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GILL SPECIALIZATIONS IN HIGH-PERFORMANCE PELAGIC TELEOSTS, WITH REFERENCE TO STRIPED MARLIN (*TETRAPTURUS AUDAX*) AND WAHOO (*ACANTHOCYBIUM SOLANDRI*)

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ABSTRACT

Analysis of the gill structure of striped marlin, *Tetrapturus audax* (Philippi, 1887), and wahoo, *Acanthocybium solandri* (Cuvier, 1832), demonstrates similarities to tunas (family Scombridae) in the presence of gill specializations to maintain rigidity during fast, sustainable swimming and to permit the O_2 uptake required by high aerobic performance. For ram-gill ventilators such as tunas, wahoo, and striped marlin, a rigid gill structure prevents lamellar deformation during fast water flow. In tunas, lamellar fusions bind adjacent lamellae on the same filament to opposing lamellae of the neighboring filament. Examination of striped marlin and wahoo gill structure demonstrates a previously undescribed inter-lamellar fusion which binds juxtaposed lamellae on the same filament, but does not connect to opposing lamellae of the adjacent filament. Lamellar thicknesses and the water-blood barrier distances in striped marlin and wahoo are comparable to those of tunas and among the smallest recorded. Vascular replica casts reveal that striped marlin lamellar vascular channels are similar to tunas in having a diagonal progression that reduces lamellar vascular resistance. Wahoo lamellar channels, however, have a linear pattern similar to most other teleosts.

Tunas, bonitos, mackerels (family Scombridae), and billfishes (families Istiophoridae, Xiphiidae) are highly specialized for fast, continuous swimming. Both groups are ram ventilators [i.e., their nonstop movement forces water over the gills thus replacing active gill ventilation (Jones and Randall, 1978; Roberts and Rowell, 1988)] which, at faster swimming speeds, reduces drag associated with cyclic jaw movements for respiration (Brown and Muir, 1970; Freadman, 1981).

The gill design of tunas (tribe Thunnini) epitomizes specializations for ensuring rigidity required by ram ventilation and for meeting increased oxygen demands associated with high aerobic performance (Muir and Kendall, 1968; Hughes, 1970). Tunas possess lamellar fusions that bind the respiratory lamellae to create a rigid gill sieve (Fig. 1; Muir and Kendall, 1968). In addition, tunas of the genus *Thunnus* have filament fusions that support elongate gill filaments (Fig. 1A). Both fusion types prevent gill deformation during ram ventilation and thereby minimize anatomical dead space (i.e., the separation of filaments and lamellae due to the force of high-velocity water flow) (Muir and Kendall, 1968; Hughes, 1984). Tunas also have large gill areas that enhance gas exchange and microvascular specializations [slender lamellae and thin lamellar walls (the water-blood barrier distance)] that decrease diffusion distances (Muir and Hughes, 1969; Hughes, 1972; Hughes and Morgan, 1973; Hughes, 1984). In addition, the diagonal orientation of the vascular channels in tuna lamellae (Fig. 1B) increases the number of respiratory blood channels in parallel in the gills and conserves vascular pressure drop across the lamellae by minimizing blood-flow distance (Muir, 1970; Muir and Brown, 1971; Olson et al., 2003).

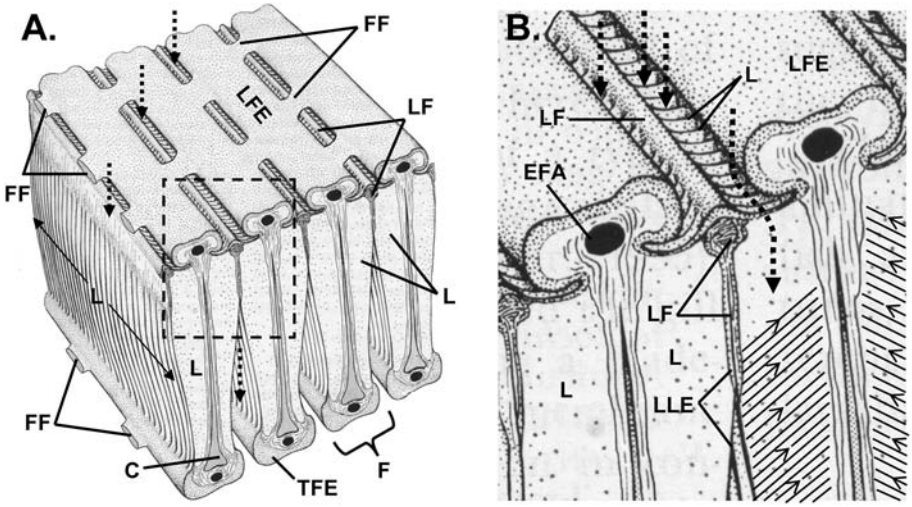

Figure 1. **A:** Cross-section through four adjacent gill filaments (F) depicting the filament and lamellar fusions of some ram-ventilating teleosts. Filament fusions (FF) connect adjacent filaments on the leading and trailing filament edges. **B:** Magnified view of the dashed box in A detailing the lamellar fusion and blood-flow pathways (as described for tunas) through the lamellae. Lamellar fusions (LF) connect the leading lateral (outer) edges of juxtaposed lamellae (L) on the same filament to the opposing lamellae of the adjacent filament. The lateral lamellar edges (LLE) are not usually bound along their entire length. Blood through tuna lamellae flows in a diagonal progression (solid arrows) from the lateral lamellar edge toward the filament base. Direction of water flow is indicated by dotted arrows. Other abbreviations: C: cartilaginous filament rod, EFA: efferent filament artery; LFE, leading filament edge; TFE, trailing filament edge. Figure modified from Muir and Kendall (1968).

While it is generally assumed that other high-performance fishes including billfishes and non-tuna members of the Scombridae have gill specializations similar to tunas, this has not been fully investigated. Filament fusions that connect adjacent gill filaments on the same hemibranch have been noted in gill descriptions of the istiophorids, swordfish, *Xiphias gladius* Linnaeus, 1758, and wahoo, *Acanthocybium solandri* (Cuvier, 1832) (Lütken, 1880; Trois, 1883; Kishinouye, 1923; Bevelander, 1934; Conrad, 1938; Muir and Kendall, 1968; Johnson, 1986). However, the status of lamellar fusions in these fishes is unclear. Muir and Kendall (1968) originally reported that billfishes and wahoo lack lamellar fusions, but Muir (1969) later affirmed their presence in the striped marlin, *Tetrapturus audax* (Philippi, 1887). Also lacking are details on gill microstructure and lamellar blood-flow pattern in billfishes and wahoo.

The objective of this study was to use scanning electron microscopy, light microscopy, and vascular replica casting to document structural and microvascular specializations in both striped marlin and wahoo. For comparison, we also examined the gills of yellowfin tuna, *Thunnus albacares* (Bonnaterre, 1788). This report reviews relevant literature on the gill structure of these fishes and documents several specializations related to ram ventilation, including the first description of a novel form of lamellar fusion.

Methods

Fish Collection.—Four striped marlin, seven wahoo, and seven yellowfin tuna were caught by hook and line off the coast of Baja California, Mexico, for the acquisition of gill tissue and preparation of gill vascular replica casts. All specimens were collected under the authorization of la Comisión Nacional de Acuacultura y Pesca, México, Permiso de Pesca de Fomento No. DGOPA/13308/210905/ and euthanized by surgically severing the spinal cord in accordance with Protocol S00080 of the Animal Care and Use Committee (University of California, San Diego). Striped marlin specimen weights were estimated at sea. For wahoo and yellowfin, fish fork lengths were measured, and specimen weights were subsequently calculated using length-weight regression equations (Chatwin, 1959; Beerkircher, 2005).

Tissue Fixation.—Following euthanization, gill tissue was removed from one striped marlin (25 kg), four wahoo (14.6, 18.5, 19.4, 24.2 kg), and six yellowfin tuna (11.0, 13.4, 15.3, 33.0, 39.9, 49.2 kg) and placed in 10% formalin buffered with seawater within 10 min of capture. The gills of a 70 kg striped marlin were extracted and placed in formalin approximately 3 hrs after capture. Gills from a 35 kg striped marlin were removed and placed on ice until immersed in formalin, also approximately 3 hrs post capture. Finally, gill tissue from a 45 kg marlin was excised and placed in formalin after the specimen had first been perfused with vascular casting solution.

Vascular Casting.—Vascular replica gill casts were made for one striped marlin (45 kg), three wahoo (12.8, 15.3, 19.4 kg), and one yellowfin tuna (4.3 kg). Specimens were euthanized and placed ventral side up in a V-shaped cradle, and the gills were ventilated with aerated seawater from a hose placed in the mouth. The heart was exposed by mid-line incision, a catheter inserted, and the specimen perfused with heparinized teleost saline (Brill and Dizon, 1979) followed by microvascular casting material (Mercox Resin, Ladd Research, Williston, Vermont). The casting solution was allowed to harden for several hours, after which the specimens were frozen until examination. In the laboratory, the specimens were thawed and the excised gill casts were macerated in washes of 20% KOH until all tissue was removed.

Gill Structure Analysis.—Striped marlin, wahoo, and yellowfin tuna gill tissue was examined using both light and scanning electron microscopy (SEM). For light microscope preparation, fixed tissue was embedded in paraffin, and semi-thin sections (5 μm) were mounted on slides and stained with hematoxylin and eosin. For SEM, fixed tissue was rinsed with deionized water and slowly dehydrated to 100% ethanol (20% increments over 24 hrs). The tissue was critically-point-dried, sputter coated with gold-palladium, and viewed under high-vacuum mode using a FEI Quanta 600 SEM. Cross-sections through critically-point-dried lamellae were used to estimate lamellar thickness and the water-blood barrier distance. Vascular replica casts were examined using low-vacuum mode SEM.

Results

Inter-lamellar Fusions.—Comparative SEM and light microscopy images of striped marlin, wahoo, and yellowfin tuna gills are shown in Figs. 2–5. A significant finding is that the majority of the gill lamellae in the striped marlin specimens examined are not bound by lamellar fusions. Rather, most lamellae are bound by a previously undescribed structure, which we have termed the inter-lamellar fusion. The inter-lamellar fusion connects the leading lateral edge of adjacent lamellae on the same filament (Fig. 2B–M). This differs from the lamellar fusion of tunas in that the inter-lamellar fusion does not extend to the opposing lamellae on the adjacent filament (compare Fig. 2D with Fig. 4A). The inter-lamellar fusion is thickest on the leading lamellar edge and quickly thins as it continues along the lateral edge of the

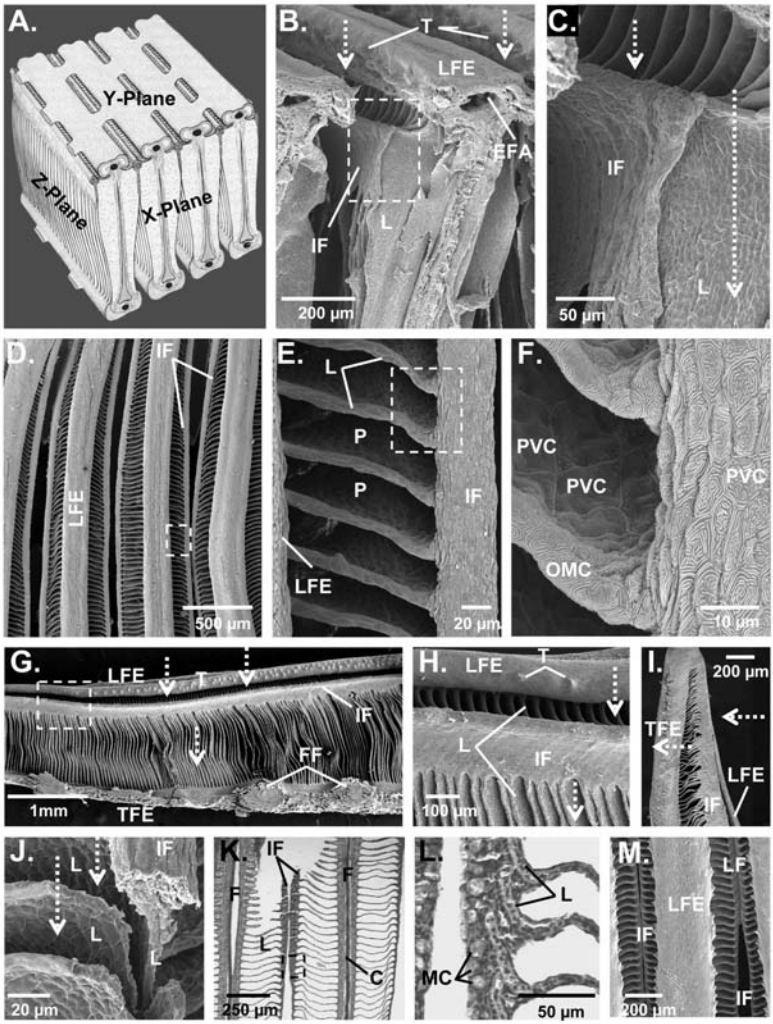

Figure 2. SEM and light microscope images of the inter-lamellar fusion in striped marlin (*Tetrapturus audax*). **A:** Reference diagram showing the three planes at which SEM and light microscope images were taken. See Figure 1 for additional diagram details. **B:** Cross-section through the leading (efferent) edge of adjacent gill filaments (x-plane). **C:** Enlarged area of the dashed box in B detailing the inter-lamellar fusion. **D:** View of the leading edge of adjacent gill filaments (y-plane). **E:** Magnified image of the dashed box of D showing the pores formed by the inter-lamellar fusion. **F:** Augmented view of E depicting the microridged pavement cells of the inter-lamellar epithelium and outer marginal channel, as well as the smooth pavement cells of the lamellar respiratory surface. **G:** Extension of the inter-lamellar fusion along the length of a filament (z-plane). **H:** Enlarged area of the dashed box in G. **I:** Inter-lamellar fusion along the filament tip (z-plane). **J:** Cross-section through the inter-lamellar fusion showing its thick leading edge and attachment to the background lamella (x-plane). **K:** Cross-section through the leading edge of two adjacent filaments (y-plane). **L:** Higher magnification of the dashed box in K showing the embedment of the lateral lamellar tips in the inter-lamellar fusion. **M:** Two inter-lamellar fusions from adjacent filaments growing together to form a complete lamellar fusion similar to that of tunas. Dotted arrows show the direction of water flow. Water flow is into the page in D–F, K–M. Images B–L are from a 45 kg striped marlin; M is from a 25 kg specimen. Abbreviations: C, cartilaginous filament rod; EFA, efferent filament artery; F, filament base; FF, filament fusion; IF, inter-lamellar fusion; L, lamellae; LF, lamellar fusion; LFE, leading filament edge; MC, mucous cell; P, lamellar pore; PVC, pavement cell; OMC, outer marginal channel; T, teeth of the epithelial toothplates; TFE, trailing filament edge.

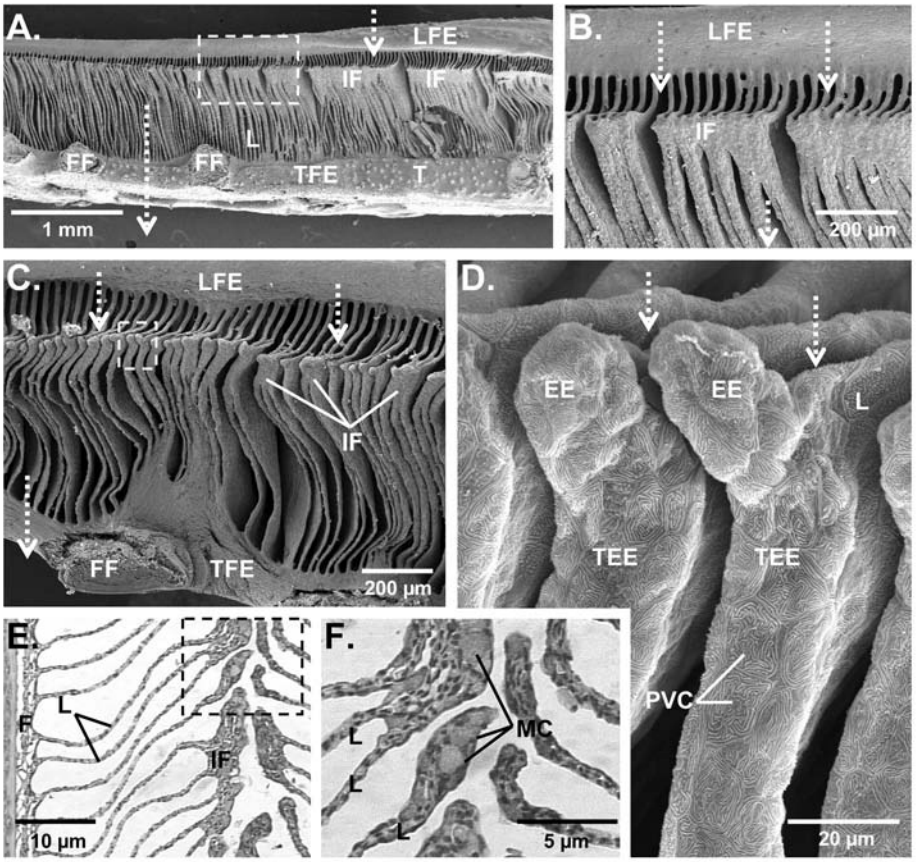

Figure 3. SEM and light microscope images depicting the positioning and structure of the inter-lamellar fusion in wahoo (*Acanthocybium solandri)*. **A:** View of the filament showing the irregularity of the inter-lamellar fusion (z-plane, refer to Fig. 2A). **B:** Magnified image of the inter-lamellar fusions in A. **C:** Image showing the thickened lateral lamellar edges of non-fused lamellae (z-plane). **D:** Magnified image of the lateral lamellar edges in C, detailing extensions of the lamellar epithelium which appear to be bridging the inter-lamellar spaces. **E:** Cross-section through fused and non-fused lamellae (y-plane). **F:** Magnified view of dashed box in E. Dotted arrows indicate the direction of water flow. Water flow is into the page in E and F. A–D are from a 19.4 kg wahoo, E,F are from a 14.4 kg specimen. Abbreviations: EE, epithelial extension; F, filament base; FF, filament fusion; IF, inter-lamellar fusion; L, lamellae; LFE, leading filament edge; MC, mucous cell; PVC, pavement cell; T, teeth of the epithelial toothplates; TFE, trailing filament edge; TEE, thickened epithelial edge.

lamellae (Fig. 2B,C,J). The fusion extends the full length of the filament (Fig. 2G) and at the filament tip it covers nearly the entire length of the lamellae (Fig. 2I).

SEM images of the fusion epithelial surface reveal the typical "fingerprinting" of pavement (squamous) cell microridges (Fig. 2F). These microridges [also observed on the striped marlin gill filament (not pictured) and lamellar outer marginal channel (Fig. 2F)] are characteristic of non-respiratory gill epithelia (Olson, 1996), while thin, non-ridged pavement cells comprise the lamellar respiratory surface (Fig. 2F). Stained cross-sections of the inter-lamellar fusion show that the lateral lamellar edges turn nearly 90° [toward the distal end of the filament (filament tip)] to approach neighboring lamellae, where they are connected by non-differentiated epithelial cells (Fig. 2L). These lateral lamellar edges therefore form the majority of the fusion's in-

ner wall (Fig. 2L). A thicker epithelium covers the outer edge of this lamellar bridging and forms the bulk of the fusion (Fig. 2L). The thick leading edge of the fusion is composed mainly of mucous (goblet) cells (Fig. 2L); as the fusion thins along the lateral lamellar edge it is composed solely of non-differentiated epithelial cells.

Inter-lamellar fusions are also present in wahoo (Fig. 3A–F), although they are somewhat irregular and less uniform than in striped marlin. Figure 3A–C shows both fused and non-fused lamellae along the length of a filament. No apparent pattern was noted for the presence or absence of inter-lamellar fusions in relation to gill arch number or position in the flow stream. Non-fused lamellae have a thick lateral lamellar edge, and in many cases, extensions of this thickened epithelium appear to reach toward adjacent lamellae (Fig. 3C,D). These epithelial extensions uniformly point toward the filament tip (Fig. 3C–F).

Like striped marlin, the wahoo inter-lamellar fusion is composed of stratified epithelium with a high concentration of mucous cells on its leading edge (Fig. 3E,F). Mucous cells are also present in the thickened epithelium of non-fused lamellae (Fig. 3F). The inter-lamellar fusion in wahoo is thinner than in striped marlin (compare Fig. 3E with 2L) and wahoo lateral lamellar edges (although turned toward the distal end of the filament) do not appear to form the inner wall of the fusion (Fig. 3F)

Lamellar Fusions.—The lamellar fusions of yellowfin tuna connect juxtaposed lamellae on the same filament to opposing lamellae on the adjacent filament (Fig. 4A,B). The fusion epithelial surface has microridged pavement cells surrounding mucous cell pores (Fig. 4C). Cross-sections through the fusion show that the leading edge is predominately composed of mucous cells, while the bulk of the fusion is composed of non-differentiated epithelial cells. The lamellar lateral edges are embedded in the fusion and curve toward the distal end of the filament, but not to the extent seen in striped marlin and wahoo (Fig. 4E).

In striped marlin, the majority of the lamellae are bound by inter-lamellar fusions; however, in some cases, inter-lamellar fusions from adjacent filaments appear to grow together to form complete lamellar fusions as described for tunas (Fig. 2M). Striped marlin sample size was not adequate to determine any significant patterns in the frequency of the completed lamellar fusions in relation to body size or position within the flow stream. In contrast, complete lamellar fusions were not observed in wahoo.

Microvascular Specializations.—Gill microvascular measurements for striped marlin, wahoo, and yellowfin tuna determined from critically point-dried gill tissue and vascular corrosion casts are shown in Table 1. The lamellar thickness (6.29 ± 1.36 μm) and water-blood barrier distance (0.531 ± 0.153 μm) of striped marlin are comparable to measurements determined for yellowfin tuna (5.88 ± 0.99, 0.537 ± 0.092 μm) in this study and previously reported values (Hughes, 1970; Muir and Brown, 1971). Wahoo lamellar thickness (5.16 ± 1.21 μm) is also similar, although the water-blood barrier thickness (0.860 ± 0.170 μm) is significantly greater ($P < 0.001$) than both striped marlin and yellowfin.

Yellowfin tuna vascular casts (Fig. 5B) confirm previous descriptions (Muir, 1970; Muir and Brown, 1971; Olson et al., 2003) of the blood-flow pattern through tuna lamellae. Blood leaving the afferent lamellar arteriole enters directly into outer marginal channels (OMC) extending along the lateral edge of the lamellae. From the OMCs, blood flows across the lamellae through diagonal channels (formed by pillar cells) that

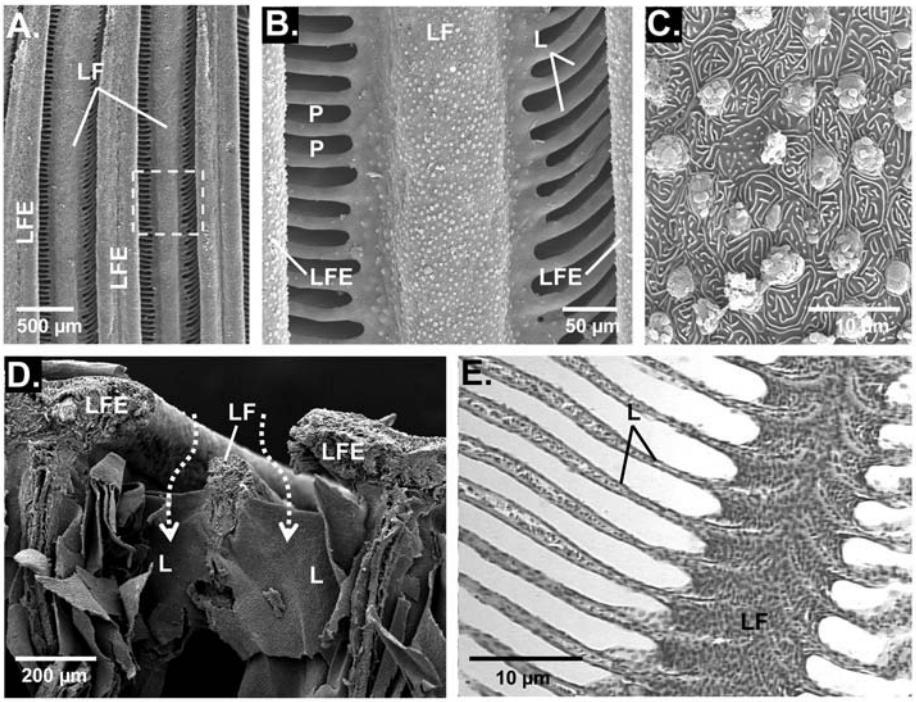

Figure 4. SEM and light microscope images of the lamellar fusion of yellowfin tuna (*Thunnus albacares*) **A:** View of the leading edge of adjacent filaments (y-plane, refer to Fig. 2A). **B:** Magnified view of the dashed box in A revealing the pores formed by the lamellar fusion. **C:** Further magnification of the lamellar fusion showing the high concentration of mucous cell pores on the leading epithelial surface. **D:** Cross-section through the leading edges of adjacent filaments showing the lamellar fusion (x-plane). **E:** Cross-section through the lamellar fusion revealing the lateral lamellar edges embedded in the fusion (y-plane). Dotted arrows show the direction of water flow. Water flow is into the page in A–C, E. A–C are from a 15.3 kg yellowfin, D,E are from a 49.2 kg specimen. Abbreviations: L, lamellae; LF, lamellar fusion; LFE, leading filament edge; P, lamellar pore.

have an angle of approximately 60° relative to the long-axis (Fig. 5B, Table 1). These diagonal channels empty into a single inner marginal channel (IMC) (Fig. 5B).

Striped marlin possess diagonal lamellar vascular channels similar to those of tunas; however, blood entry into the lamellae is unique (Fig. 5C). The afferent lamellar arteriole bifurcates and forms both the inner and outer marginal channels. Blood channels leaving the IMC flow toward the lateral edge, where some channels follow the OMC along the lateral edge, while others return inward and recombine with the IMC (Fig. 5C). For the majority of the lamellar length, blood leaves the OMC and flows medially inward at about 40° to the long-axis (Fig. 5C, Table 1); this angle is greater than that determined for a single specimen by Muir and Brown (1971). In contrast to yellowfin and striped marlin, wahoo have a more typical teleost design; blood flows through lamellar vascular channels that run parallel to the lamellar long-axis (Fig. 5D). Wahoo pillar cells are also spaced farther apart forming larger vascular channels (Fig. 5D).

Table 1. Comparison of microvascular characteristics in the gills of three high-performance teleosts and the rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792). Rainbow trout data are from Muir and Brown (1971)[a] and Hughes (1972)[b]. Measurements are ± standard deviation. *Measured from a 4.3 kg specimen.

Species	W (kg)	Lamellar thickness (μm)	Water-blood barrier distance (μm)	Average angle of lamellar blood flow	# of lamellae per mm filament
Tetrapturus audax	45	6.29 ± 1.36	0.531 ± 0.153	40°	25.47 ± 2.05
Acanthocybium solandri	19.4	5.16 ± 1.21	0.860 ± 0.170	0°	32.21 ± 2.14
Thunnus albacares	14.5	5.88 ± 0.99	0.537 ± 0.092	60°*	33.37 ± 1.15
Oncorhynchus mykiss	–	35[b]	6[b]	0°[a]	17[b]

Discussion

This study describes structural specializations in the gills of striped marlin and wahoo. While we note general similarities for these fishes and tunas in terms of adaptations for enhancing gas exchange across the lamellar surface and sustaining gill rigidity during ram ventilation, we document a fundamentally different inter-lamellar fusion in striped marlin and wahoo.

Filament Fusions.—Previous reports on gill structure noted fusions connecting adjacent filaments on the same hemibranch in billfishes, wahoo, and in the tuna genus *Thunnus* (Lütken, 1880; Trois, 1883; Kishinouye, 1923; Bevelander, 1934; Conrad, 1938; Muir and Kendall, 1968; Johnson, 1986). Muir and Kendall (1968) reported that these filament fusions, which usually occur on both the leading and trailing filament edges (Fig. 1A), are formed by extensions of the filament epithelium. However, Johnson (1986) subsequently showed that in billfishes and wahoo, filament fusions on the trailing edge are strengthened by cartilaginous extensions of the filament rods. Johnson (1986) further showed that billfish and wahoo filaments and filament fusions on both the leading and trailing edges are covered by bony epithelial toothplates (Fig. 2B,G,H), which may further stiffen the filaments and reinforce the fusions. In contrast, *Thunnus* filament fusions are solely composed of epithelial tissue and are not strengthened by cartilaginous fusions of the filament rods or by bony toothplates (Johnson, 1986).

Lamellar Fusions.—In tunas, lamellar fusions connect the leading edge of juxtaposed lamellae on the same filament to opposing lamellae on the adjacent filament (Figs. 1, 4; Muir and Kendall, 1968). Lamellar fusions thus maintain the distance between adjacent lamellae (pore width) which, as suggested by hydrodynamic models for skipjack tuna gills, is optimized for effective O_2 extraction while minimizing drag (Brown and Muir, 1970; Stevens and Lightfoot, 1986). Because lamellar fusions incorporate lamellae from adjacent filaments, they also prevent filaments from being separated during ram ventilation; this is particularly important for the smaller body-sized tuna genera (i.e., *Auxis*, *Euthynnus*, and *Katsuwonus*), which completely lack filament fusions (Muir and Kendall, 1968; Muir, 1969). Lamellar fusions may also play an important role in securing adjacent filaments in *Thunnus*, as the filament fusions on the leading edge generally do not extend to the filament tips, and appear absent on the leading edge in both blackfin, *Thunnus atlanticus* (Lesson, 1831) (Muir and Kendall, 1968) and albacore, *Thunnus alalunga* (Bonnaterre, 1788) (Wegner, unpubl. data).

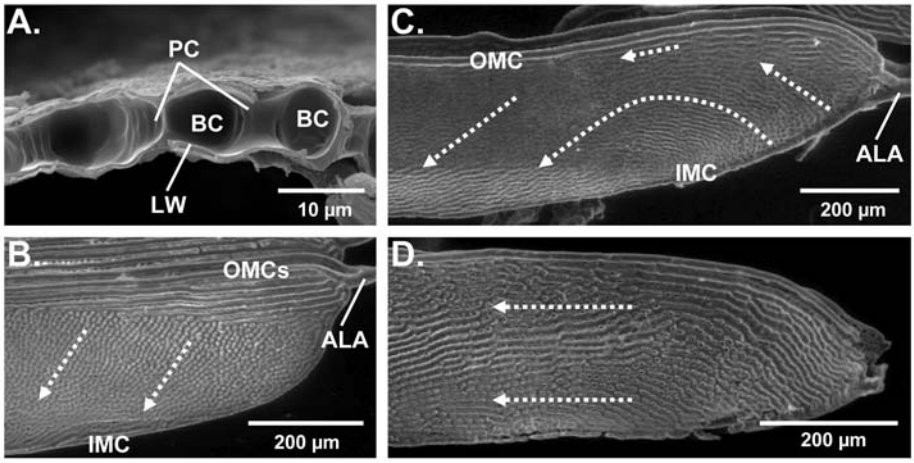

Figure 5. SEM images of the lamellar vasculature. **A:** Cross-section through a striped marlin (45 kg) lamella showing the blood channels formed by pillar cells. Vascular replica casts of the lamellae are shown for **B:** a 4.3 kg yellowfin tuna, **C:** a 45 kg striped marlin, and **D:** a 15.3 kg wahoo. Dotted arrows show the direction of blood-flow through the exchange surfaces. Water flow is from left to right in B–D. Abbreviations: ALA, afferent lamellar arteriole; BC, blood channel; IMC, inner marginal channel; LW, lamellar wall (water-blood barrier distance); OMC, outer marginal channel; PC, pillar cell.

Inter-lamellar Fusions.—The inter-lamellar fusion of the striped marlin extends along the leading lateral edge of adjacent lamellae on the same filament and secures their relative position (Fig. 2). This creates rigid pores similar to those formed by lamellar fusions (compare Fig. 2E with 4B). Although wahoo lamellae are not always completely fused and are thus less rigid, the thick lateral lamellar edges (Fig. 3C,D) may still help to maintain pore integrity.

The major difference between inter-lamellar and lamellar fusions is that inter-lamellar fusions do not connect with the lamellae on the adjacent filament (compare Fig. 2D with 4A). Thus, the inter-lamellar fusion serves solely to secure lamellar pore width, while the lamellar fusion also secures the filament-to-filament distance. Because striped marlin and wahoo have stronger filament fusions [supported by both cartilage and bony epithelial toothplates (Johnson, 1986)], a complete lamellar fusion may be less crucial to secure adjacent filaments. How tuna, striped marlin, and wahoo filament, lamellar, and inter-lamellar fusion patterns compare in their effect on water resistance through the gill sieve is unknown.

The structural composition of inter-lamellar fusions in striped marlin and wahoo is nearly identical to that of tuna lamellar fusions. Both fusions are composed of stratified epithelium with mucous cells comprising the leading edge. A mucus covering of the gill epithelia likely reduces water drag through the gill sieve (Daniel, 1981) and would thus be important for lamellar and inter-lamellar fusions that turn and guide water flow into lamellar pores. Nutrient supply to lamellar and inter-lamellar fusions appears to be from the outer (lateral) lamellar vascular channels, which near the leading (efferent) edge, are embedded within the fusion (Figs. 2L, 3F, 4E).

Our findings suggest that the lateral lamellar edges may play a role in the formation of lamellar and inter-lamellar fusions. In all three species examined, the lateral lamellar edges embedded in the fusion are turned toward the distal end of the

filament, minimizing the distance between neighboring lamellae (Figs. 2L, 3F, 4E). In wahoo, the leading lateral edges of non-fused lamellae are turned toward their neighbors and often possess epithelial extensions, which appear to be bridging the inter-lamellar space (Fig. 3C,D). If the inter-lamellar fusions of wahoo are in the formation process this may explain their irregularity along the length of the filaments. However, we did not observe any notable differences in the frequency of fused lamellae with an increase in wahoo body mass (14.6–24.2 kg). Examination of a larger size range of wahoo may reveal a trend in lamellar fusing and body size.

Finally, some remarks are warranted regarding the apparent similarities between lamellar fusions in tunas and those which occur in the primitive, freshwater, air-breathing bowfin, *Amia calva* Linnaeus, 1766 (Bevelander, 1934; Daxboeck et al., 1981; Olson, 1981). In *Amia*, lamellar fusions appear to maintain gill rigidity during air exposure. *Amia* lamellar fusions are structurally different than those of tunas in that the lateral lamellar edges are bound for their entire length, not just near the leading edge. Also, the outer lamellar vascular channels embedded in the fusion do not appear to turn toward the tip of the filament (Olson, 1981) as seen in tunas.

Tuna Microvasculature.—Tunas have thin lamellae and thin lamellar walls that minimize diffusion distances for effective gas exchange (Table 1; Hughes, 1970; Muir and Brown, 1971). Unlike most teleosts, the configuration of tuna pillar cells forces blood through the lamellae in a unique diagonal progression (Fig. 5B; Muir, 1970; Muir and Brown, 1971; Olson et al., 2003). Pressure drop (δp) through a tube can be minimized by increasing channel diameter or decreasing channel length as shown by the Hagen-Poiseuille equation:

$$\delta p = (32\mu Vl)/d^2 \text{ (dynes cm}^{-2}\text{)}$$

where μ is viscosity, V equals the mean velocity, l is the channel length, and d equals the channel diameter. Muir and Brown (1971) proposed that the short, diagonal vascular channels of tuna lamellae minimize vascular pressure drop while maintaining small channel diameters (short diffusion distances). Other relatively large teleosts (Atlantic cod, *Gadus morhua* Linnaeus, 1758, and Atlantic salmon, *Salmo salar* Linnaeus, 1758) appear to minimize pressure drop though large lamellae by increasing the diameter of the vascular channels, which consequently increases diffusion distances and likely decreases gas exchange efficiency (Muir and Brown, 1971).

The diagonal progression of tuna lamellae also increases the number of respiratory blood channels in parallel. Assuming the blood is fully saturated in these short, oblique channels, this ultimately increases functional gill area for gas exchange (Muir, 1970). Even though lamellar blood flow is diagonal, Brown and Muir (1970) suggested that contours influencing the flow pattern of water passing between adjacent lamellae may still result in a counter-current flow. However, this has not been substantiated.

Striped Marlin and Wahoo Microvasculature.—The thickness of striped marlin and wahoo lamellae and lamellar walls (the water-blood barrier distance) are comparable to measurements determined for yellowfin and other tuna species (Table 1; Hughes, 1970) and are much thinner than those documented for most other fishes (Hughes and Morgan, 1973). Striped marlin have converged with tunas for diagonal lamellar vascular channels, although this characteristic appears more fully developed in the latter (compare Fig. 5B with 5C). Thin lamellar walls may be particu-

larly important to tuna and striped marlin because their oblique channels reduce red blood cell (RBC) residence times through the lamellae (Olson et al., 2003). Although wahoo lamellae appear as thin as those of tunas and striped marlin (Table 1), they do not possess shortened diagonal channels (Fig. 5D). The added resistance of channel length appears to be compensated by larger spaces between the pillar cells. Increased RBC residence times through longer wahoo lamellar channels may account for the slightly thicker lamellar walls than those found in striped marlin and yellowfin (Table 1).

If the main function of oblique lamellar blood flow is to minimize blood pressure drop while maintaining thin lamellae (short diffusion distances) and to increase the number of respiratory blood channels in parallel, it remains unclear why diagonal channels occur in Atlantic mackerel, *Scomber scombrus* Linnaeus, 1758 (Muir and Brown, 1971), but not in wahoo (which possess much larger lamellae and likely have greater aerobic demands). Also unexplained is why yellowfin and striped marlin have diagonal channels in the short lamellae near the filament tips. A more detailed comparative study of channel design in these and other high-performance fishes may provide additional insight into these discrepancies.

Tunas possess the largest relative gill surface areas documented (Muir and Hughes, 1969), and preliminary data indicate that the gill areas of striped marlin and wahoo are also relatively large compared to most marine teleosts (Wegner, unpubl. data). The increase in gill surface area for these high-performance fishes is largely the result of their reduced lamellar thickness which increases the number of lamellae per length filament (Table 1). Thus, the combination of increased gill surface areas, thin lamellae, and thin lamellar walls, greatly enhances gas exchange in these high-performance fishes.

Phylogeny.—The relationship of *Acanthocybium* and the billfishes within the suborder Scombroidei has been controversial, and only a brief account will be presented here. Early osteological studies showed that *Acanthocybium* is more similar to the Scombridae (tunas, bonitos, Spanish mackerels, mackerels) than the billfishes (Istiophoridae + *Xiphias*) (Gregory and Conrad, 1937; Conrad, 1938). Similarly, based on morphological characters, Collette et al. (1984) placed the wahoo within the Scombridae as sister group to *Scomberomorus* (Spanish mackerels) and placed the billfishes as sister group to the Scombridae. Johnson (1986) subsequently proposed an alternative hypothesis, in which billfishes are the sister group to *Acanthocybium* and included in the Scombridae (wahoo + billfishes are proposed as the sister group to *Scomberomorus*). Johnson (1986) based his hypothesis on several synapomorphic traits of *Acanthocybium* and the billfishes including: an elongate beaklike snout, the loss of gill rakers, cartilaginous gill filament fusions, and the investment of the gill filaments with bony epithelial toothplates. Johnson (1986) argued that these characters (with the exception of the loss of gill rakers) are unique among perciforms, and are less likely to occur within separate lines than the skeletal changes that separate the billfishes from the wahoo. The inter-lamellar fusion documented in this study is thus another synapomorphy that links the Istiophoridae to *Acanthocybium*. Despite this finding, recent studies based on both morphological and genetic data call for billfishes to be placed in a separate suborder from the Scombroidei (Orrell et al., 2006).

If the billfishes are in fact the sister group to wahoo, the inter-lamellar fusions of striped marlin and wahoo may be of the same origin as the lamellar fusions of tu-

nas. To our knowledge the status of lamellar and inter-lamellar fusions in the genera separating *Acanthocybium* from the tunas has never been documented. However, we note that lamellar fusions (similar to those of tunas) are present for the Eastern Pacific bonito, *Sarda chiliensis* (Cuvier, 1832) (Wegner, unpubl. data).

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