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Hydraulic control of tuna fins: A role for the lymphatic system in vertebrate locomotion

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Abstract

The lymphatic system in teleost fish has genetic and developmental origins similar to those of the mammalian lymphatic system, which is involved in immune response and fluid homeostasis. Here, we show that the lymphatic system of tunas functions in swimming hydrodynamics. Specifically, a musculo-vascular complex, consisting of fin muscles, bones, and lymphatic vessels, is involved in the hydraulic control of median fins. This specialization of the lymphatic system is associated with fish in the family Scombridae and may have evolved in response to the demand for swimming and maneuvering control in these high-performance species.

The lymphatic system of vertebrate animals plays a key role in osmoregulation and immune response. Although developmental and molecular markers indicate genetic origins similar to those in mammals (1, 2), the lymphatic system in teleost fish, the most diverse class of vertebrates, might perform additional functions (3–8). A variety of experiments have described the lymphatic system in teleost fish, illustrating that it is a separate, parallel circulatory system (1, 5, 7, 9, 10). Current knowledge of the lymphatic system in fish assumes that its presence and distribution do not appear to be exclusively linked to phylogenetic position, but rather to the physiological adaptations of the species (4, 6). The lymphatic system has been hypothesized to be more extensive in tunas than in other teleost fish (3, 4), owing to their elevated blood pressures (11), high metabolic rates in some species (12, 13), and high gill surface areas (14) that likely increase osmoregulatory demands (12, 15). However, the lack of data on the morphology of the lymphatic system in tunas makes it

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SUPPLEMENTARY MATERIALS

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Materials and Methods

Figs. S1 to S9

Tables S1 to S5

Movies S1 and S2

Reference (37)

difficult to determine its function. Here, we use a multi-disciplinary approach, applying vascular injection, as well as histological, cytological, and immunological methods, video observations of swimming, computer-aided design (CAD), and computer fluid dynamics, to study the role of the lymphatic system of Pacific bluefin (*Thunnus orientalis*) and yellowfin tunas (*Thunnus albacares*).

Dissections of tuna fin musculature and vasculature revealed a large vascular sinus (VS) located at the base of both the second dorsal and anal fins (Fig. 1A). Perfusion of a casting compound (Microfil) into the VS exposed a musculo-vascular complex not previously described in morphological descriptions of tuna fins (Fig. 1 and figs. S1 and S2). Morphologically, the sinus creates a chamber formed by the bone and cartilage tissues. The dome of the chamber is formed by the proximal parts of the fin rays, whereas the ventral surface is formed by the adjacent proximal parts of pterygiophores supporting the fin (Fig. 1A). The junction between the fin rays and pterygiophores restricts lateral movements of the fin rays. The VS is connected with vessels that form a vascular network around the fin and channels located between the fin rays (Fig. 1, B and C). The vascular network of the second dorsal fin is connected with large vascular channels around the first dorsal fin (Fig. 1, C to E).

This common structure of the musculo-vascular complex was observed in both second dorsal and anal fins: The inclinator muscles contact the soft-walled vessels that are connected with the VS that extends into the expandable channels between the fin rays (Fig. 1, A and C, and figs. S1 to S3). The second dorsal and anal fin erection is hypothesized to be under the control of the musculo-vascular complex, including involvement of the erector, depressor, and inclinator muscles, as well as vascular vessels. We hypothesize that contraction of inclinator muscles causes inflow of fluid from the soft-walled vessels to the incompressible fin sinus and then to the expandable fin channels (Fig. 1B). Increase of pressure in the fin channels facilitates the effort of the erector muscles and counteracts the work of the depressor muscles, thus providing fine adjustments of the fin erection (Fig. 2, A and B, and movie S1). To test this hypothesis, we pumped saline solution into the fin sinus of a recently deceased tuna to simulate the contraction of the inclinator muscles and alter pressures in fin channels. Increased pressure in the range of 17 to 48 kPa caused fin erection and decreased fin sweep angle (Λ) by up to 14° (movie S2 and fig. S4). The pressure-driven fin erection in the absence of erector muscle effort is evidence of the hydraulic effect in the fins, although the contribution of specific muscles in fin movement remains unclear.

To understand how the pressure-driven fin erection corresponds to the range of fin movements and behavior of living fish, we recorded and analyzed videos of swimming tunas in a captive facility. Video data were categorized by the behavior patterns of bluefin and yellowfin tunas before, during, and after feeding periods and by measured sweep angle of the second dorsal fin (fig. S4) that moves synchronously with the anal fin. The fin erection depended on swimming behavior and varied from $\Lambda = 73.9 \pm 4.3^\circ$ (mean $\pm$ SD) during rectilinear movement when no feeding occurred to $\Lambda = 60.3 \pm 7.2^\circ$ (mean $\pm$ SD) during frequent changes of movement direction during feeding times [$P < 0.001$, repeated measures analysis of variance (ANOVA); fig. S5 and table S1]. Additional analyses of free-swimming tunas at a large exhibit tank in the Monterey Bay Aquarium revealed that the maximal

second dorsal fin erection was significantly associated with turning maneuvers (table S2). Together, these data suggest that median fin erection increases from cruising behavior with predominant rectilinear movement to searching and feeding behavior with frequent changes of movement direction. The results also indicate that the pressure-driven fin erection in the in vitro experiment (movie S2) falls within the natural range of fin erection in swimming tuna.

Median fins in fishes are typically oriented in the vertical plane and involved in control of posture and swimming trajectories (16). Median fins of tunas are analogous to thin, symmetrical hydrofoils generating sideways lift force (17) when the fin plane makes an angle to the fluid flow direction (Fig. 2, C to E). Movable fins, capable of changing their area and shape, are associated with expanded capabilities for producing hydrodynamic stabilizing forces (16). To understand how fin erection alters its hydrodynamic performance, we constructed CAD models of the Pacific bluefin tuna with erected and depressed second dorsal and anal fins and tested them with a computer fluid dynamics program for a range of speeds (2, 6, and 10 m/s) and yaw angles (1° to 20°) (fig. S6). Erected fins had improved lift within a range of yaw angles from 1° to 8° , which increased with increased simulated speed (table S5). This increment of lift resulted in improved lift-to-drag (L/D) ratios, i.e., the best wing performance (18) up to 22 and 15% for the dorsal fin and anal fin, respectively, at increased speeds from 2 to 6 m/s (fig. S6 and table S5). The L/D increment for both fins depressed at the same conditions was 11%. This L/D ratio variation with wing sweep is comparable to the experimental data of morphing aquatic micro air vehicle (19). The improved performance of erected median fins may be advantageous at turning maneuvers (20–22), which is in agreement with video analyses of swimming tuna (fig. S5 and tables S1 and S2). Both depressed and erected fins had maximal L/D ratios within a small range of yaw angles, from 4° to 7° ; this effect was reversed above 9° . As tuna are highly specialized in cruising, with a relatively large turning radius compared to other species (23), this range may indicate possible optimization of median fins for swimming control at small inclinations from the axial movement.

To determine the origin of the vascular system within the fin sinuses, we performed vascular injections, as well as histological, cytological, and immunological assays. Injection of Microfil into the VS revealed separation between the vessels connected to the VS and the arteries and veins (Fig. 3A and fig. S7). The Microfil was present on hematoxylin & eosin (H&E)–stained sections only in a subsection of vessels with veinlike morphology (Fig. 3A)—a characteristic of the lymphatic system (1, 7, 24). Pigmented aggregates resembling melano-macrophage centers (MMCs) were observed on the endothelium of the VS and connected vessels (Fig. 3, A and B, and fig. S7). In other teleosts, MMCs contain lymphocytes and macrophages and have been shown to facilitate antigen presentation, suggesting a function similar to that of lymph nodes in other vertebrates (25, 26). Isolation of cells revealed that red blood cells (RBCs) were the dominant cells in the blood ($>95\%$), whereas RBCs constituted less than 40% of the cells in the sinus liquid (Fig. 3C); the rest of the cells had a lymphocyte and myeloid morphology. The spleen in fish is a lymphoid organ and considered to be the reservoir for the blood system (26, 27) and, as such, the leukocytes fraction in the spleen was higher than in the blood but lower than in sinus liquid (Fig. 3C).

To compare the different cell components of the sinus liquid and blood, we used flow cytometry (FACS, fluorescence-activated cell sorting) analysis. FACS analysis revealed about 96% RBCs, 1% lymphocytes, and 3% myeloid cells in blood, thus supporting our microscope observations. By contrast, the sinus liquid samples revealed a composition of about 35% RBCs, 20% lymphocytes, and 40% myeloid cells (Fig. 3E). In addition, hematocrit was significantly lower in sinus liquid compared to blood (Fig. 3D). Likewise, hemoglobin and mean cell hemoglobin concentrations were reduced in sinus fluid, as was plasma lactate content (table S3). We used a LysoTracker marker to test whether leukocytes that were observed on a morphological level contained acidic vesicles. LysoTracker-positive leukocytes contain acidic vesicles, which are typically associated with lysosomal compartments in immune cells. LysoTracker was observed in myeloid cells at high levels and to a lesser extent in some of the lymphocytes but was not observed in RBCs (fig. S7H). We then performed a fluorescent beads phagocytosis assay to test whether the leukocytes in the sinus liquid would function immunologically (24, 27). The percentage of phagocytic cells was higher (>4-fold) in the sinus fluid compared to the blood (Fig. 3, F and G), thus confirming the functionality of the leukocytes in the sinus liquid. To validate phagocytosis, we performed a confocal microscopy three-dimensional analysis of beads internalization, and engulfment and fusion with lysosomes of *Staphylococcus aureus* bioparticles (fig. S8). Cytoskeleton inhibition significantly diminished phagocytosis of *S. aureus*, showing that this process is an active function of phagocytic cells in the VS fluid (fig. S8). Together, the results suggest that the VS located at the base of median fins is connected to the lymphatic system of tuna and filled with lymphatic fluid.

We describe a musculo-vascular complex of median fins in Pacific bluefin tuna, where the inclinator muscles and lymphatic vessels indicate hydraulic control of fins. We also found this complex in other scombrids, including yellowfin tuna, Pacific bonito, and Spanish mackerel, but it was absent in Pacific mackerel (Fig. 4A). Although the phylogeny of the Scombridae family tribes has been questioned (28), our finding corroborates a more recent whole-mitochondrial genome analysis (29) showing the proximity of tuna, bonito, and Spanish mackerel tribes (Fig. 4B). The apomorphy of the lymphatic network in median fins may have evolved as a response to the demand for swimming control in cruising specialist species, possibly related to pressure distribution around their bodies (30–32) (fig. S9). Differences in secondary vascularization in scombrid fishes may also be related to variability in metabolic rates associated with species specialization (6, 33). Constant fine adjustment of the sweep of the fins or wings can be observed in other animal classes and appears as part of swimming or flight control involved in lift-based propulsion (34). The discovered mechanism described here may also inspire further development of biomimetic propulsive robots with enhanced motion control.

The use of fluids, in combination with muscular arrangements, for force transmission has been described in a variety of invertebrate animals, including worms, mollusks, arthropods, and coelenterates, as well as in penises in mammals (35, 36). Unlike other known examples of animal hydraulics, the musculo-vascular complex described here for tuna fins is formed by the integration of the lymphatic vessels and muscles having skeletal support. The complex is composed of the three elements of a canonical hydraulic system: muscles that may serve as a hydraulic pump to pressure the lymphatic fluid, vascular vessels to guide and

control the system, and fin rays acting as actuators to convert pressure energy into mechanical energy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Morphology of the musculo-vascular system in Pacific bluefin tuna median fins
(A) Cross section of the second dorsal fin (SDF) sinus. Top left inset: A dissecting scope image of the SDF cross section. Bar, 1 mm. Abbreviations: fin rays (FR), expandable vascular channels (VC), incompressible chamber of the vascular sinus (VS), scales (SC), inclinator muscles (IM) surround lateral vessels connected with VS, epaxial musculature (EPM), proximal parts of pterygiophores (PT), erector muscles (ERM), and depressor muscles (DM). **(B)** Cross section of the depressed (left) and erected (right) SDF shows collapsed and expanded VC between fin rays. **(C)** Three-dimensional structure of the musculo-vascular system in SDF. PT and some FR have been removed for illustrative purposes. FR, connective tissue (CT) sheath between skin and IM, EPM, and compressible vascular channels (CVC) continue around the first dorsal fin (FDF). **(D)** Cross section of the FDF. FR of the folded FDF in the groove on the fish's back, CVC beneath the SC layer, IM, ERM and DM, and EPM. **(E)** Blue Microfil injection into the incompressible chamber of the VS at the base of the SDF; skin with the scales has been removed. Bar, 2 cm. FR of the erected FDF, CVC around the FDF, and connection of the CVC with the VS incompressible chamber at the base of the SDF; blue Microfil compound injected into the sinus reveals the expanded vascular channels between FR of the SDF.

Fig. 2. Biomechanics and hydrodynamics of tuna median fins

(A and B) Schematic of the hydraulic effect in the second dorsal fin (SDF) of tuna. (A) Depressed fin: compressible vascular channels (CVC), fin rays (FR), expandable vascular channels (VC) between the FR, relaxed inclinators (IM), and incompressible chamber with vascular sinus (VS). (B) Erected fin: solid arrows indicate collapsed CVC caused by contraction of the IM. Dashed arrow indicates outflow of liquid from CVC to the VS and VC. (C) Isometric view of a CAD model of tuna shows the simulated flow pattern associated with the SDF and anal fin (AF) at 6 m/s and a yaw angle of 11° . (D and E) Top view of a tuna CAD model shows the velocity V , lift vector L , and drag vector D ; α is the yaw angle formed by the fin plane and the velocity vector. (D) Enlarged view of the second dorsal fin without associated flow.

Fig. 3. Vascular sinuses of median fins are connected to the lymphatic system

(A) Blood and lymphatic vessels after blue Microfil injection into the VS of the anal fin. Internal lining of the peritoneal cavity (left), H&E histological section of the lining of the peritoneal cavity (right). Examples of arteries, veins, and vessels staining positive for Microfil; melano-macrophage centers (MMCs) are labeled. Bars, 1 cm (left panel); 150 μ m (right panel). (B) Image of longitudinal section of VS. Veins and MMCs in the endothelium of the VS are labeled; ruler in mm. (C) Cells were isolated from the VS, blood, and spleen. Upper panels, live cells; lower panels, methanol-fixed and Giemsa-stained cells. Examples of the main three cell types are labeled: red blood cells, lymphocytes, and myeloid cells. Bars, 40 μ m. (D) Hematocrit analysis of the VS liquid (left) and blood (right). Plasma, hematocrit (RBCs), and buffy coat (white blood cells and platelets) are labeled. (E) Cells were isolated from blood (left) and the VS (right) and analyzed by flow cytometry using forward scatter (FSC) and side scatter (SSC) for populations of RBCs, lymphocytes, and myeloid cells. (F and G) Cells were cocultured with beads and analyzed by green

fluorescence for phagocytosis and FL4 (unstained). Representative analysis of blood samples with gated positive cells (left) and VS (right). (G) Normalized analysis of fold change of cells positive for phagocytosis from three different experiments. * $P < 0.005$, single-factor ANOVA.

Fig. 4. The lymphatic sinus in the Scombridae family

(A) Dissecting scope images of sections taken from the base of the second dorsal fin from Pacific mackerel, Spanish mackerel, bonito, and yellowfin tuna. The vascular sinus (VS) is labeled in Spanish mackerel, bonito, and yellowfin tuna with a black arrow but is not detected in Pacific mackerel. Bars, 1 mm. (B) Hypothesized development of the VS in the Scombridae family cladogram. The cladogram of the four scombridae tribes is reproduced with permission from a whole-mitochondrial genome analysis (22), based on 18 species of scombroid fishes. Hypothesized development of the vascular sinus based on the detection of VS in two species of Thunnini tribe (*Thunnus albacares* and *Thunnus orientalis*), one species of Sardini tribe (*Sarda chiliensis*), and one species of Scomberomorini tribe (*Scomberomorus sierra*) and the lack of the VS in one species of Scombrini tribe (*Scomber australasicus*).