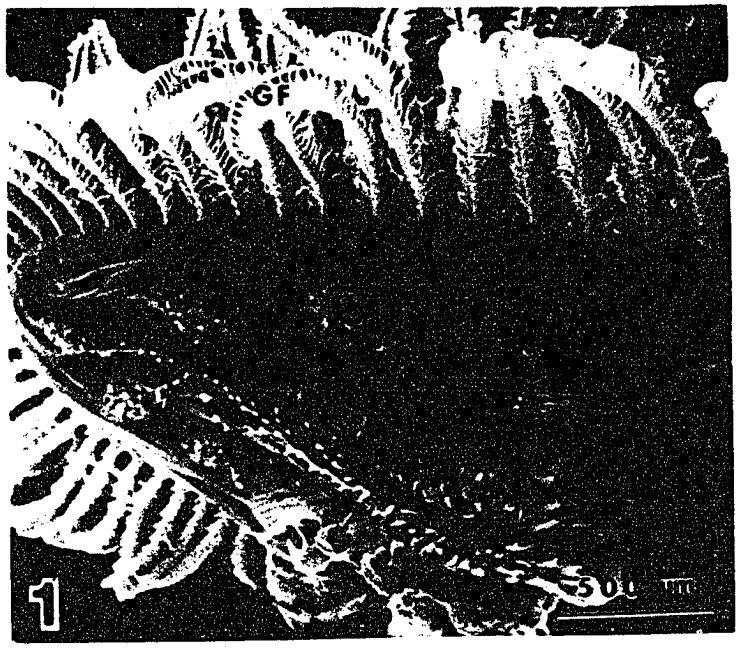
With procedure 4 most of the normal morphology of the epithelium is well preserved, but occasionally the epithelium is broken and the lateral cell borders of chloride cells (Fig. III-10) and mucous cells (Fig. III-11) are visible. The epithelium of the short and long gill rakers seems more easily dissociated than that of the filaments. The basal lamina, of the rakers including that under the

TABLE III-I. Summary of procedures used to achieve microdissection of striped bass gills by sonication.

Procedure	Fixatives Used: 2.5% glutaraldehyde 1.8% paraformaldehyde 0.1% cacodylate-HCl pH 7.2	Fixatives Used: 1% boric acid (H_3BO_3)	Fixatives Used: 2% OsO_4 in 0.1 M cacodylate-HCl pH 7.2	Dehydration	Sonication (50/60 Hz)
1	—	—	48-72 h, 4°C	acetone	0-20 min
2	—	5 h	48 h, 4°C	acetone	0-1 min
3	—	5 h	—	acetone	5-20 min
4	2 h, 20-24°C or overnight, 4°C	—	24-48 h, 4°C	ethanol or acetone	5-25 min
5	—	—	+ 0.1% Triton X-100 2-4 h, 20-24°C	ethanol or acetone	0-1 min

Fig. III-1 Lateral view of gill arch II of a 5 cm striped bass. The epithelium has been removed to expose the basal lamina of the gill filaments (GF) and the arch proper. Note the location of the short gill rakers (SGR). Procedure 3.

Fig. III-2 Gill arch II showing the epithelium (arrows) removed from the gill arch proper and the short gill rakers (SGR). Note the spine(s) of the SGR. Gill filaments (GF); and respiratory lamellae (RL). Procedure 2; sonicated 2 min.



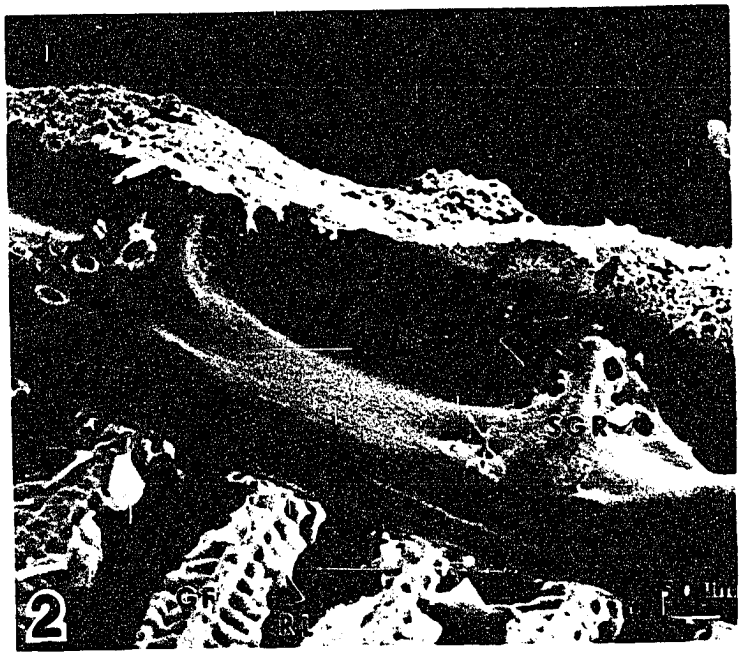
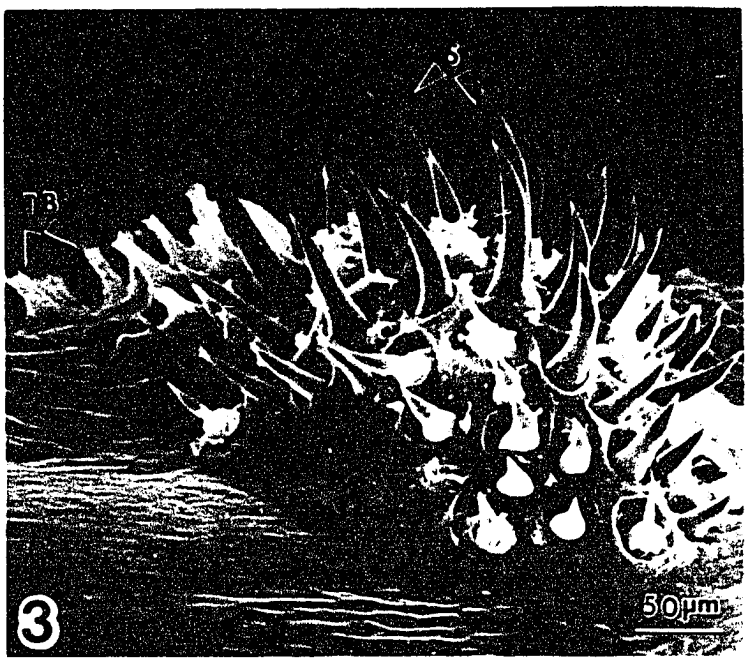


Fig. III-3 Short gill raker of gill arch II with epithelium removed. Note the spines (S) and the basal lamina of the taste buds (TB). Procedure 3; sonicated 10 min.

Fig. III-4 Basal lamina underlying taste bud (TB). Procedure 3; sonicated 10 min.

Fig. III-5 Light micrograph of a taste bud on short gill raker. Notice the shape of the basal lamina and the connective tissue underlying the taste bud as well as the light (LC) and dark (DC) cells of the taste bud. The extent of the epithelium (E) is shown. Mucous cell (MC).



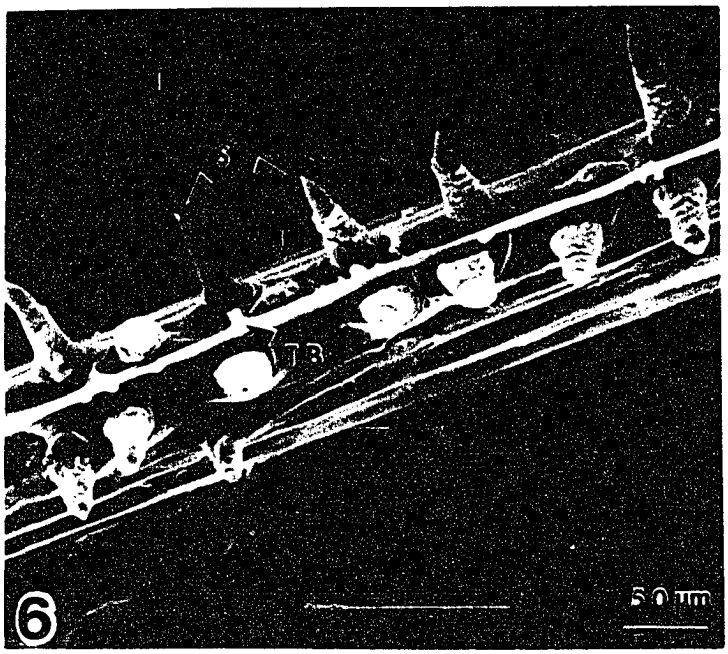
3

4

5

Fig. III-6 Long gill raker with some epithelium remaining on the spines (S) Notice the centrally located row of columns representing the basal laminae of taste buds (TB). Procedure 4; graded acetone series; sonicated 5 min.

Fig. III-7 Columnar basal lamina of taste bud from the long gill raker of arch I. Procedure 4; graded acetone series; sonicated 5 min.



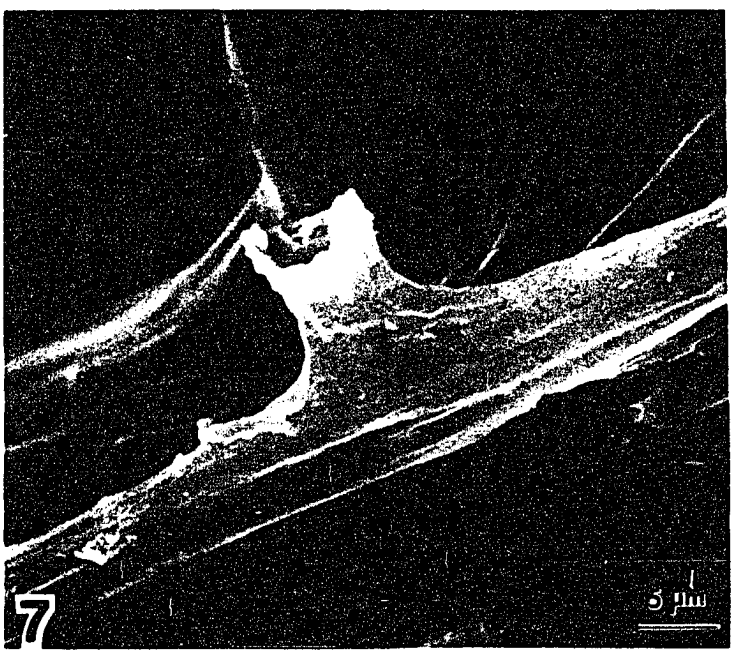
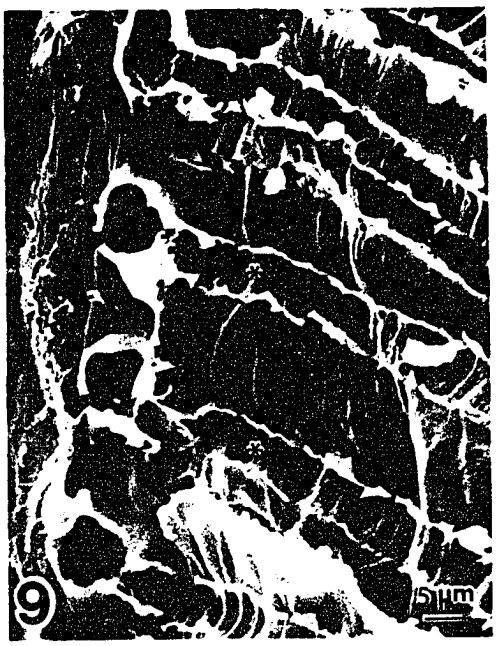


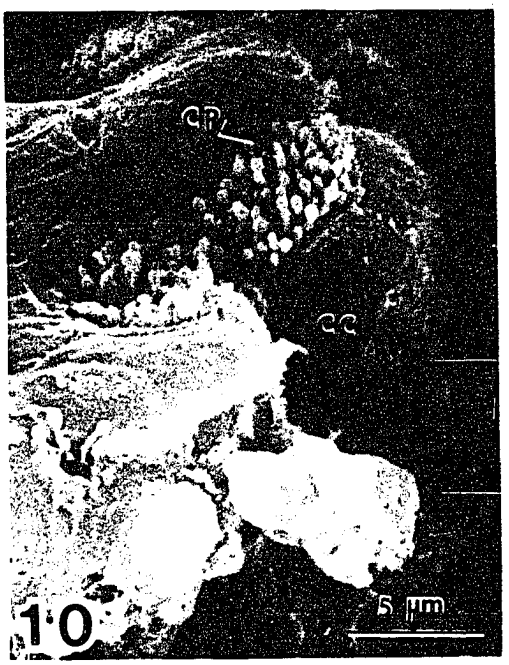
Fig. III-8 Basal lamina of respiratory lamellae (RL). Note the basal lamina overlying the marginal vessel (MV). Procedure 3; sonicated 5 min.

Fig. III-9 Collagen of filament proper showing the basal channel exposed by removal of respiratory lamellae. Asterisks indicate where the respiratory lamellae would normally be. Arrows point to openings of the afferent lamellar arterioles. Procedure 4; graded acetone series; sonicated 5 min.

Fig. III-10 Chloride cells (CC) from the afferent surface of the gill filament with the lateral cell walls exposed. Note the cellular microvillous projections (CP). Procedure 4; graded ethanol series, sonicated 5 min.

Fig. III-11 Mucous cell (MC) adjacent pavement cells. Procedure 4; graded acetone series; sonicated 10 min.





taste buds was often exposed (Fig. III-7), while the pharyngeal teeth remained covered (Fig. III-6). No difference was observed between alcohol and acetone dehydrated specimens.

Discussion

Aldehyde fixation is known to inhibit cellular dissociation (Vial and Porter, 1975), and also microdissection of epithelia by sonication (Low and McClugage, 1984). Osmium tetroxide (OsO_4) is used as a post-fixative in many tissues, including fish gills (Hossler et al., 1986b) to preserve the lipoprotein membranes. Postfixation with OsO_4 of striped bass gills for scanning electron microscopy or transmission electron microscopy normally requires a 2 h exposure to 2% OsO_4 in 0.1 M cacodylate-HCl, pH 7.2, at 4°C (Chapter 1; Olson, 1985). Excessive exposure to OsO_4 is known to produce brittleness (Low, 1954). Procedure 4 consists of the normal fixation of gill tissues with aldehydes (2.5% glutaraldehyde, 1.8% paraformaldehyde in 0.1% cacodylate-HCl, pH 7.2; Chapter 1, Hossler et al., 1986b), followed by prolonged osmication (24-48 h at 4°C). This combination of fixatives was used to make the tissue brittle enough to selectively remove the epithelium surrounding the chloride cells when sonicated, while preserving the morphology of the intact cells. Procedure 4 produces identifiable chloride cells and mucous cells which are revealed by removing the adjacent epithelium. Problems arise with the procedure, however. Due to the randomness of the procedure, much time and effort, as well as the preparation of a large number of specimens, are required to find epithelial breaks at the desired location. This method is excellent for observing the basal lamina under

the taste buds on the long gill raker. The epithelium of the long gill raker is easier to remove than that of the filaments and the arch proper, possibly due to the greater exposure of the projecting rakers during sonication.

The addition of detergent (Triton X-100) during OsO_4 fixation is thought to increase OsO_4 penetration (see Highison and Tibbitts, 1986), but in the present study no noticeable differences between specimens fixed with OsO_4 and detergent (Procedure 5), and OsO_4 alone (Procedure 1) were noted, probably because the gill epithelium is readily penetrated by OsO_4.

Boric acid treatment (5 h in a 1% aqueous solution) is known to dissociate tissue (Vial and Porter, 1975), and in the present study (Procedure 3) it removes all of the epithelia without sonication. Borate treatment is so effective that epithelial removal is probably achieved in part simply by the changing of solutions during normal preparation of specimens for SEM. The basal lamina of the short gill rakers and the spines and taste buds are best revealed by this method. When boric acid is combined with osmication (Procedure 2), the degree of epithelial removal is directly proportional to the length of sonication. Borate dissociates tissue (Vial and Porter, 1975), but OsO_4 tends to maintain some degree of cellular adhesion.

In future experiments on fish gills the required procedures may vary according to the species and the degree and location of cellular removal desired. Many factors such as specimen size, contact with the sonication solution, and freedom to vibrate contribute to the degree of epithelial removal. The greater the contact between the specimen and the sonication solution, the more extensive the microdissection. Epithelial removal is more easily accomplished for areas that can be forced against each other during sonication

such as the free distal tips of filaments. Such contact can even break away respiratory lamellae at the distal portion of the filament while leaving the epithelium intact on the proximal, less flexible aspect of the filament. Because of the variety of contributing factors, trial and error are necessary to achieve the desired results (Highison and Low, 1982).

The lateral cellular borders of the chloride cells can be exposed by any of the procedures except Procedure 3 which removes all of the epithelium. Changes in the basolateral plasma membranes of chloride cells could not be observed due to the small number and size of the chloride cells present. This made it difficult to obtain breaks in the epithelia in the region of the chloride cells and to find cells free of debris when the surrounding epithelium was removed. Perhaps the opercular epithelium would be a better tissue than gills for the study of chloride cells using microdissection by sonication, since in some species at least chloride cells make up 50-70% of all the cells in that tissue (Karnaky and Kinter, 1977).

In conclusion, microdissection by sonication can be used to examine the histological and ultrastructural features of gills; and as observed by others, this procedure is especially useful in revealing the form and extent of the basement membrane. However, sample size, contact of the sample with the sonication solution, the amount of microdissection desired, and the structures to be viewed must be taken into consideration in future experiments to determine the optimal conditions.

CHAPTER 4

THE TIME COURSE OF SEA WATER ACCLIMATION OF STRIPED BASS: NA,K-ATPASE, CHLORIDE EFFLUX, PLASMA CORTICOSTEROIDS, AND ELECTROLYTE DYNAMICS

Introduction

Sea water adaptation of euryhaline teleosts is accompanied often by increases in the amount and activity of gill sodium-potassium adenosine triphosphatase (Na,K-ATPase). Na,K-ATPase activity can be measured by the amount of inorganic phosphate released upon hydrolyzation of ATP in the presence of magnesium, sodium, and potassium, or upon hydrolyzation of p-nitrophenylphosphate (PNPP) in the presence of magnesium and potassium. The amount of Na,K-ATPase present can be measured by ^{3}H-ouabain binding (see Hossler et al., 1979c; Hossler, 1980). Ouabain binds to the catalytic peptide of Na,K-ATPase on a one-to-one basis (Jorgensen, 1974; Schwartz et al., 1975). Therefore, the number of binding sites is a measure of the number of enzyme molecules (see the General Introduction of this manuscript). Sea water teleosts have gill Na,K-ATPase activities that are two to six times higher than those of freshwater teleosts (Epstein et al., 1967; Kamiya and Utida, 1969; see Kirschner, 1980, and the General Introduction). These changes in enzyme activity and number are accompanied by changes in Na^{+} and Cl^{-} efflux (see Motais et al., 1966; Motais, 1967; Evans et al., 1973; Forrest et al.,

1973a,b; Karnaky et al., 1977; Hossler et al., 1979c; Epstein et al., 1985), plasma electrolyte and cortisol levels (see Threadgold and Houston, 1964; Kamiya, 1967; Ball et al., 1971; Bornancin and De Renzis, 1972; Utida et al., 1972; Johnson, 1973; Scheer and Langford, 1976; Epstein et al., 1980; Jacob and Taylor, 1983), and chloride cell numbers and morphology (see Chapter 1).

Cortisol is the major corticoid in most teleosts (Chester Jones et al., 1969; Henderson et al., 1970). Mayer et al. (1967) found that eels with interrenal tissue removed could not tolerate sea water unless cortisol injections were administered. Epstein et al. (1967), Pickford et al. (1970), Langford (1971), Butler and Carmichael (1972), and Hendler et al. (1972) found that hypophysectomy causes a decrease in the activity of gill Na,K-ATPase, which is corrected by cortisol injections. Cortisol administered to freshwater-adapted animals increases gill Na,K-ATPase activity (Epstein et al., 1971), chloride cell number (Doyle and Epstein, 1972; Foskett et al., 1981), and chloride cell differentiation (Doyle and Epstein, 1972), and it causes pigmentation changes in eels resembling those that occur during migration to sea water (Epstein et al., 1971). However, cortisol does not increase Na^+ and Cl^+ efflux until the animals are placed in sea water (Epstein et al., 1971; Foskett et al., 1981). Doyle and Epstein (1972) found that cortisol injections increase the number of fully differentiated chloride cells, but the apical membranes of these cells do not contact the external environment until the animal is placed in sea water. It appears that cortisol acts as a hormonal mediator for sea water adaptation (Epstein et al., 1967, 1971; Mayer et al., 1967; Cynamon et al., 1969; Hirano, 1969; Pickford et al., 1970; Butler and Carmichael, 1972; Doyle and Epstein, 1972; Kamiya, 1972a; Evans et al., 1973; Forrest et al., 1973a,b; Porthe-Nibelle

and Lahlou, 1974; Epstein et al., 1980; Strange and Schreck, 1980; Foskett et al., 1981; Redding et al., 1984; Nichols et al., 1985; Abo Hegab and Hanke, 1986; Dange, 1986) by conditioning the fish for osmotic stress (Epstein et al., 1971; Doyle and Epstein, 1972; Forrest et al., 1973b; Foskett et al., 1981).

Time course studies have been reported for osmotic parameters and Na,K-ATPase in Anguilla anguilla (Bornancin and De Renzis, 1972), Dormitator maculatus (Evans and Mallery, 1975), and Oncorynchus kisutch (Boeuf et al., 1978). The only time course studies which monitored both cortisol and Na,K-ATPase changes were done on Anguilla rostra (Forrest et al., 1973a,b) and Fundulus heteroclitus (Jacob and Taylor, 1983).

In the present study, the time course of sea water acclimation in striped bass (Morone saxatilis) is described. Changes in branchial Na,K-ATPase (amount and activity), chloride efflux, plasma osmolality, plasma chloride, and plasma corticosteroids are measured after transfer of freshwater-adapted fish to sea water.

Materials and Methods

Striped bass (Morone saxatilis) were obtained and maintained as reported in Chapter 1 and by King and Hossler (1986). The fish were held for at least 3 days before experimentation. For acclimation studies, fish were transferred directly from freshwater (FW) to 3% salt water and then sampled after the time period designated in each experiment (either 1.5 h, 3 h, 6 h, 12 h, 24 h, 3 days, 7 days, or 14 days). All sampling was done at approximately 2:00 p.m. (except for the 12 h studies which were done at 8:00 a.m. or 8:00 p.m.) to avoid any variation due to circadian rhythm (see Simpson, 1978; Spieler, 1979; Bulger, 1986).

Na,K-ATPase Assay

This method for measuring Na,K-ATPase activity has been described previously (Zaugg, 1982). Striped bass, 18.5-22.0 cm long (54.0 - 124.5 g), were killed by decapitation, and the gill arches were dissected free. The filaments were cut from the arches and immersed in 1.5 ml of a solution (SEI) containing 0.3 M reagent grade sucrose, 0.02 M disodium ethylenediamine tetraacetate (Na_2EDTA), and 0.1 M imidazole, adjusted to a final pH 7.1 with HCl. The filaments were frozen at -50°C for up to 3 weeks until Na,K-ATPase activity was assayed. Zaugg (1982) reported no loss of enzyme activity in samples frozen for up to six weeks at -23° or colder.

The samples were thawed, placed on ice, and homogenized manually in a ground glass homogenizer with a glass pestle until all the filaments were distintegrated. The homogenate was centrifuged (10 min at 3100 rpm), and the supernatant solution was decanted and discarded. The tubes were inverted to drain. Pellets were suspended in 1 ml of SEID (SEI containing 0.1% sodium deoxycholate), homogenized manually in a ground glass homogenizer with a glass pestle, and centrifuged (8 min at 3100 rpm). The supernatant was withdrawn and assayed for Na,K-ATPase activity.

Two stock solutions were prepared. Stock solution A contained 28 mM $MgCl_2$, 155 mM NaCl, 75 mM KCl and 115 mM imidazole (pH 7.0). Stock solution B was identical to stock solution A but contained 0.58 mM ouabain.

A 10 µl aliquot of each enzyme preparation was placed into a test tube (in ice water) containing 0.65 ml of solution A, and another 10 µl aliquot of the enzyme preparation was placed in a test tube (in ice water) containing 0.65 ml of solution B. One hundred microliters of Na_2ATP (0.03 M, pH 7.0) was added to each tube. The tubes were removed from the ice bath, shaken, and then placed in a 37°C water bath for 10 min. The tubes were shaken gently during

the first minute of the incubation period. After incubation, the tubes were placed in ice water and shaken for 1 min.

The inorganic phosphate released was measured by the method of Fiske and SubbaRow (1925). One milliliter of 5% TCA (trichloroacetic acid) was added to each tube, and the tubes were centrifuged to remove the protein. To one ml of the supernatant were added 0.38 ml H_2O, 0.50 ml molybdate reagent (2.5% ammonium molybdate in 2.5 N H_2SO_4) and 0.12 ml reducing reagent (0.25% 1,2,4-aminonaphtholsulfate acid containing 0.5% Na_2SO_3 and 15% $NaHSO_3$). The tubes were incubated in a 37°C shaking water bath for 10 min, cooled in running water, and the phosphate content was determined by absorbance at 660 nm.

The difference between the amount of phosphate liberated by enzyme preparations with solution A (ouabain sensitive plus ouabain insensitive ATPase activity) and solution B (ouabain insensitive ATPase activity) is the amount of Na,K-ATPase activity. Enzyme activity is expressed as micromoles of inorganic phosphate liberated per milligram of protein per hour.

Protein was determined colorimetrically by the method of Lowry et al. (1951) with bovine serum albumin as a standard.

Paranitrophenyl Phosphatase (PNPPase) Assay

This method for measuring K^+-dependent phosphatase activity is based on that described by Judah et al. (1962). Striped bass, 4-20 cm long, were killed by decapitation. The gill arches were dissected free and the filaments were cut from the arches and placed in 0.25 M sucrose, 0.1 M hydroxymethyl-amino-

methane (Tris), pH 6.8. The filaments were blotted, weighed, placed in a glass homogenizer (on ice) with homogenizing solution (0.25 M sucrose, 5 mM Na_2EDTA, 30 mM Tris, 0.1% sodium deoxycholate, pH 6.8) at a level of 50 mg wet weight/ml and homogenized with a glass pestle. The homogenate was filtered through a wire mesh and 0.05 ml (2.5 mg tissue wet weight) of homogenate was added to control and experimental tubes. The control tubes contained 60 mM Tris-HCl, 1 mM ouabain, and 3 mM $MgCl_2$ (pH 7.3 at 37°C). The experimental tubes contained 10 mM KCl, 50 mM Tris-HCl, and 3 mM $MgCl_2$ (pH 7.3 at 37°C). The enzymatic reaction was started by the addition of paranitrophenyl phosphate (PNPP) to give a final concentration of 3 mM (final volume of 1 ml); the tubes were shaken in a 37°C water bath for 15 min, and the reaction was stopped by placing the tubes on ice and adding 3 ml of 0.1 N NaOH. After centrifugation, the absorbance of the supernatant was determined at 420 nm on a Model 100-40 spectrophotometer (Hitachi, Ltd., Tokyo, Japan). The amount of PNPP hydrolyzed was determined as follows: 0.62 O.D. units = 0.2 µmoles. Protein determinations were made by the Lowry method (Lowry et al., 1951).

^3H Ouabain Binding

This procedure is based on the method of Hossler (1980). The fish (15.5-22.0 cm long; 40.5 - 107.0 g) were killed by decapitation; the gill arches were carefully dissected out; and the filaments were cut from the arches and rinsed in 0.1 M Tris - HCl (pH 7.5) containing 0.25 M sucrose. The filaments were blotted, weighed, and homogenized in a ground glass tube with a glass pestle at 10 mg wet wt/ml. The incubation medium contained 0.25 ml of homo-

genate, 3 mM ATP, 6 mM $MgCl_2$, 100 mM NaCl, 50 mM Tris-HCl (pH 7.5 at 37°C), and 1 x 10^{-6} M ^{3}H-ouabain (7 Ci/mmol) in a total volume of 0.5 ml. The control medium lacked ATP and $MgCl_2$ and contained 5 mM EDTA, 10 mM KCl, and 145 mM Tris-HCl (pH 7.5 at 37°C). Incubation was for 15 min at 37°C with agitation. The binding reaction was stopped by placing the incubation tubes on ice. Unbound ^{3}H-ouabain was removed by filtering and washing on glass-fiber filters (Gelman type A-E; Gelman Instrument Company, Ann Arbor, Michigan) with 5 aliquots (about 10 ml each) of an ice-cold wash solution containing 6 mM $MgCl_2$, 100 mM NaCl, and 50 mM Tris-HCl (pH 7.5). After washing, the filter disks were dried overnight in an oven (GCA Corporation) and placed in toluene-Triton X-100 scintillation fluid. The bound isotope was counted in a Beckman LS 9800 scintillation counter (which had a counting efficiency for ^{3}H of 50%; Beckman Instruments, Irvine, CA). Counting efficiency was determined by using an internal standard.

^{36}Chloride Efflux

Gill ^{36}Cl efflux was measured in intact unanesthesized specimens as described previously (Hossler et al., 1979c; Epstein et al., 1985). Striped bass weighing 10.5 - 91.5 g (9.5-21.0 cm long) were injected intraperitoneally with 1-2 µl of ^{36}Cl, immersed in 1000 ml of oxygenated freshwater or sea water, and allowed to equilibrate for 20 min before taking a 1 ml sample of the bath was taken (CPM_0). Another bath sample was taken after 1 h (CPM_1), and the fish was bled with a heparinized syringe via a heart puncture. The blood was centrifuged and 20 µl plasma was placed into 1 ml distilled water. Both bath samples and the plasma sample were placed in 10 ml toluene-Triton X-100 scintillation fluid and counted by liquid scintillation. The plasma clearances of

^{36}Cl were calculated from the radioactivity excreted into the bath and the residual radioactivity in the plasma as follows:

$$\text{plasma clearance (ml/g/h)} = \frac{(CPM\ )\ (1000)}{(CPM_S)\ (50)\ (W)}$$

$CPM = CPM_1 - CPM_0$

CPM_S = counts per min/20 µl plasma

W = weight of the fish in grams

All of the radioisotope excreted under these conditions is thought to be excreted by the gills (Epstein et al., 1985).

Plasma Osmolality, Chloride, and Corticosteroids

The fish were maintained for at least 3 days before use in a 12 h light/12 h dark cycle at room temperature before experimentation. This allowed for adaptation to the light cycle and temperature which are known to affect hormone levels (see Fivizzani et al., 1984) and permitted cortisol levels to re-equilibrate after the stress of handling, confinement, and transportation (Wedemeyer, 1972; Barton et al., 1980; Tomasso et al., 1980). Food was withheld for 24 h before the beginning of the experiments to prevent plasma cortisol changes (Speiler and Noeske, 1981).

The 13-25 cm (22-159 g) striped bass were anesthetized with 0.02% MS-222 (tricaine methanesulfonate) and bled within 5 min to avoid

corticosteroid hormone changes due to stress (Davis et al., 1982). Blood taken with a sodium heparinized syringe via a heart puncture was centrifuged and the resulting plasma was stored frozen (-50°C) until analyzed. Plasma chloride concentrations were determined by amperometric-coulometric titration with an Aminco Analytical Chloride Titrator (American Instrument Company, Silver Spring, MD). Total plasma corticosteroids were measured with a cortisol radioimmunoassay kit (Serono Diagnostics, Inc., Braintree, MA) and a Prias Liquid Scintillation Counter (Packard Instrument Company, Inc., Downers Grove, IL). Plasma osmolality was measured with a Model 5500 Wescor vapor pressure osmometer (Logan, Utah).

Statistics

The data were evaluated by analysis of variance ($p < 0.05$) for overall significance. Comparisons between experimental groups and the freshwater group (0 h) were evaluated by Dunnett's test ($p < 0.025$; Winer, 1971).

Results

All of the data obtained is presented in Figs. IV-1 to IV-7. Due to the stress placed on the fish during transport from the hatchery and during confinement in the holding tanks, this data can be considered only preliminary. Many long term (over one week) studies were not feasible due to disease and stress. These factors may have affected the results obtained in the short term studies. Because of the high variability between fish and the availability

of fish from the hatcheries, all time periods for each experiment could not be attempted. Time periods found to be significant in previous studies on other species were selected in order to gain an overall picture of adaptation to sea water during the first few hours and days after transfer.

Figures IV-1 and IV-2 show the time course of changes in Na,K-ATPase and PNPPase activity when striped bass are transferred directly from freshwater to sea water. Both assays are used as a measure of Na,K-ATPase activity (Skou, 1975; Mayahara et al., 1983; see General Introduction) and were measured over a period of at least one week to allow time for possible Na,K-ATPase activity changes (Forrest et al., 1973a,b; Jacob and Taylor, 1983). Although there is a decrease in PNPPase activity after 24 h, Na,K-ATPase activity remains at or above freshwater levels. Thereafter both enzyme activities increase and reach maximum levels 3 days after transfer. At day 3, Na,K-ATPase and PNPPase activities are 1.6 and 1.4 times greater than the freshwater levels, respectively, but statistically the increases are not significant. After 3 days in sea water both enzyme activities fluctuate, possibly due to the development of disease in some fish.

The optimal pH for PNPPase activity in striped bass gills under the described assay conditions was found to be 7.3, which is similar to that found for Na,K-ATPase activity in other species (e.g., pH 7.5, Dendy et al., 1973; pH 7.5, Kamiya and Utida, 1968; and pH 7.2, De Renzis and Bornancin, 1984).

Ouabain binding (Fig. IV-3) is used as a measure of the amount of Na,K-ATPase present (Schwartz et al., 1975; Hossler et al., 1979c; Hossler, 1980). Ouabain binding studies were done only over the first 24 h after

transfer to determine if the amount of Na,K-ATPase increased within the first few hours. No significant difference was found between the FW (0 h), 3 h, and 24 h specimens.

Chloride efflux (Fig. IV-4) gradually increases during the first 24 h. The values from fish that have been in sea water for 24 h and 7 days are 5 and 26 times, respectively, that of the freshwater values.

Plasma osmolality and chloride values (Figs. IV-5 and IV-6) increase within 3 h after transfer. Plasma osmolality stays elevated even after a week in sea water. Plasma chloride returns to freshwater levels 3 days after transfer, coinciding with the peak in Na,K-ATPase and PNPPase activities.

Thirty-one percent of the plasma corticosteroid measurements obtained in the adaptation study were out of the range (1-60 µg/dl) of the RIA kit used. Only the data obtained within the range is illustrated in Fig. IV-7. All of the actual values are listed in the legend of Fig. IV-7.

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; potassium 3 mg/L; chloride 29 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; total dissolved solids 180 mg/L; conductivity 284 µmho/cm; pH 7.3. The calcium level fluctuates somewhat due to the water source. Ion concentration in the hatchery water at the Southeastern Fish Cultural Laboratory (Marion, Alabama) are as follows: carbon dioxide 6.4 ppm; total alkalinity 106.5 ppm; total hardness 106.6 ppm; calcium hardness 81.1 ppm; ammonia 0.15 ppm; nitrite 0.012 ppm; nitrate 0.01 ppm; phosphate 0.029 ppm; chloride 1.40 ppm; sulfate 3 ppm; sodium 1.9 ppm; potassium 3.6 ppm; calcium

Fig. IV - 1 Changes in Na,K-ATPase activity of striped bass gills following transfer of freshwater-acclimated fish to sea water. Values represent the mean (M) ± SE. The 0-hour sample was taken prior to the transfer.

The actual values are as follows:

0 h	4.78 ± 0.57 (5)
3 h	6.25 ± 0.82 (5)
1 day	5.78 ± 0.99 (6)
3 days	8.91 ± 0.92 (2)
7 days	7.97 ± 2.43 (5)

The numbers in parentheses are the number of fish sampled. No significant difference was found between the groups.

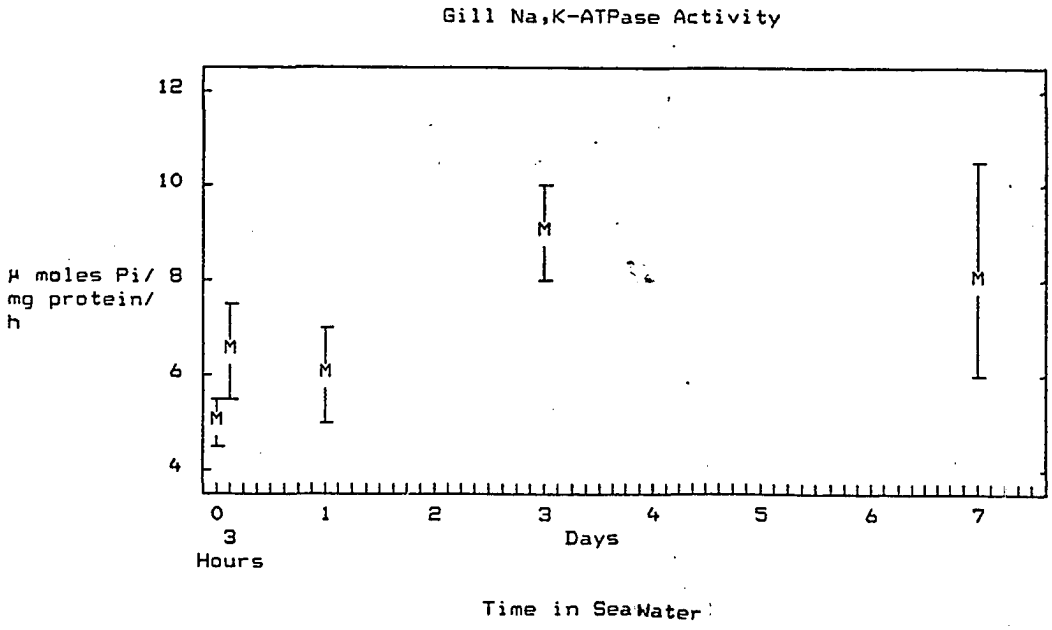


Fig. IV - 2 Changes in PNPPase activity of striped bass gills following transfer of freshwater acclimated-fish to sea water. Values represent the mean (M) ± SE. The hour sample was taken prior to the transfer.

The actual values are as follows:

0 h	0.96 ±0.10	(9)
3 h	1.04 ±0.17	(6)
6 h	0.86 ±0.14	(5)
12 h	0.88 ±0.10	(6)
1 day	0.65 ±0.15	(6)
2 days	0.97 ±0.24	(3)
3 days	1.35 ±0.35	(4)
4 days	0.83 ±0.16	(7)
7 days	1.32 ±0.16	(7)
14 days	0.97 ±0.18	(5)

The number in parentheses represent the number of fish sampled. No significant difference was found between the groups.

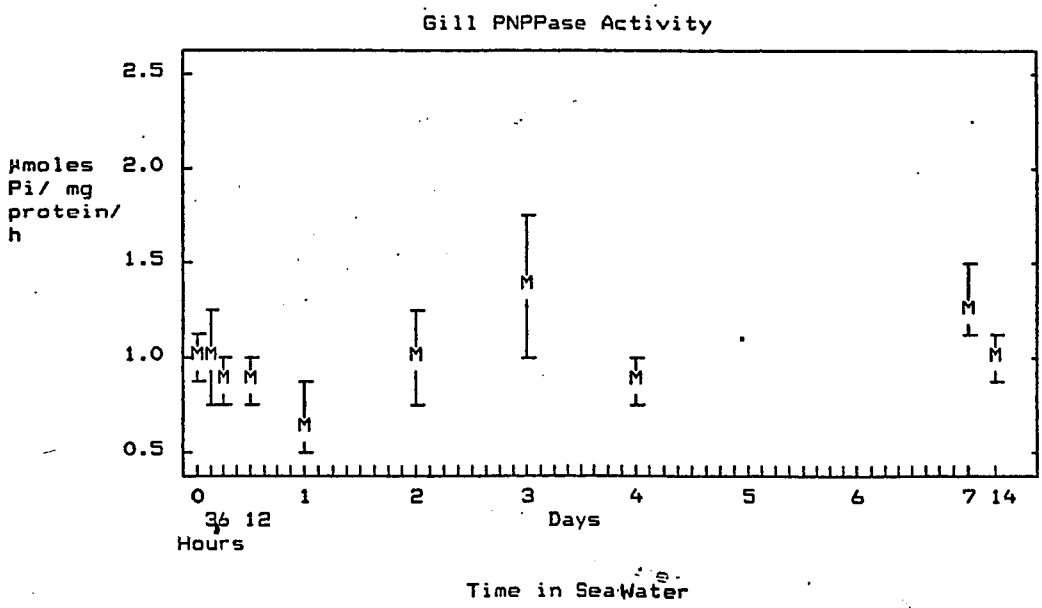


Fig. IV - 3 Changes in ouabain binding of striped bass gills following transfer of freshwater-acclimated fish to sea water. Values represent the mean (M) ± SE. The 0-hour sample was taken prior to the transfer.

The actual values are as follows:

0 h	0.58 ± 0.17 (5)
3 h	0.78 ± 0.17 (5)
1 day	0.63 ± 0.09 (4)

The numbers in parentheses represent the number of fish sampled. No significant difference was found between the groups.

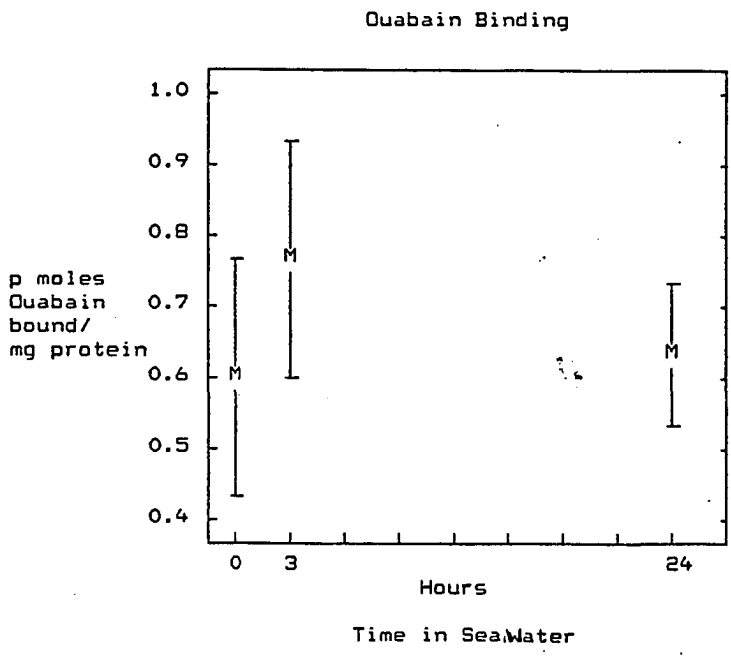


Fig. IV - 4 Changes in ^{36}chloride efflux of striped bass following transfer of freshwater-acclimated fish to sea water. Values represent the mean (M) ±SE. The 0-hour sample was taken prior to the transfer.

The actual values are as follows:

0 h	0.01±	0.01	(5)
1.5 h	0.02±	0.01	(4)
3 h	0.02±	0.01	(4)
6 h	0.03±	0.01	(5)
12 h	0.04±	0.02	(3)
1 day	0.05±	0.01	(3)
7 days	0.17±	0.02	(4)*

The numbers in parentheses represent the number of fish sampled. The * represents a significant difference between the group indicated and the 0 h group as determined by Dunnett's test (p , 0.025; Winer, 1971).

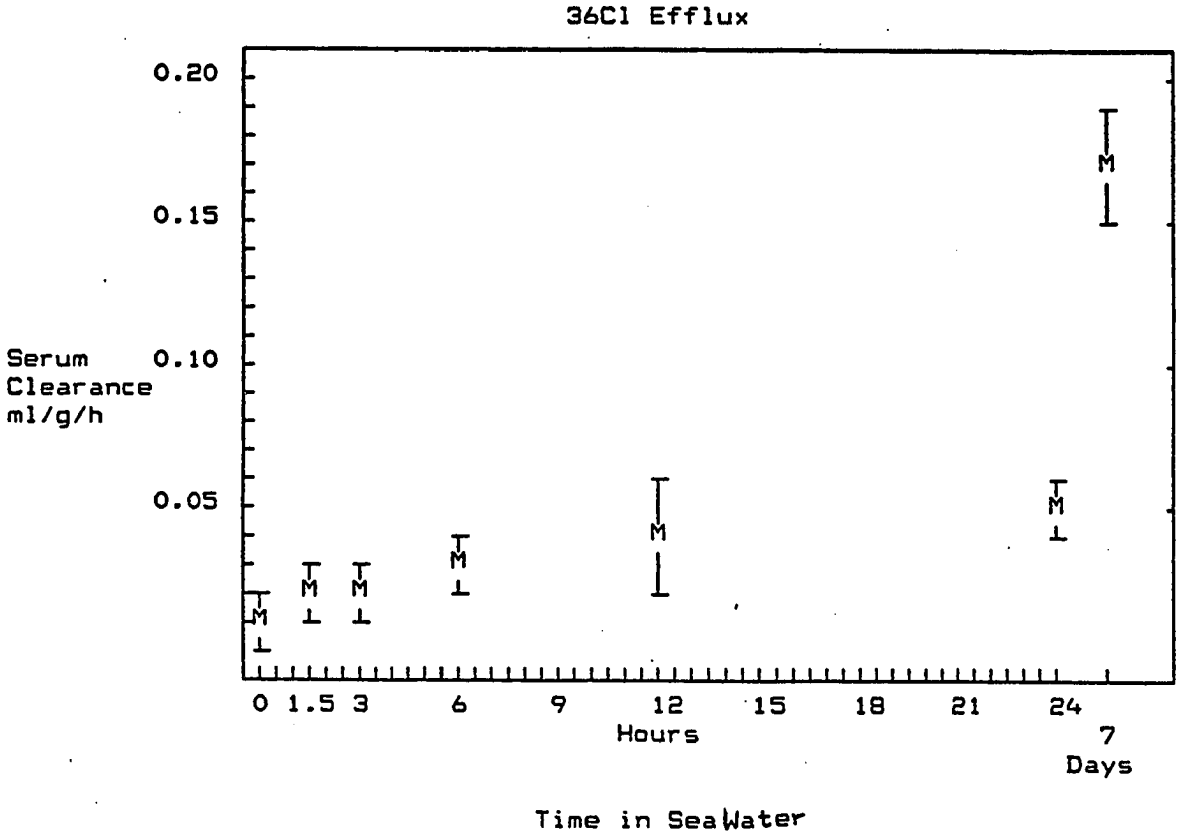


Fig. IV - 5 Changes in plasma chloride concentrations of striped bass following transfer of freshwater-acclimated fish to sea water. Values represent mean (M)± SE. The 0-hour sample was taken prior to the transfer.

The actual values are as follows:

0 h	162.2 ± 4.6 (29)
3 h	204.4 ± 9.6 (12)
6 h	194.3 ± 5.8 (14)
1 day	197.7 ± 8.5 (21)
3 days	170.8 ± 4.2 (16)
5 days	206.2 ± 24.2 (2)

The numbers in parentheses represent the number of fish sampled. No significant difference was found between the groups.

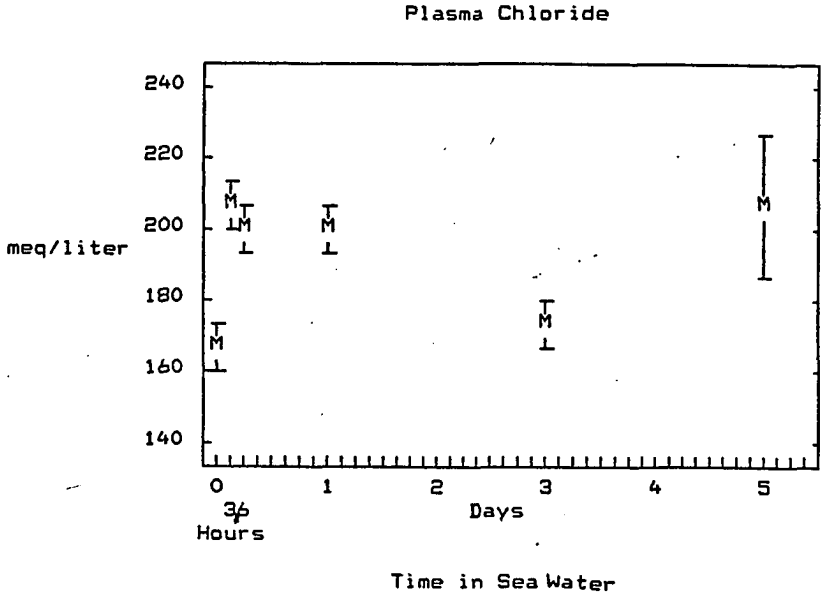


Fig. IV - 6 Changes in plasma osmolality of striped bass following transfer of freshwater-acclimated fish to sea water. Values represent the mean (M) ± SE. The 0-hour sample was taken prior to the transfer.

The actual values are as follows:

0 h	327.2 ±	6.6	(14)
3 h	418.8 ±	10.9	(13)*
6 h	414.2 ±	9.3	(12)*
1 day	410.3 ±	14.4	(13)*
3 days	409.2 ±	11.0	(13)*
5 days	464.5 ±	32.5	(2)*
7 days	405.7 ±	11.4	(3)*

The numbers in parentheses represent the number of fish sampled. The * represents a significant difference between the group indicated and the 0 h group as determined by Dunnett's test ($p < 0.025$; Winer, 1971).

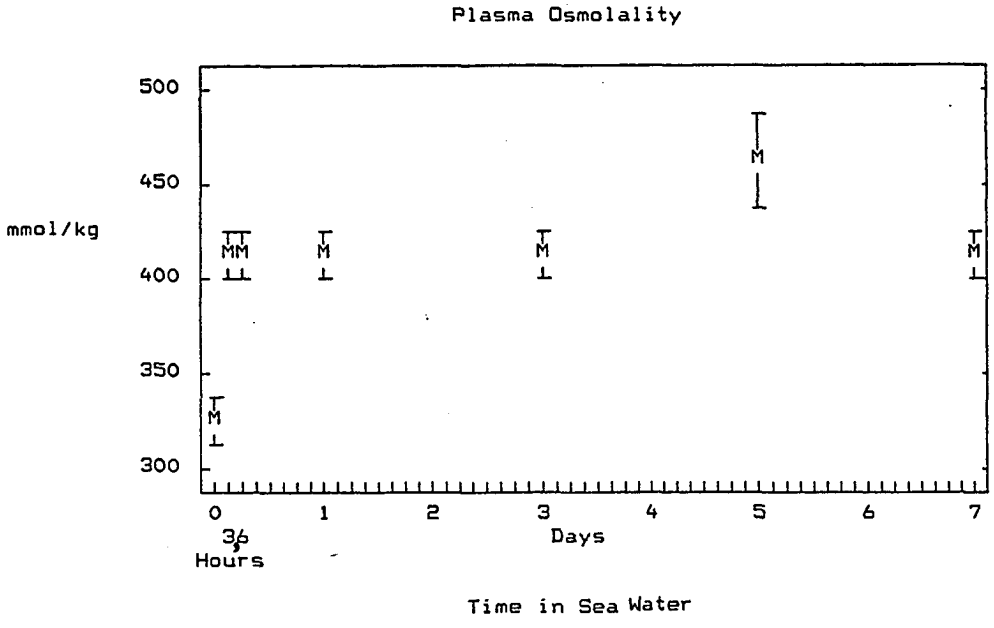


Fig. IV - 7 Changes in plasma corticosteroids of striped bass following transfer of freshwater-acclimated fish to sea water. Values represent the mean (M) ± SE. The 0-hour sample was taken prior to the transfer.

The actual values are as follows:

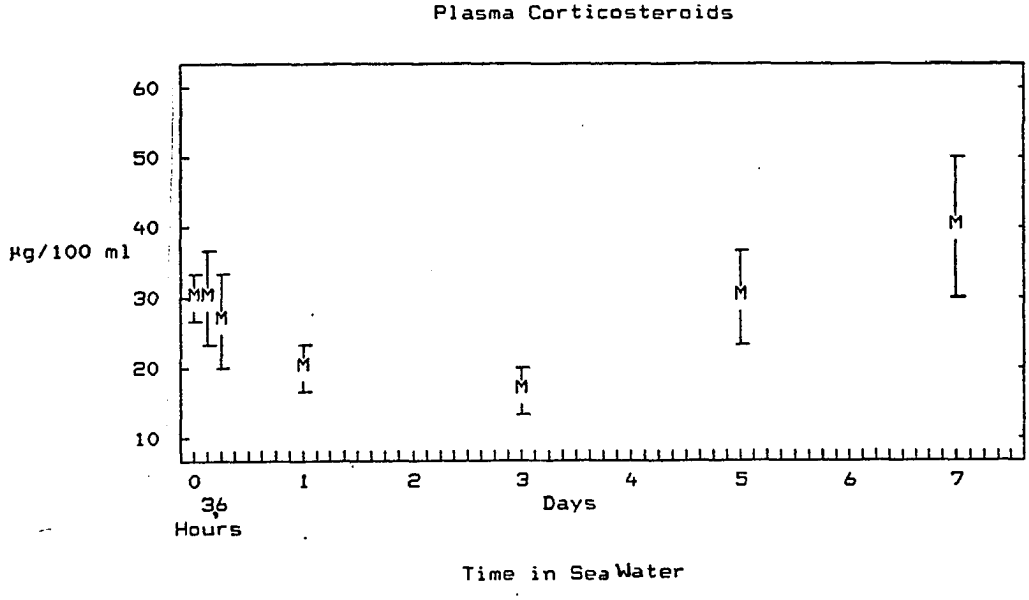
0 h	28.2 ± 4.7	(15)
3 h	30.3 ± 6.4	(8)
6 h	26.4 ± 7.8	(8)
1 day	20.6 ± 4.6	(10)
3 days	18.1 ± 3.4	(14)
5 days	31.1 ± 7.5	(2)
7 days	40.2 ±10.0	(2)

The numbers in parentheses are the number of fish sampled. No significant difference was found between the groups.

If the values obtained within the assay range as described above are combined with the values obtained in this study outside the 1-60 µg/dl range the results are as follows:

0 h	27.6 ± 21.4	(18)
3 h	69.5 ± 45.8	(15)
6 h	43.4 ± 28.3	(13)
1 day	52.6 ± 48.1	(16)
3 days	35.7 ± 35.8	(18)
5 days	31.1 ± 7.5	(2)
7 days	48.0 ± 13.7	(3)

Statistical analysis was not applied to this data.



41.3 ppm; magnesium 5.5 ppm; manganese 0.12 ppm; zinc 0.005 ppm; copper 0.01 ppm; ferrous iron 0.08 ppm; total iron 0.90 ppm; conductivity 92 µmho/cm; pH 7.5.

Discussion

The results from the Na,K-ATPase and PNPPase studies are difficult to evaluate because seasonal variations (Zaugg and McLain, 1970; Boeuf et al., 1978; Lassere et al., 1978), water temperature, animal age/size (Courtois, 1976; De Renzis and Bornancin, 1984; McCormick and Naiman, 1984), disease, and stress may affect measurements. Also, the assay conditions may not have been optimal for striped bass gill Na,K-ATPase and PNPPase activity, since the concentrations used were derived from experiments on other species. Possible inhibition of Na,K-ATPase by zinc, copper, and aluminum in the hatchery and laboratory water were examined. The concentrations present were below those known to cause inhibition (Lorz and McPherson, 1976; Stagg and Shuttleworth, 1982; Crespo and Karnaky, 1983; Staurnes et al., 1984a,b).

Both the Na,K-ATPase and PNPPase assays require sodium deoxycholate, a detergent, as part of the homogenizing solution. Detergents and freezing (Na,K-ATPase assay) are thought to increase Na,K-ATPase activity of homogenates because exposure of the enzyme active sites to the substrate is enhanced (Skou, 1961; Bonting, 1970; Jorgensen and Skou, 1971; Rostgaard and Moller, 1971; Zaugg, 1982). Zaugg's method (Zaugg, 1982) for measuring Na,K-ATPase activity includes a partial purification of the gill homogenate, and specific activities measured by this method are generally higher than those obtained from crude homogenate preparations such as in the PNPPase assay. The results from both assays show similar trends, although specific activities using the Na,K-ATPase assay are higher for each time period tested.

Na,K-ATPase activity in eels (Forrest et al., 1973a,b) and killifish (Jacob and Taylor, 1983) appear to take several days after exposure to sea water to reach maximal levels. In eels Na,K-ATPase activity starts increasing after 3 days in sea water and is maximal after 2 weeks. Killifish gill Na,K-ATPase activity decreases for 2 days after transfer and then reaches its maximum 3 days after transfer and remains high. Striped bass also reach maximal recorded activity 3 days after transfer, but activity fluctuated considerably. Statistically, there is no significant difference between the freshwater (0 h) and the sea water activities. This may be due to the small sample sizes and the high variability between fish.

The decrease in gill PNPPase activity observed in striped bass during the first 24 h in sea water is similar to the reduction in Na,K-ATPase activity in eel gills reported by Utida et al. (1971) and Bornancin and De Renzis (1972). Bornancin and De Renzis (1972) proposed that the initial reduction may represent the freshwater form of Na,K-ATPase being turned off before a sea water form is turned on (Motais, 1970a,b; Pfeiler and Kirschner, 1971; Gallis et al., 1979).

The increase in striped bass gill Na,K-ATPase activity during acclimation to sea water (3% salt water) is not as dramatic as in other species (see General Introduction). This may be due to suboptimal assay conditions. It is also possible that striped bass have the capability of osmoregulation at even higher salinities as do Cyprinodon variegatus (200% seawater, Karnaky et al., 1976a). If so, 3% salt water may only require the activation of the transport mechanisms of a fraction of the chloride cells. This may be represented ultrastructurally by the alteration of only some of the apical crypts during acclimation to sea water (see Chapter 1). Also, the opercular epithelium of striped bass may be responsible for much of the ion regulation, as proposed by

Payan and Girard (1984) for other species. In Fundulus, chloride cells comprise only 6% of the cells in the gill epithelium but make up 50-70% of the opercular epithelial cells (Karnaky and Kinter, 1977).

Ouabain binding increases with increasing salinity in some species (Karnaky et al., 1976b; Hossler et al., 1979b,c; Hossler, 1980) and may begin increasing within the first 24 h after transfer to sea water (Hossler, 1980). No change occurs during the first 24 h after transfer of striped bass to sea water. The lack of change may be due to suboptimal assay conditions or it may represent a difference between the maximum catalytic rates in mullet (Hossler, 1980) and striped bass. Perhaps mullet Na,K-ATPase complexes are working near maximum efficiency in freshwater. Transfer to sea water may require an immediate increase in Na,K-ATPase molecules to handle the osmotic stress. Towle et al. (1977) hypothesized that short-term salinity changes involve catalytic rate modifications rather than changes in the number of Na,K-ATPase molecules. This is may be true for striped bass.

Na^+ and Cl^- efflux rates have been used in a variety of species as a measure of osmoregulation (Maetz et al., 1969; Epstein et al., 1980, 1985; Evans et al., 1973; Forrest et al., 1973a,b; Bath and Eddy, 1979; Hossler et al., 1979c; Karnaky et al., 1977). Maetz et al. (1969) have shown that Na^+ efflux in eels starts increasing within the first 5 h after transfer to sea water, while it takes approximately 10 h in rainbow trout (Bath and Eddy, 1979). Chloride efflux in striped bass starts increasing within 3-6 h after transfer. If chloride efflux in striped bass is an active process as it is in other species (see General Introduction), an energy dependent enzyme complex must be required. Since Na,K-ATPase activity does not appear to increase significantly, another enzyme may be responsible for the efflux at this early time.

Plasma osmolality levels for sea water fish are reported to be 20% higher than those of freshwater fish (Johnson, 1973). In striped bass, the plasma osmolality for a fish acclimated to sea water for 7 days is 24% higher than for the freshwater adapted fish. Leray et al. (1981) reported that plasma osmolality reached a maximum 2-4 h after rainbow trout were transferred to sea water but returned to freshwater levels in 6-10 days. In striped bass, plasma osmolality is highest after 3 h in seawater and remains elevated for the first week. No long term osmolality studies were done.

Davis et al. (1982) reported plasma chloride levels for freshwater striped bass to be 118.8 ± 5.2 meq/L ($\bar{x}$ ±SE). We found plasma chloride levels for freshwater striped bass to be 36% higher than those reported by Davis et al. Because of the small volume of plasma obtained from the fish used in this study, evaporation may have occurred resulting in more concentrated plasma chloride. Striped bass plasma chloride is maximal after only 3 h in sea water, as compared to 72 h and 2 days in other species (Threadgold and Houston, 1964; Epstein et al., 1980).

Davis et al. (1982) reported plasma corticosteroid concentrations of "undisturbed" striped bass to be 2.99± 0.71 µg/100 ml ($\bar{x}$ ±SE). The freshwater values obtained in this study (28.2 ± 4.7 µg/100 ml; $\bar{x}$ ± SE) are 10 times higher. This could have resulted from stress due to disease. Because of the high corticosteroid values for the freshwater fish used in this study, the effects of sea water adaptation caused many of the values obtained to be above the range of the assay. This assay must be repeated with fish under considerably less stress to obtain results that reflect adaptation to sea water and not just general stress.

Long-term studies of these parameters are needed to fully understand striped bass adaptation to sea water. Also, other transport or cotransport systems such as the $Na^+/K^+/2Cl^-$ cotransport system (Epstein et al., 1983; Burnham et al., 1985; Geck and Heinz, 1986; Jorgensen, 1986; Silva et al., 1986) should be investigated for their possible role in striped bass osmoregulation.

GENERAL DISCUSSION

The objective of this study has been to investigate the adaptation of striped bass to sea water with special emphasis on the chloride cell of the gills. Ultrastructural studies suggest that exposure to sea water (3% salt water) for one week induces an increase in chloride cell number, size, and mitochondrial content, as well as an amplification of the basolateral plasma membrane of these cells. The apical crypts start to undergo morphological changes within the first 3-6 h after transfer and increase in number 32.5% above freshwater fish after a two-week period. In fish that have been exposed to sea water for 7 days, the apical crypts exhibit morphological diversity; some contain obvious cellular extensions and others are pit-like in structure. Other species such as mullet (Hossler, 1980) exhibit exclusively the deep, pit-like crypts in sea water. Since chloride cells have been shown to be the site of gill Na,K-ATPase activity (Mizuhira et al., 1970; Kamiya, 1972b; Sargent et al., 1975; Hootman and Philpott, 1978; Naon and Mayer-Gostan, 1983) and chloride concentration (Copeland, 1948; Philpott, 1965; Petrik, 1968; Bierther, 1970; Masoni and Garcia Romeu, 1973), it is hypothesized that they are responsible for the active chloride secretion of the gills (see review by Karnaky, 1986). It has been proposed that the deepening of the apical pit increases surface area for ion transport and allows access to the external environment for all the chloride cells which share an apical crypt (Hossler et al., 1979b). The morphological changes in crypts that occur in striped bass during adaptation to sea water may reflect the recruitment of a fraction of the chloride cells for active chloride extrusion. Activation of all of the chloride cells may not be necesary during

adaptation to sea water (3% salt water) since striped bass may be able to tolerate even higher salinities as can Cyprindon variegatus (200% seawater, Karnaky et al., 1976a).

Na,K-ATPase has been localized to the basolateral plasma membrane tubular system of chloride cells (Mizuhira et al., 1970; Shirai, 1972; Silva et al., 1977; Karnaky et al., 1976b; Hootman and Philpott, 1979). In many species Na,K-ATPase activity increases by 2-6 fold upon adaptation to sea water (see General Introduction). The Na,K-ATPase pump is thought to supply the gradient necessary for salt extrusion (see Karnaky, 1986). Na,K-ATPase activity of striped bass gill filaments does not seem to increase during the first 24 h after transfer to sea water but is highest 3 days after transfer. The fact that chloride efflux increases within 3-6 h after transfer to sea water and that there is initially (during first 24 h) no increase in Na,K-ATPase activity suggests that other transport mechanisms are responsible for short term osmoregulation.

The increase in Na,K-ATPase activity during acclimation to sea water (1.4-1.6 times freshwater levels) is not as dramatic as in other species (2-6 times freshwater levels; see General Introduction). This may be due to fish stress and disease or to suboptimal assay conditions. It is feasible that the opercular epithelium may be responsible for a great deal of the osmoregulatory capabilities in striped bass. It is also possible that other transport mechanisms, such as the $Na^+/K^+/2Cl^-$ coport are more active than Na,K-ATPase in osmoregulation of striped bass gills. Further studies should investigate whether inhibitors of $Na^+/K^+/2Cl^-$ coport such as bumetanide, and inhibitors of Na,K-ATPase such as ouabain, decrease chloride efflux.

Ouabain binding studies of striped bass gill filament homogenates indicate that no increase in Na,K-ATPase enzyme complexes occurs during the first 24 h after transfer to sea water. Some species such as mullet (Hossler, 1980) have a significant increase in the number of Na,K-ATPase molecules (ouabain binding sites) during the first 24 h of acclimation to sea water. Towle et al. (1977) hypothesized that short term salinity changes involve catalytic rate modifications rather than changes in the number of Na,K-ATPase molecules, which may be true for striped bass. It is also possible that mullet Na,K-ATPase complexes are working near their maximum efficiency in freshwater. Transfer to sea water may require an immediate increase in Na,K-ATPase molecules to handle the osmotic stress. Striped bass Na,K-ATPase complexes may be working at submaximal activity in freshwater. Exposure to sea water only increases enzyme efficiency. Long term ouabain binding studies are needed to determine if Na,K-ATPase complex increase after 24 h in sea water.

Essential to osmoregulation mechanisms is the blood flow in the gills and that is described here using vascular corrosion casting (see Chapter 2). The control mechanism for possible blood flow changes in striped bass gill filaments during increased osmotic stress may occur at a variety of places and may include shunts, sphincters, and cellular contraction. The constrictions of the afferent filamental arteries (see Chapter 2 and the Appendix) may represent sites of control of blood flow to the whole filament. Interlamellar blood distribution mechanisms could be controlled by sphincters in the afferent lamellar arterioles. Intralamellar distribution mechanisms may include shunting through the marginal vessel or basal channel and be affected by pillar cell contraction. Sphincters in efferent filamental arteries (see Chapter 2 and the Appendix) may force blood into the central venous sinus via the arterio-venous

anastomoses. Chloride cells have been associated with both the central venous sinus with its arterio-venous network, and with the basal channel of the respiratory lamellae. The elaborate central venous sinus-accessory companion vessel complex may provide a counter-current mechanism of blood flow in the chloride cell area, giving it a possible role in osmoregulation.

Although this study has established that many morphological, enzymatic, and hormonal changes occur during the adaptation of striped bass to sea water, many questions remain unanswered. Future studies must focus on (1) the roles of transport and coport mechanisms other than Na,K-ATPase in osmoregulation; (2) the initiation and control of long term versus short term adaptation mechanisms; (3) cell junctional complex changes during osmotic adaptation; (4) the function of the central venous sinus complex and its response, if any, to increased salinity; and (5) the possible differences in osmoregulatory capabilities between striped bass that are progeny of freshwater adapted fish and those that are progeny of sea water fish.

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APPENDIX

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The gill arch of the striped bass, *Morone saxatilis*. I. Surface ultrastructure

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SUMMARY - The surface ultrastructure of the four gill arches of the striped bass, *Morone saxatilis*, are described anatomically and morphometrically using routine scanning electron microscopic techniques. The gill arches are structurally similar to those of other euryhaline teleosts. Filaments are free for most of their length, have convex afferent surfaces and flat efferent surfaces, and support rows of well developed respiratory lamellae. The latter are triangular and leaf-like, conform to the shape of the filament, and have a wide leading edge exposed on the efferent filament surface. Pavement cells, with obvious, concentrically arranged surface microplicae cover most surfaces of the gill arches. The microplicae are much less obvious or absent on the pavement cells of the respiratory lamellae. Apical crypts of chloride cells are abundant on the afferent and interlamellar filament surfaces, but are absent elsewhere on the gill arch. Gill rakers on the anterior row of the first gill arch are long, well developed processes, but rakers in the posterior row of the first gill arch and in both rows of the other gill arches are reduced to short, spine-studded, raised areas of the epithelium. Taste buds are abundant on the pharyngeal surfaces of the gill arches and on all gill rakers. The highly developed gill arch structure is typical of a moderately active, euryhaline teleost.

KEY WORDS *striped bass - Morone saxatilis - gill - scanning electron microscopy - chloride cell*

INTRODUCTION

There has been increasing interest in the biology of the striped bass, *Morone saxatilis*, in recent years because of the value and extent of the sports fishery of this species in both marine (St. Laurence Seaway to Florida; Gulf of Mexico; and California coast) and freshwater environments (see Pfuderer *et al.*, 1975), and because of the recent decline in the numbers of this species as a result of pollution of these environments (Hazel *et al.*, 1971; Pfuderer *et al.*, 1975; Dawson, 1982; Mehrle, 1982; Hall *et al.*, 1982, 1984; Wright *et al.*, 1985). In addition because striped bass are an anadromous species and are known to tolerate abrupt changes in salinity (Tagatz, 1961; Otwell and Merriner, 1975; Hossler, unpublished observations) they provide a useful model system for studies of the rate of osmotic adaptation and the mechanism of osmoregulation. Most studies of striped bass to date have dealt with culture conditions, growth rates, and distribution (see bibliography compiled by Pfuderer *et al.*, 1975; and reviews by Kerby *et al.*, 1983, and Geiger and Parker, 1985) but very few reports have dealt with the physiology, biochemistry, and anatomy (Groman, 1982) of this species. In preparation for studies on the mechanism of osmoregulation we present here a description of the surface ultrastructure of the gill arches of the striped bass. A preliminary account of this study has appeared in abstract form (Harpole and Hossler, 1983).

MATERIALS AND METHODS

Striped bass, *Morone saxatilis*, 3-20 cm in length were donated by the Eagle Bend Fish Hatchery and Morristown State Fish Hatchery, Tennessee Wildlife Resources Agency. Fish were maintained in aerated, 100 l tanks (20-24 °C) in tap water or 0.1% sea salts (w/v; Instant Ocean Salts, Aquarium Systems, Mentor, Ohio) at least one week before use. Fish were fed processed, trout fry pellets (Silver Cup Fish Feed, Murray Elevators, Murray, Utah) and kept on a cycle of approximately 11 h of subdued light and 13 h of dark.

Fish were killed by decapitation, the opercula were spread laterally, and each gill arch was carefully removed, rinsed free of blood with 0.9% NaCl, and placed in freshly prepared fixative for 2 h at 20-24 °C or overnight at 4 °C. Fixative consisted of 2.5% glutaraldehyde and 1.8% paraformaldehyde in 0.1M cacodylate-HCl buffer (pH 7.2). After fixation gill arches

were rinsed for 1 h with 3 changes of excess buffer (0.2M cacodylate-HCl, pH 7.2), then post-fixed for 2 h at 4 °C in 2% OsO_4 in 0.1M cacodylate-HCl (pH 7.2). Gill arches were rinsed in buffer as before, then dehydrated in a graded ethanol series during a period of 1-2 h. Gill arches were placed in a critical point drying apparatus (model E3000, Polaron Instruments, Inc., Hatfield, PA) in 100% ethanol, dried with liquid CO_2, affixed to specimen stubs with silver paste, coated with a thin layer of gold or gold-palladium in a sputter coating apparatus (model Desk 1, Denton Vacuum, Inc., Cherry Hill, NJ), and observed in a model S430, scanning electron microscope (Hitachi Scientific Instruments, Mountain View, CA). Measurements were made from electron micrographs.

For light microscopy gills were fixed as described above, dehydrated first in a graded ethanol series then in propylene oxide, and embedded in eponaraldite (Mollenhauer, 1964). Sections were cut (2 µm thick), mounted on glass slides, stained with toluidine blue (1% in 1% borate), and photographed with a Zeiss standard light microscope.

RESULTS

Striped bass are especially subject to shock during transport and handling. The mortality rate is usually reduced by covering tanks and adding up to 0.1% sea salts to the water during transport, and by maintaining fish in tanks partially covered with translucent shields or cardboard and located in low-traffic, low-light areas of the laboratory.

Gill arch structure is similar to that of other teleosts (see Hughes, 1984; Laurent, 1984). Two rows of filaments (anterior and posterior hemibranches) extend posterolaterally and two rows of rakers extend anteromedially from each of the four arches, designated I, II, III and IV, rostral to caudal (Figs. 1 and 2). Both rows of filaments curve slightly posteriorly *in vivo*, and are free for most of their length (i.e., are perciform type teleost gills; Dornescu and Miscalencu, 1968). Filament length varies with different gill arches and with different locations on a given arch. Filaments of the posterior hemibranch are longer than those of the anterior hemibranch on the dorsal aspects of arches I and II. Filaments of the anterior hemibranch are longer than those of the posterior hemibranch on the ventral aspects of arches II, III and IV. Elsewhere the filaments of each hemibranch are similar in length.

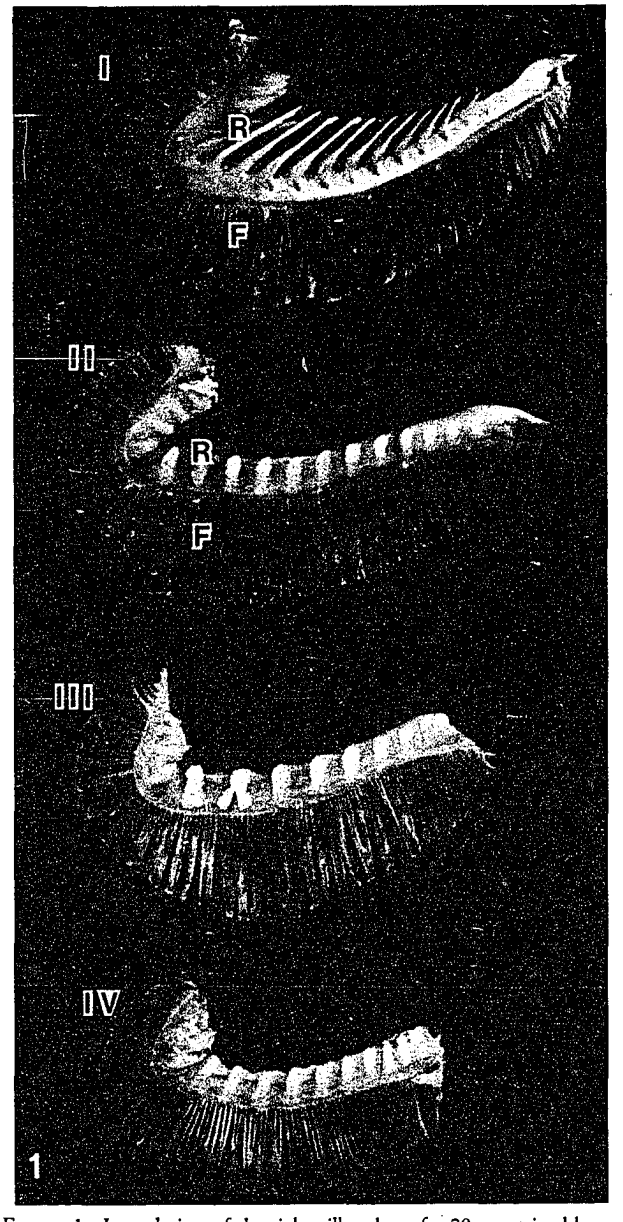
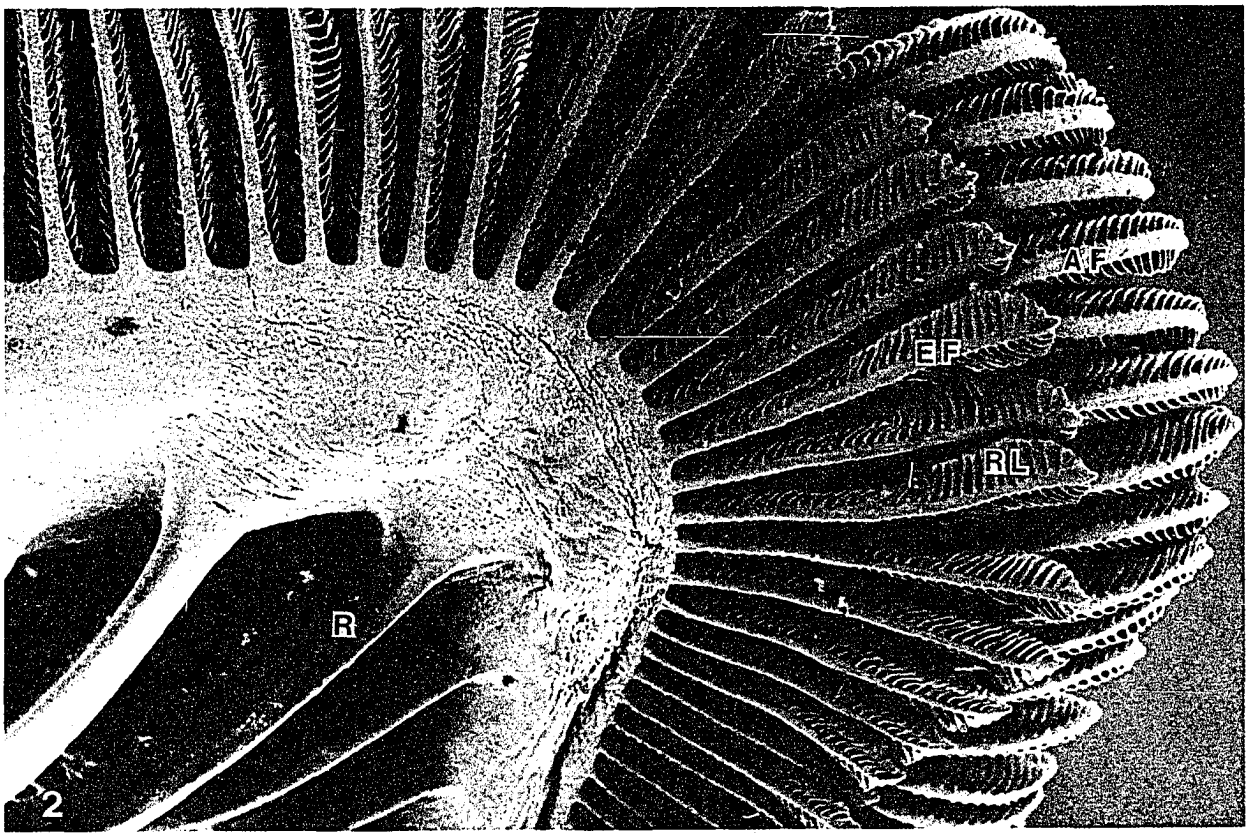
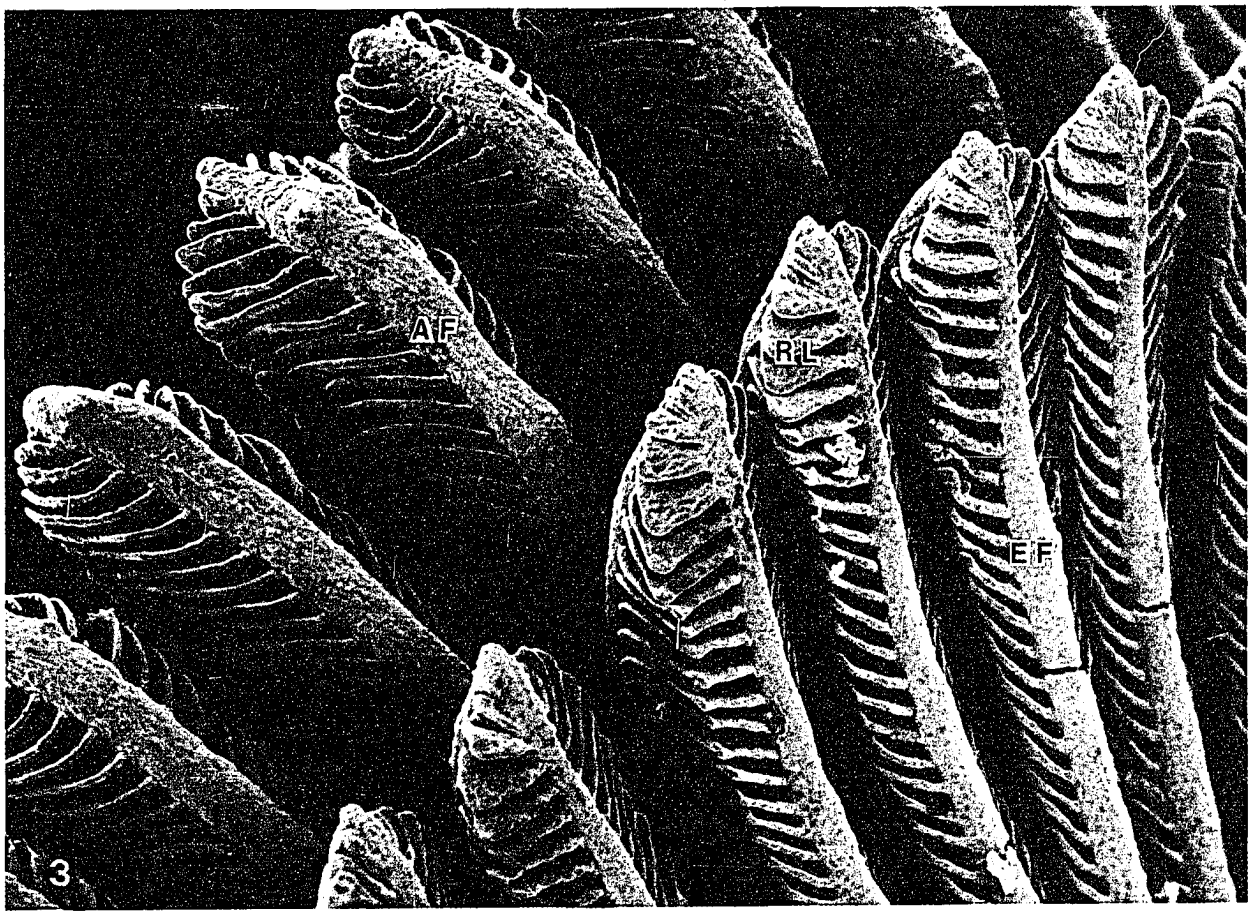


FIGURE 1 Lateral view of the right gill arches of a 20 cm striped bass. Arches are designated I, II, III, and IV, rostral to caudal. Photographed with a Leicaflex SL 35mm camera with a 100mm macro lens. R: rakers; F: filaments. × 2.5.

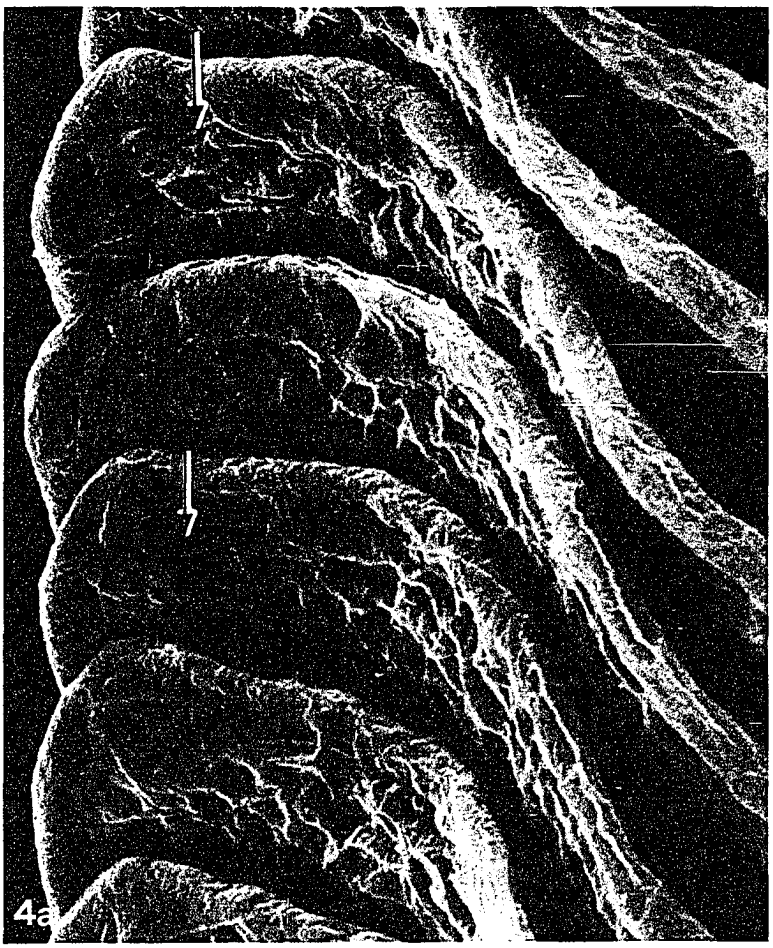
FIGURE 2 Lateral view of gill arch I. R: rakers; AF: afferent filament surface; EF: efferent filament surface; RL: respiratory lamellae. × 78.

FIGURE 3 Structure of filaments and respiratory lamellae. AF: afferent filament surface; EF: efferent filament surface; RL: respiratory lamellae. × 214.





Surface ultrastructure of striped bass gill



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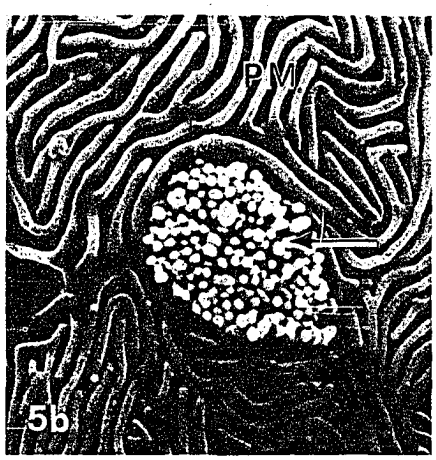
4b



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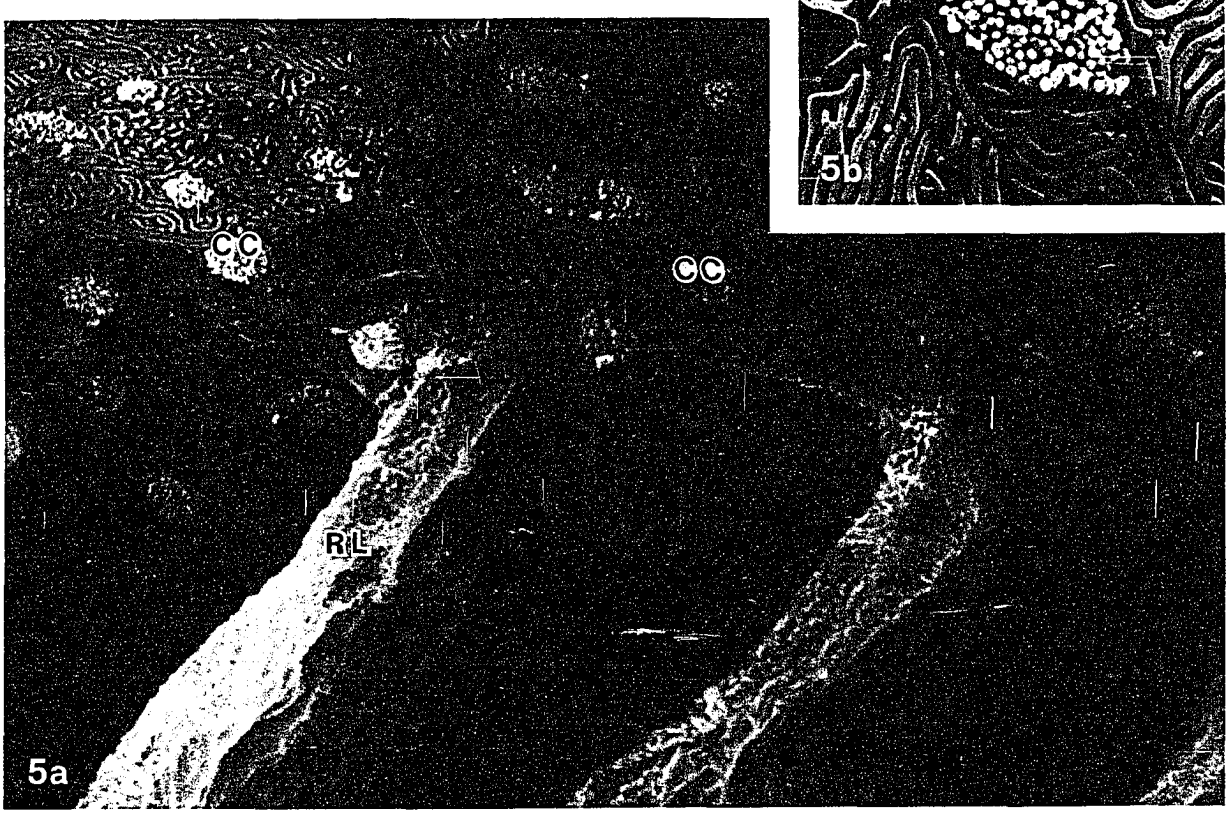

5a

TABLE 1

Measurements* on gill arches of 10cm striped bass

Gill arch	Length (cm.)	Number of filaments		Number of rakers	
		Ant. hemibranch	Post. hemibranch	Ant. hemibranch	Post. hemibranch
I.	15.4 ± .55 (10)	118.4 ± 8.4 (11)	116.8 ± 9.6 (12)	23.8 ± 2.4 (12)**	19.2 ± .75 (11)
II.	15.4 ± .55 (10)	107.4 ± 11.3 (11)	102.8 ± 11.2 (11)	15.1 ± 1.4 (13)	14.6 ± 1.4 (12)
III.	12.4 ± .55 (10)	93.4 ± 13.1 (10)	84.5 ± 8.7 (10)	12.7 ± 1.3 (10)	12.6 ± 1.2 (10)
IV.	12.2 ± .75 (10)	83.8 ± 11.5 (6)	82.2 ± 7.9 (6)	8.8 ± .75 (6)	8.8 ± .75 (6)

* Values expressed as mean ± standard deviation (N); ** long gill rakers.

Filaments in the two hemibranches of the same arch are not paired but rather alternate and interdigitate somewhat basally. Distally, the tips of filaments of one hemibranch lie close to those of the adjacent hemibranch on the next arch. Similarly, the pharyngeal surfaces of adjacent arches are closely aligned with each other such that the rakers of one arch could mesh with those of an adjacent arch to form a seal between the pharyngeal and gill cavities.

Both the length and number of filaments and rakers per arch increase with the growth of the fish. But as an example, we present counts obtained from typical fish, 10 cm in length, in Table 1.

Each filament supports two rows of triangular, leaflike, respiratory lamellae, one row on each side of the filament (Figs. 3 and 4*a*). In the center of gill arch I on a typical 10 cm fish there are about 40-50 lamellae per mm on each side of a filament. In cross section, filaments are rounded on their afferent surfaces but more flattened on their efferent surfaces (Fig. 3). The respiratory lamellae conform to the shape of the filament, are present from its base to its apex, and greatly increase its surface area. Each of the triangular-shaped lamellae, has its leading, free edge aligned with the efferent surface of the filament.

With few exceptions all surfaces of each gill arch are covered by a mosaic of flattened, polygonal pavement cells (Copeland, 1948) which exhibit concentrically-arranged microplicae (Andrews, 1975) on their surfaces (Fig. 5*a,b*). Cell borders can usually be discerned by changes in the surface pattern or by prominent intercellular ridges. These cells measure 7.5 ± 2.7 μm in diameter, and the surface microplicae measure 0.2 ± 0.3 μm in width. On the interlamellar surfaces, the surface microplicae are often discontinuous or replaced by short surface projections (Fig. 5*a*). On the respiratory lamellae, however, the microplicae disappear, giving rise to smooth-surfaced pavement cells, whose borders are distinctly outlined by rows of short surface projections (Fig. 4*a,b*).

On the afferent surfaces of filaments, on the interlamellar

FIGURE 4*a,b* (*a*) Respiratory lamellae. (*b*) Polygonal, smooth-surfaced pavement cells on respiratory lamellae. Note the absence of microplicae, but the presence of short surface projections especially along intercellular borders (arrow). (*a*) × 1429, (*b*) × 3064.

FIGURE 5*a,b* (*a*) Afferent and interlamellar filament surfaces. (*b*) Apical crypt of chloride cell. RL: respiratory lamellae; CC: apical crypts of chloride cells; PM: pavement cell microplicae (compare with Fig. 4*b*); arrow: apical extensions of chloride cells. (*a*) × 2086; (*b*) × 7440.

surfaces, and around the bases of respiratory lamellae the pavement cell epithelium is interrupted by the apical crypts of chloride cells (Figs. 5*a,b* and 6). These apices open along the borders of adjacent pavement cells, measure 3.16 ± 1.05 µm in diameter, and consist of tufts of short, cellular projections (Fig. 5*b*). Chloride cell apices are not observed on the efferent filament surface (Fig. 6), on the raker surfaces (Figs. 7 to 11) or on any other surface of the gill arch.

The anterior row of rakers on gill arch I consists of long, finger-like appendages which span the slit between gill arch I and the operculum and thus separate the pharyngeal cavity from the opercular cavity (Fig. 8). The anterior surface of the rakers is convex, but the posterior (pharyngeal) surface (Fig. 10) is flattened and contains a single row of spines along each edge. The posterior row of rakers on gill arch I (Fig. 8) and both rows of rakers on the remaining gill arches (Fig. 9) are reduced to short, raised areas generously studded with spines.

Taste buds are observed in a single row along the center of the pharyngeal surface of each gill arch (Figs. 8 and 9) and along the center of each of the long rakers (Figs. 7 and 10), and are also distributed randomly among the spines on each of the short rakers (Figs. 11 and 12). Taste buds are located on raised areas of the epithelium (Figs. 7 and 12) and consist of tufts of large and small microvilli which project above the epithelial surface along the lateral borders of adjacent, pavement cells (Figs. 12 and 13). Taste buds usually contain about 9 ± 4 large microvilli and 163 ± 56 small microvilli, and measure about 3.06 ± 1.1 µm in diameter (Fig. 13).

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FIGURE 6 Light micrograph of cross section of filament. Note presence of clear, columnar chloride cells (arrows) on afferent (A) and interlamellar, but not efferent (E) filament surfaces. RL: respiratory lamellae. × 761.

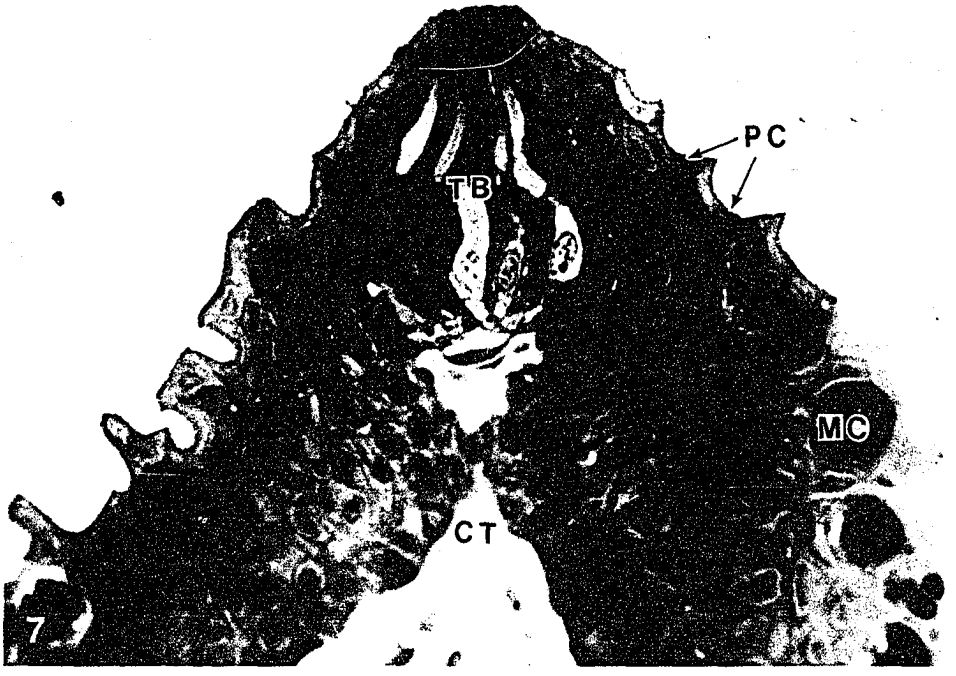
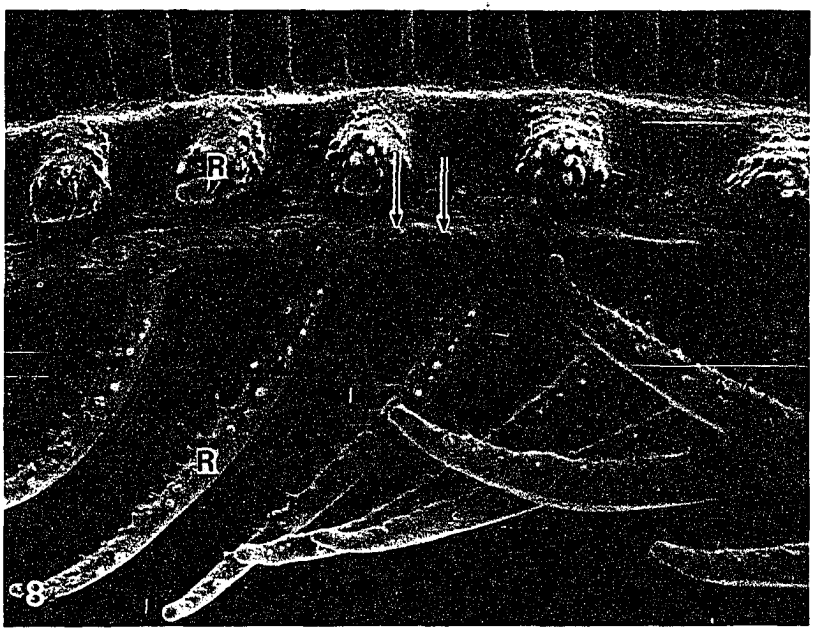


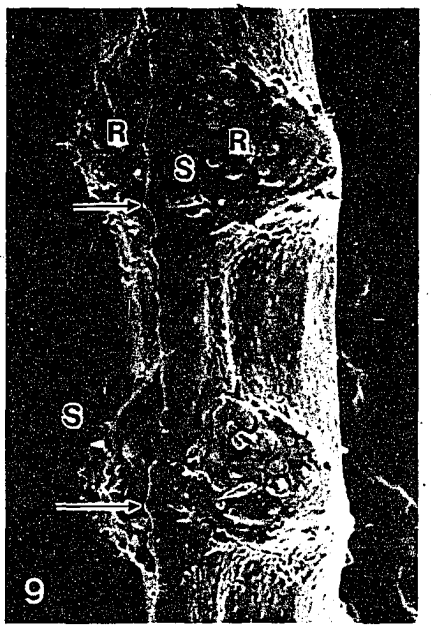
FIGURE 7 Light micrograph of pharyngeal surface of a long gill raker near its base. Note taste bud (TB) with its characteristic dark and light cells on a raised area of the epithelium. PC: pavement cells; MC: mucous cells; CT: connective tissue core of raker. × 1092.

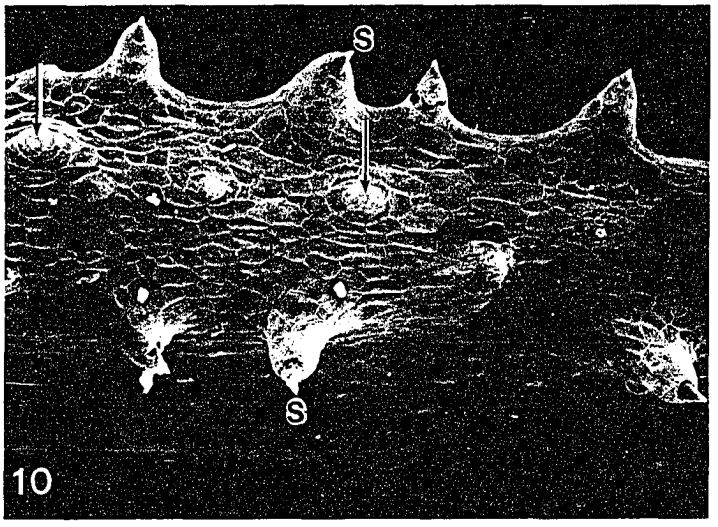
DISCUSSION

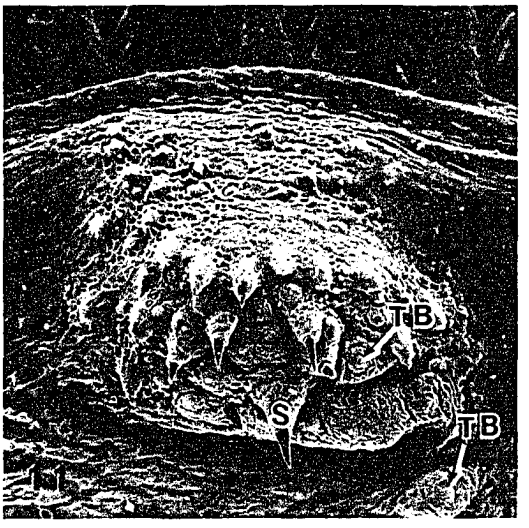
The gill arches of the striped bass, *Morone saxatilis*, are typical of arches of teleosts of intermediate activity according to Gray (1954) and as discussed by Hughes (1984). All four gill arches are highly developed, exhibiting relatively long filaments the full length of each arch. The respiratory lamellae are numerous and of average size when compared with other fish of intermediate activity (e.g., mullet: Hossler *et al.*, 1979; and killifish: Hossler *et al.*, 1985). However, the number of lamellae per millimeter (40-50) on each side of the filament is high compared to the average value (18-25) reported by Hughes (1984) for fishes of intermediate activity. This could indicate that these fish should be classified near the upper range of activity of this group. However, these high values could result from our use of immature fish in this study.

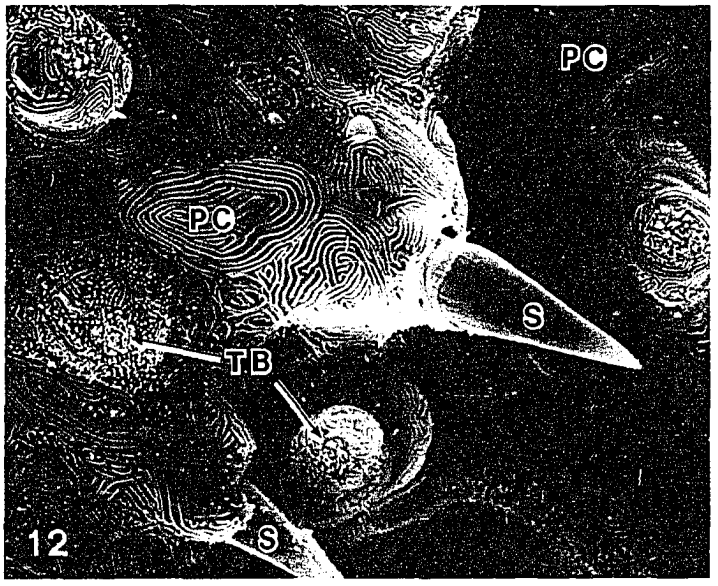
With the exception of its importance in feeding mechanisms (see discussion by Lander, 1983), gill raker anatomy has received little attention in the literature. As is the case with some other teleosts (e.g., killifish, Hossler *et al.*, 1985) only the anterior row of rakers on the first gill arch of striped bass is highly developed. These rakers bridge the gill slit between the operculum and the first gill arch and likely prevent food or ingested debris from entering the opercular cavity and causing possible damage to the respiratory lamellae. This first gill slit would seem to be potentially the largest opening in the gill and could function in expelling water during feeding — hence the importance of this first row of rakers and their well-developed pharyngeally directed spines. All of the remaining rows of rakers in the striped bass gill, although heavily laden with spines, are reduced to short, raised areas along the pharyngeal surface of each arch. Their positioning, however, allows

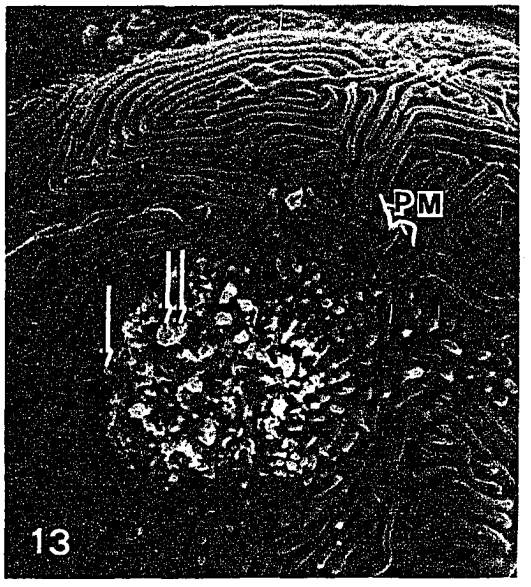












juxtaposed rakers on adjacent arches to interdigitate when the arches are approximated thus forming a tight seal between the pharyngeal and gill cavities. Lander (1983) has demonstrated very elegantly in bluegill sunfish that such a seal is essential for the formation of negative pressure in the pharyngeal cavity during suction feeding.

During respiration, however, the slits between gill arches are open, and the primary source of resistance to waterflow is provided by the close approximation of filaments (especially at their tips) and their attached respiratory lamellae (see discussion in Hughes, 1984). This arrangement insures that water entering the gills will flow across the lamellar surface. The respiratory function would also be enhanced by orienting the triangular lamellae such that one broad surface of the triangle would face the incoming water on the efferent surfaces of the filaments as observed here. The reduction in surface sculpturing on the pavement cells of the respiratory lamellae seen here has been observed in other species (trout, Hughes, 1979; mullet, Hossler *et al.*, 1979; *Tilapia*, Fishelson, 1980; killifish, Hossler *et al.*, 1985). Although it is possible that pavement cell surface ridges at this site are masked by a covering of mucus, we feel the ridges are truly absent for several reasons. First, the cell to cell junctions with their microvilli remain distinct on the lamellae. And second, preliminary transmission electron micrographs of lamellae of striped bass (unpublished) have failed to reveal the surface ridges. Because surface sculpturing is believed to act as an anchoring site for mucus (Hughes and Wright, 1970; Sperry and Wassersug, 1976; Hughes, 1979), its absence in this area might serve to reduce the thickness of the blood-water barrier.

Of special interest in this study was the location and anatomy of the apical crypts of chloride cells because our intent is to use striped bass as a model system for studies on the mechanism of osmoregulation. As with most other euryhaline teleosts (see discussion in Laurent, 1984) chloride cells in striped bass are limited to the epithelium of efferent and interlamellar surfaces of filaments. The diameter of the apical crypts (3.16 μm) is similar to that of other euryhaline teleosts adapted to freshwater (e.g., mullet, about 4 μm, Hossler, 1980; and killifish, 2.03 μm, Hossler *et al.*, 1985). As with other fish the crypts appear as slight depressions between adjacent pavement cells through which project numerous chloride cell surface extensions. Preliminary observations of seawater adapted fish (not reported here) indicate that the anatomy of crypts is altered by changes in salinity, but these results will be dealt with in a subsequent manuscript.

FIGURE 8 Pharyngeal surface of gill arch I. R: rakers; arrows: raised areas of epithelium containing taste buds. × 60.

FIGURE 9 Pharyngeal surface of gill arch III. R: rakers; S: spines; arrows: raised areas of epithelium containing taste buds. × 107.

FIGURE 10 Pharyngeal surface of long rakers of gill arch I. Note distinct borders of pavement cells. S: spines; arrows: raised areas of epithelium containing taste buds. × 557.

FIGURE 11 Short gill raker. Note raised areas of epithelium containing taste buds (TB) alternating with spined (S) epithelial projections. × 179.

FIGURE 12 Details of short gill raker. Note spines (S), pavement cells (PC), and raised areas of epithelium containing taste buds (TB). × 1767.

FIGURE 13 Details of taste bud. Note pavement cell microplicae (PM) and large (double arrow) and small (single arrow) microvilli of taste bud cells. × 5365.

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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 epon-araldite (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

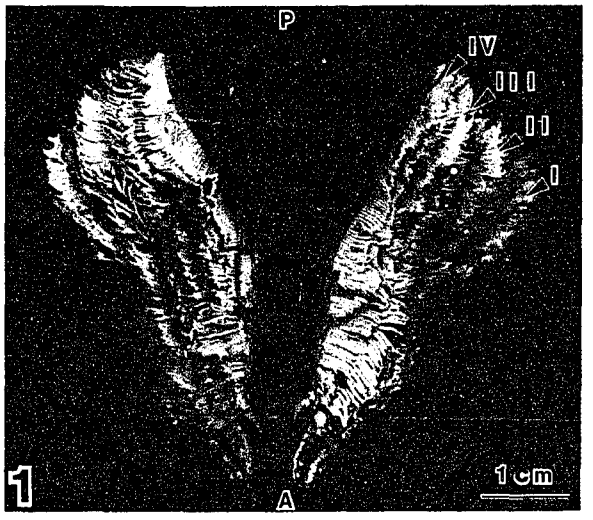


Fig. 1 Ventral view of a vascular cast of all four pairs of gill arches of a 24 cm striped bass. Photographed with a Leicaflex SL 35 mm camera with a 100mm macro lens.

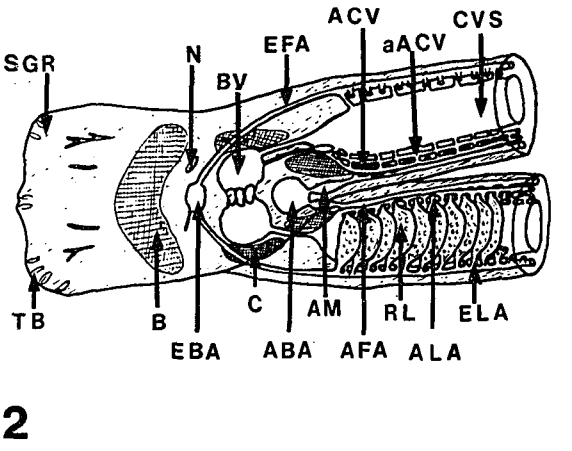


Fig. 2 Schematic cross-section of striped bass gill arch II showing vasculature. The lamellar vasculature has been removed from the upper filament.

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

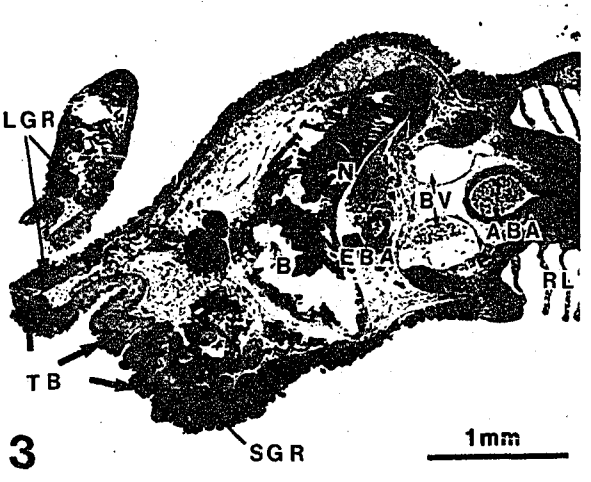


Fig. 3 Light micrograph of a cross-section of gill arch I. Compare with the vascular schematic on Fig. 2.

(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

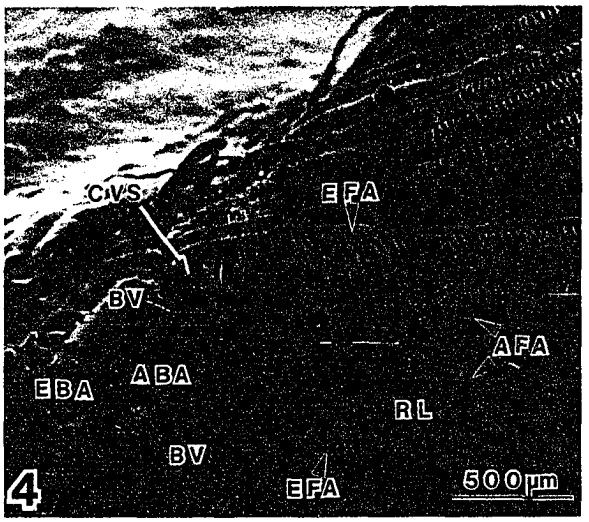


Fig. 4 Scanning electron micrograph of a cross section of a vascular cast of the second gill arch. Compare with the vascular schematic on Fig. 2.

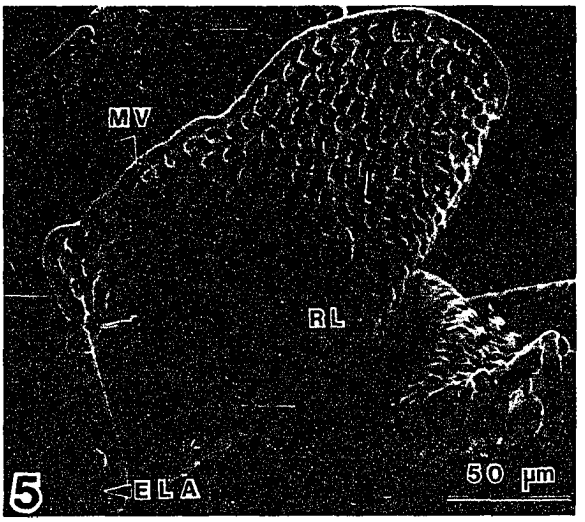


Fig. 5 Vascular network of the respiratory lamellae (RL). Note the marginal vessel (MV). The openings in the capillary network mark the positions of the pillar cells.

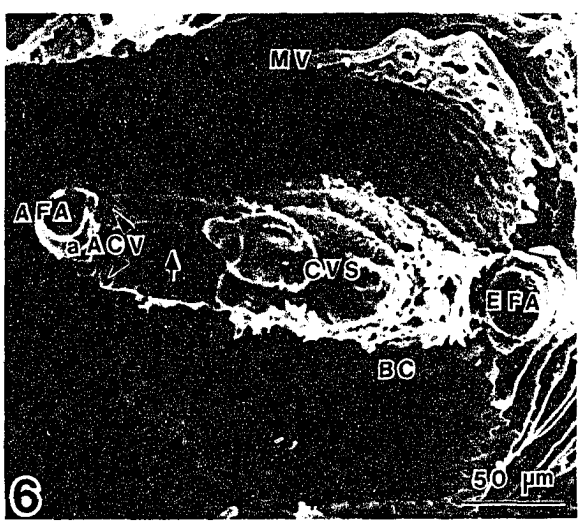


Fig. 6 Cross-section of the vasculature of the gill filament. Note the position of the central venous sinus (CVS) and the accessory afferent companion vessels (aACV). The afferent companion vessels are absent. The arrowhead marks the position where the cartilage would normally be. Note the basal charnel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

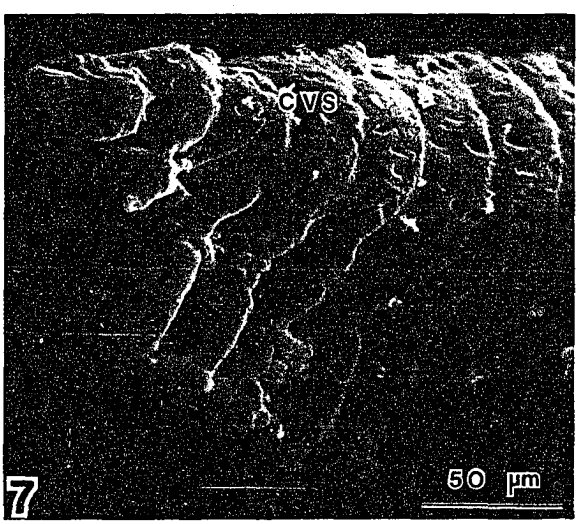
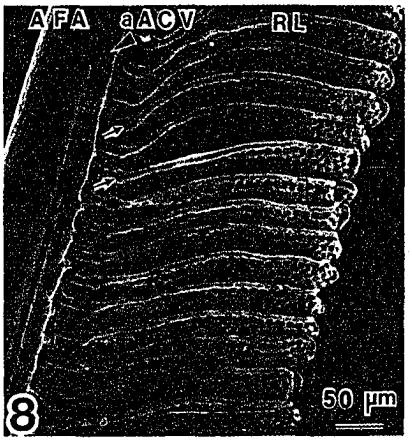


Fig. 7 Cast of central venous sinus (CVS) at apex of filament. The surrounding respiratory lamellae, afferent filamental artery, and the efferent filamental artery were removed. The afferent side of the CVS is at the bottom.

(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-



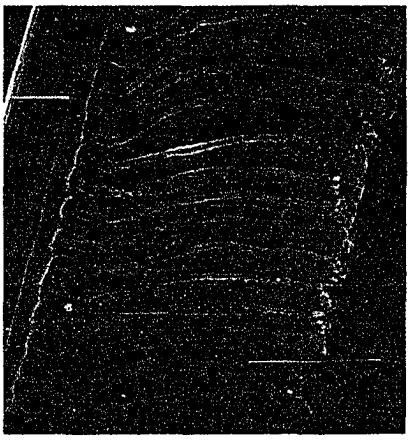


Fig. 8 Stereo pair of the afferent filamental artery (AFA), respiratory lamellae (RL), and the afferent companion vessel (ACV). Arrows: enlargements in the afferent lamellar arterioles (ALA).

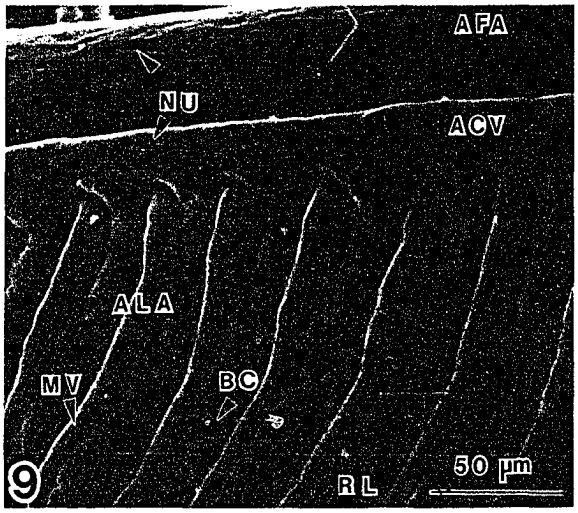


Fig. 9 Cast of blood supply of respiratory lamellae (RL) showing the marginal vessels (MV) and basal channels (BC). Note the afferent companion vessel (ACV) and its connections to the central venous sinus which alternate with the afferent lamellar arterioles (ALA).

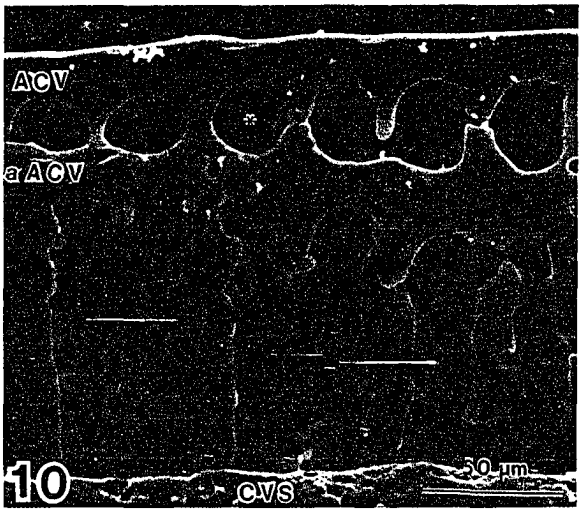


Fig. 10 Cast of "accessory" afferent companion vessel (aACV) and afferent companion vessel (ACV). The asterisk indicates the area where an ALA would exit to attach the RL to the AFA.

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

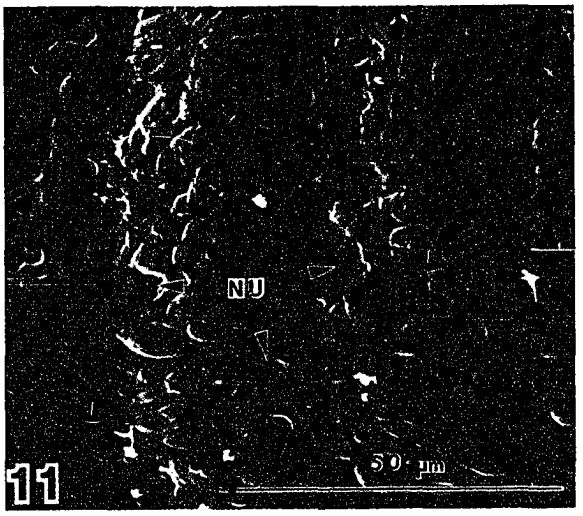


Fig. 11 Cast of the central venous sinus. Note the indentations produced by the respiratory lamellae and the impressions left by the nuclei of endothelial cells (NU).

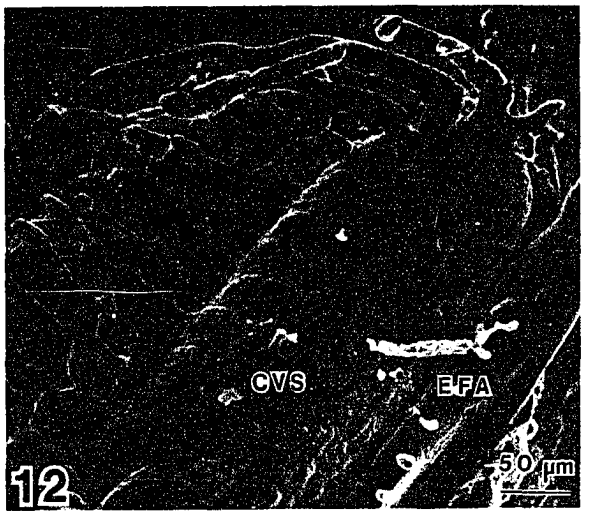


Fig. 12 Vascular cast of the proximal end of the central venous sinus (CVS). Note the CVS-ACV complex at upper left.

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.

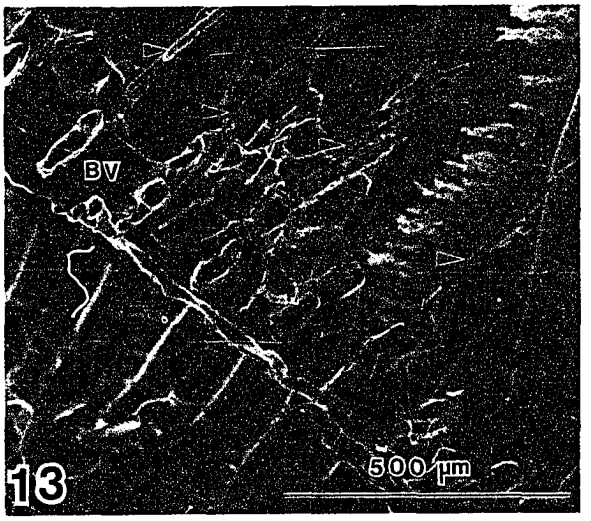


Fig. 13 Vascular cast of the branchial vein (BV) and its connections. Note the vessels from each filament and the tributaries that are interwoven with the BV. Arrowheads indicate small vessels which drain the CVS-ACV complex and the nutrient supply to the filament.

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

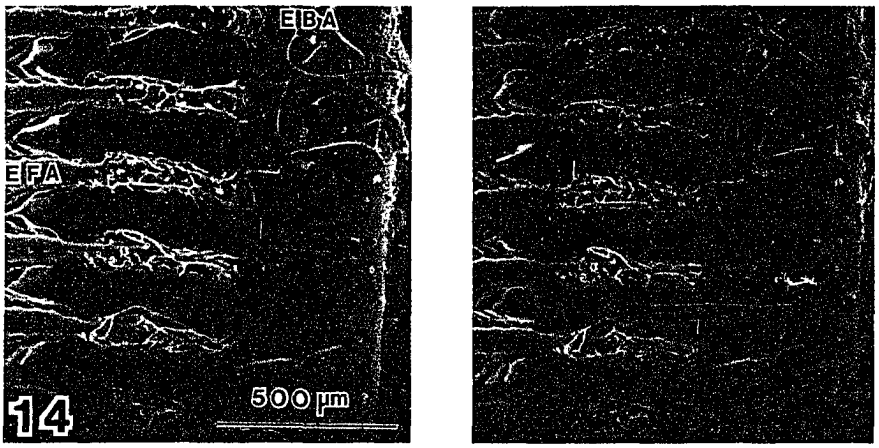


Fig. 14 Stereo pair showing nutrient vessels from the efferent branchial artery (EBA) and the efferent filamental artery (EFA). Note the coiled origins of the nutritional vessels from both the EFA and EBA, and the connections between those vessels.

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

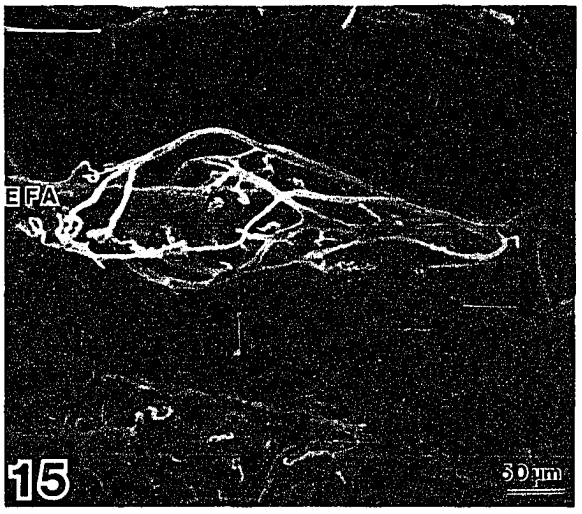


Fig. 15 Details of the vascular network around the proximal aspect of the efferent filamental artery (EFA).

(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).

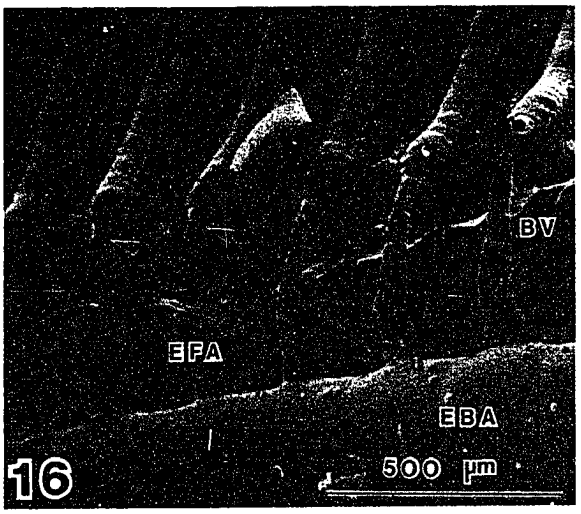

Fig. 16 Cast of the efferent filamental artery (EFA) and the efferent branchial artery (EBA). Arrowheads, possible sites of sphincters.

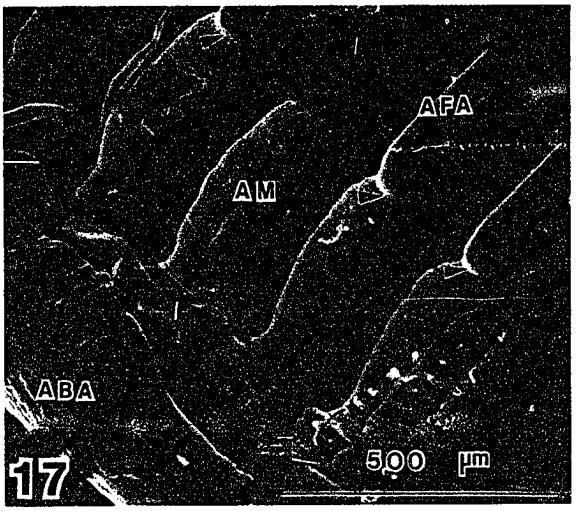

Fig. 17 Cast of the ampulla (AM) of the afferent filamental artery (AFA). Note the narrow junctions of the AMs with the afferent branchial artery (ABA), and the constrictions (arrowheads) in the AFAs.

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,

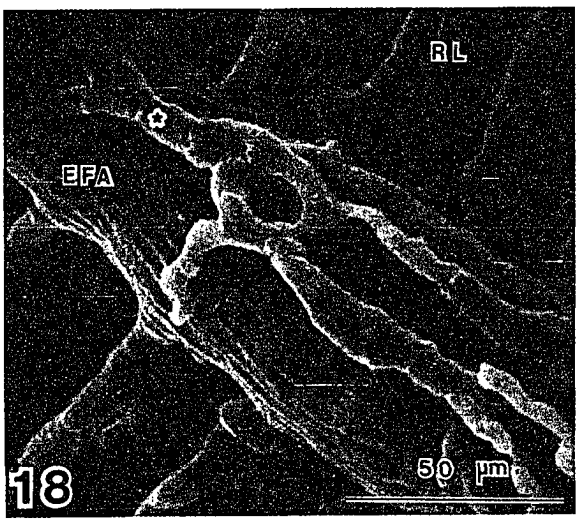

Fig. 18 Cast of the efferent filamental artery (EFA). Note nutritional vessels (asterisk) arising from the EFA and anastomosing with each other.

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).

VITA

VITA

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Science & Humanities Award	Carson-Newman College	1983
Who's Who Among Students in American Universities and Colleges	Carson-Newman College	1983
Frank G. Brooks Award	Beta Beta Beta Biological Society	April 1983
A. Edward Hughes Award (organic chemistry)	Carson-Newman College	April 1982
CRC Press Freshman Chemistry Achievement Award	Carson-Newman College	April 1981
Honors Program	Carson-Newman College	1979-1983
Valedictorian Scholarship	Carson-Newman College	1979-1983

Publications

Abstracts Presented:

King, Judy A. and Fred E. Hossler. Striped bass gill vasculature studied with vascular casting and scanning electron microscopy. (Abstract) 25th Annual Meeting of the Southern Society of Anatomists, October 1985.

King, Judy A.C. and Fred E. Hossler. Striped bass gill vasculature studied with vascular casting and scanning electron microscopy. 2nd Annual Research Forum, Quillen-Dishner College of Medicine, Johnson City, Tennessee, April 1986.

King, Judy A.C. Striped bass vasculature studied with vascular casting and scanning electron microscopy. (Abstract). Proceedings of Southeastern Electron Microscopy Society, 9: 29, 1986.

King, Judy A.C. Striped bass vasculature studied with vascular casting and scanning electron microscopy (Abstract). Scanning Electron Microscopy, May 1986.

King, Judy A.C. Microdissection by sonication: A SEM study of the gill arch of the striped bass, Morone saxatilis (Abstract). Appalachian Regional Electron Miscroscopy Society Meeting, October 1986.

Papers:

Hossler, F.E., J.H. Harpole, Jr. and J.A. King. 1986. The gill arch of the striped bass, Morone saxatilis. I. Surface ultrastructure. J. Submicrosc. Cytol. 18 (3): 519-528.

King, Judy A.C. and Fred E. Hossler. 1986. The gill arch of the striped bass, Morone saxatilis. II. Microvasculature studied with vascular casting and scanning electron microscopy. SEM/IV: 1477-1488.

King, Judy A.C. and Fred E. Hossler. The gill arch of the striped bass, Morone saxatilis. III. Microdissection by ultrasonication (in preparation).