

Innervation and structure of the adipose fin of a lanternfish

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Abstract

Adipose fins of teleost fishes have been shown to function as mechanosensory organs that respond to minute bending forces created by turbulence in fast-flowing streams. Nonetheless, adipose fins also exist in some fishes that occupy still waters, including lanternfish (Myctophidae) in the deep sea. The authors examined adipose fin structure in northern lampfish, *Stenobrachius leucopsarus*, from coastal British Columbia. After fixation, embedding and sectioning of the adipose and supporting tissue, it was evident that lanternfish adipose fins are stiffened by compound actinotrichia, acting like fin rays, that would create a higher aspect ratio. The actinotrichia converge at the base of the fin in a hinge point complex that anteriorly interacts with a cartilaginous endoskeletal rod, controlled by skeletal muscles. Afferent nerves enter the fin at this point and form fine branches as they track deeper alongside actinotrichia. The authors propose that the vertical nightly migration to surface waters, as well as predator evasion within large schools, results in microturbulence. In these circumstances, the adipose fin acts as a mechanosensor providing feedback to the caudal fin, as it occurs in salmonids and catfish.

KEYWORDS

adipose, endoskeleton, lanternfish, muscles, myctophid, nerves

1 | INTRODUCTION

Adipose fins of teleosts were for a long time considered to be vestigial and lacking innervation or obvious function (Garstang, 1931). A series of papers on adipose fins of salmonids (Buckland-Nicks, 2016; Buckland-Nicks *et al.*, 2011; Reimchen & Temple 2004) and catfish (Stewart & Hale, 2015) have recently proven this theory to be incorrect. Rather, the adipose fin, although varied in origin (Stewart *et al.*, 2014), is a highly innervated structure (Buckland-Nicks, 2016; Koll *et al.*, 2020) that can act as a sensitive mechanosensory organ for monitoring precaudal flow (Aiello *et al.*, 2016; Koll *et al.*, 2020). This was predicted by Reimchen and Temple (2004), who studied swimming responses of trout after amputation of the adipose fin. Nonetheless, the structure and innervation of adipose fins is achieved in different ways in these two groups. Salmonid adipose fins are flexible, lack muscles, fin rays or adipose tissue but have an extensive neural network interconnected with astrocyte-like glial cells linked to a collagen framework (Buckland-Nicks, 2016; Buckland-Nicks *et al.*, 2011; Koll *et al.*, 2020); whereas the catfish adipose lacks the glial cell network and relies more on information conveyed by the deformation of

afferent nerves and their fine branches (Aiello *et al.*, 2016). These proprioceptive adipose fins are passive, as they do not have any muscles or endoskeleton and their mobility is based on water motion.

Fins primitively lacked endoskeletons for muscle attachment, and therefore it was suggested that mechanosensation preceded the acquisition of fin movement (Aiello *et al.*, 2016). The first musculo-skeletal linkage in an adipose fin was discovered in the catfish *Horabragrus brachysoma*, but it is absent in adipose fins of any families related to Horabragidae (Stewart & Hale, 2013). The present paper describes, for the first time, the structure of the adipose fin of a common lanternfish (Myctophidae) which, like the adipose fin of *H. brachysoma*, is highly innervated and equipped with a musculo-skeletal linkage, permitting movement.

2 | MATERIALS AND METHODS

2.1 | Sampling

Northern lampfish individuals, *Stenobrachius leucopsarus* (Eigenmann & Eigenmann, 1890) (Myctophidae), a species of lanternfish, were caught in

a rope trawl off Texada Island in the Strait of Georgia (app. 49.6° N, 124.5° W), on 11 February 2020 at a depth of 116 m, brought to the surface and placed in labelled bags in a freezer at -20°C (lethal sampling of fish for inspection purposes, abundance estimates and other population parameters required for stock assessments are exempted from requiring an animal use protocol under Fisheries and Oceans Pacific Region Animal Care Committee protocols).

Frozen fish were thawed in the laboratory, and each adipose fin of 10 individuals was removed together with about 1×0.5 cm of dorsal tissue and processed for light and electron microscopy. Samples of unfixed frozen tissues were sent to R.J. Nelson (University of Victoria) for DNA barcoding and identification.

2.2 | Electron microscopy

For transmission electron microscopy (TEM) six adipose fins were fixed in 0.1 M phosphate-buffered 2.5% glutaraldehyde overnight at 5°C . Subsequently the fins were rinsed in buffer and post fixed with 1% osmium tetroxide in the same buffer for 1 h at room temperature. Fins were rinsed in buffer, dehydrated in an ethanol series to 100% and transferred through propylene oxide to Epox 812 resin, following standard procedures. Fins were embedded in pure resin in BEEM capsules and baked for 2 days at 60°C . Blocks were trimmed to provide both frontal and sagittal sections of the fins, and a series of $1 \mu\text{m}$ sections were cut with glass knives and stained with 1% toluidine blue made to pH 9 with sodium bicarbonate. Areas of specific interest were outlined by mesa-trimming, thin sectioned with a diamond knife (Diatome) on an LKB Ultratome II and stained with uranyl acetate and lead citrate following standard procedures.

2.3 | Immunocytochemistry

For immunocytochemistry, four fins were immersed in 4% paraformaldehyde (TAAB Laboratories Equipment Ltd., Aldermaston, Berks, UK) in phosphate-buffered saline (PBS; 140 mM NaCl and 50 mM Na_2HPO_4 , pH 7.2) overnight. Whole adipose fins were embedded in

warm 3% agar made up in PBS buffer in moulds. Cooled agar blocks were mounted on the specimen tray of a Vibratome 1000 (TPI Inc., St Louis, Missouri, USA) with Loctite Superglue Gel. The tray was filled with PBS, and a series of $150 \mu\text{m}$ sections were cut longitudinally through the fin. Sections were picked up with a wet sable brush, transferred to PBS and stored at 4°C .

Zn-12 monoclonal antibody was used to reveal the general pattern of innervation of the adipose fin. This antibody has been shown to specifically label axons in zebrafish, goldfish and other teleosts (Newton *et al.*, 2014; Trevarrow *et al.*, 1990; Varatharasan *et al.*, 2009).

Sections of adipose fins were immersed in a PBS-based blocking solution composed of 0.25% Triton X-100, 2% dimethyl sulfoxide, 1% bovine serum albumin, 1% normal goat serum (all from Sigma Aldrich, St Louis, MO, USA) for 12 h at 4°C . Unless otherwise stated this solution was also used for all subsequent washes and dilutions. Tissues were next incubated in the Zn12 primary antibody diluted 1:100 for 7 days at 4°C , washed thoroughly and then incubated for 5 days in goat anti-mouse secondary antibody, labelled with Alexa Fluor 488 (Invitrogen, Burlington, ON, Canada).

To verify the specificity of the secondary antibodies, tissues were processed for immunocytochemistry as described earlier, but the incubation in primary antibodies was replaced with incubation in the same diluent with the omission of the antibodies. No fluorescence was observed in these negative controls. Tissues were examined on a Zeiss 510 LSM, using lasers appropriate for Alexa Fluor 488 nm.

3 | RESULTS

3.1 | Fin structure

The adipose fin of the lampfish, *S. leucopsarus* (a species of lanternfish), is located just anterior to the caudal fin. A single pair of bilateral muscles attaches to a rod-like endoskeletal cartilage anterior to the adipose fin (Figures 1–4), that terminates as a rounded tip in the connective tissue at the base of the fin (Figures 1 and 2). Caudally from the attachment to the endoskeleton, the muscles taper (Figure 4) and

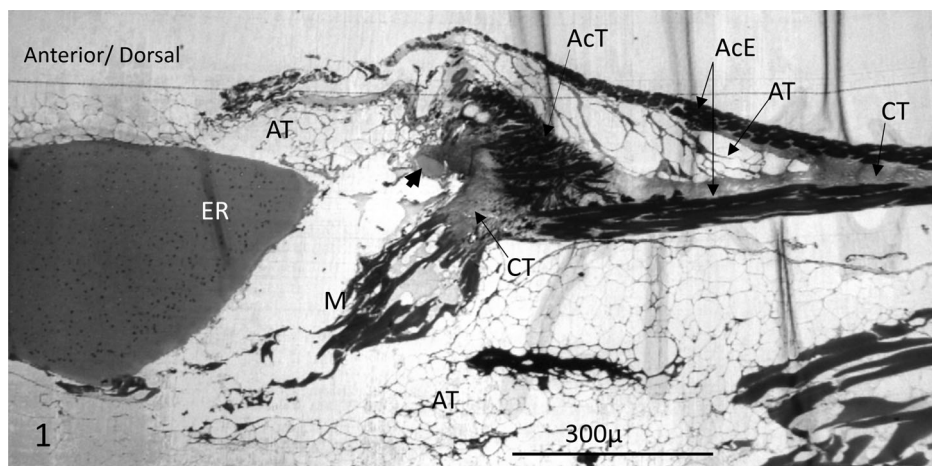


FIGURE 1 Sagittal section through adipose fin, where the endoskeletal rod (ER) terminates in a ball-like joint (arrowhead) with connective tissue (CT). Muscle fibres (M) attach between the endoskeletal rod and the CT at the base of the fin, where the compound actinotrichia terminate (AcT) near the hinge point complex. Actinotrichia reinforce the edge of the fin (AcE), inside which is adipose tissue (AT) near the base and CT near the tip. Scale bar = $300 \mu\text{m}$

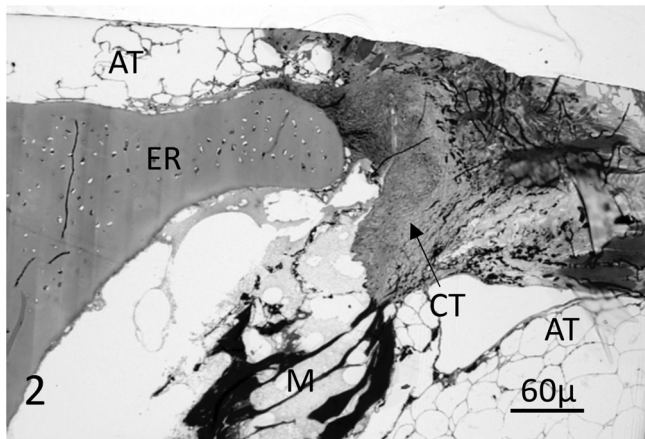


FIGURE 2 Endoskeletal rod (ER) and muscle fibres (M) terminating in connective tissue (CT) near hinge point complex. Adipose tissue (AT) can be seen surrounding the ER and at the base of fin. Scale bar = 60 μ

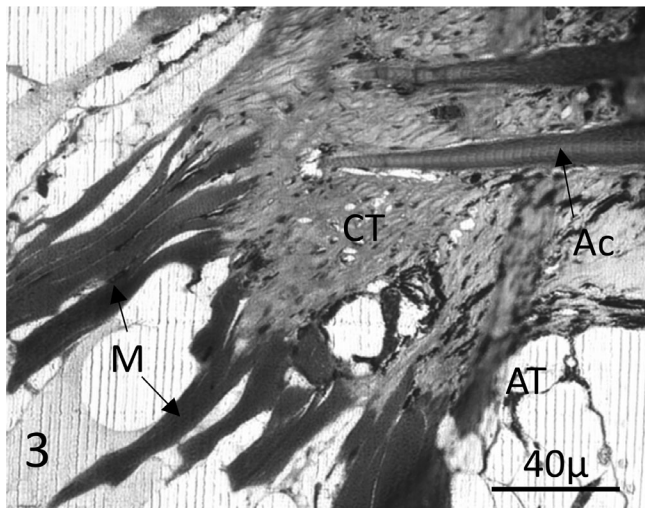
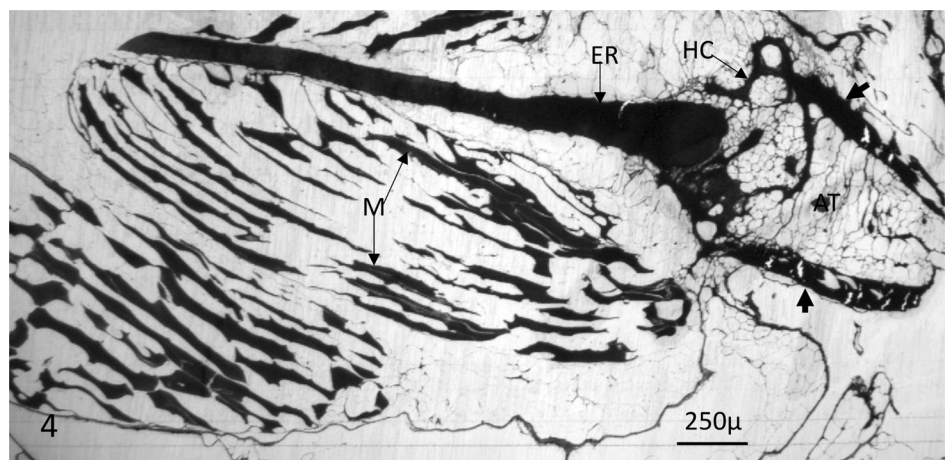


FIGURE 3 Enlarged area of Figure 1 showing compound actinotrichia (Ac) and muscle fibres terminating in connective tissue (CT) at the base of fin near hinge point complex. Adipose tissue (AT). Scale bar = 40 μ

FIGURE 4 Frontal section of fin showing endoskeletal rod (ER) connecting with hinge point complex (HC) leading into reinforced edges of adipose fin (arrowheads). Muscle fibres (M) attached to cartilage. Scale bar = 250 μ



become embedded in connective tissue anteriorly at the base of the fin (Figures 1–3). Compound actinotrichia support the fin in bundles particularly at the fin edge (Figures 1 and 4) but they converge on the same area of connective tissue at the base of the fin (Figures 1 and 5). In this way the endoskeleton and connective tissue form a “hinge-point complex” between actinotrichia and muscles (Figure 4). Each compound actinotrichium is composed of bundles of individual actinotrichia and the “hinge point” is reinforced by complex branches of them (Figure 4). Individual actinotrichia may be connected to ligaments (Figures 6) or may be embedded directly into the connective tissue adjacent to the muscle insertions (Figure 3). At the TEM level muscles are revealed to comprise typical striated skeletal muscle fibres (Figure 7). Adipose tissue is found inside the adipose fin, as well as adjacent to the endoskeletal cartilage and muscles (Figures 1–4 and 8). Individual actinotrichia are shown to be striated and may be bundled to form compound actinotrichia (Figures 9 and 10).

3.2 | Immunofluorescence

Zn-12 antibody labelled with Alexa Fluor 488 revealed several nerves at the junction between connective tissue and muscles, which entered the fin and ran alongside actinotrichia (Figure 11), and tracked deeper into the fin (Figure 12). Nerves were also associated with the muscles in this area (acting as a control for neural stain), which meet the convergence of actinotrichia near the hinge point complex (Figures 11, 13 and 14). A diagram summarizing these features is shown in Figure 15.

4 | DISCUSSION

Teleost fins evolved from simple dermal projections and later developed endoskeletal support, with muscles gradually being integrated into the system and providing active control of fin movement (Coates, 1994; Stewart *et al.*, 2019). The adipose fin, located between the dorsal and caudal fins of a wide variety of fishes, was assumed to be a vestige with insignificant function (Sandon, 1956), apparently lacking innervation and any internal skeleton or muscles. These

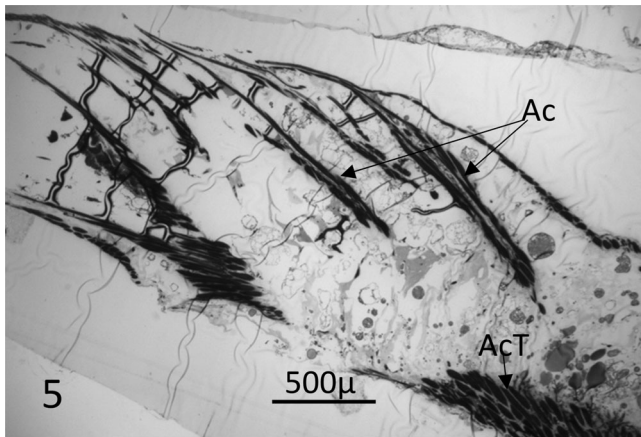


FIGURE 5 Sagittal section of adipose fin (AF) showing compound actinotrichia (Ac) supporting middle and edge of fin. Actinotrichia terminate (AcT) close together at the base of the fin. Scale bar = 500 μ

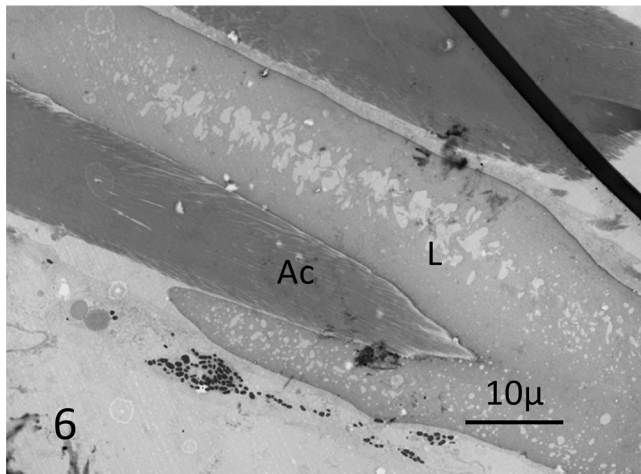


FIGURE 6 TEM of actinotrichium (Ac) terminating within a ligament (L). Scale bar = 10 μ

assumptions have since been shown to be incorrect, as detailed studies have shown that adipose fins can have hydrodynamic benefits (Reimchen & Temple, 2004), be highly innervated (Buckland-Nicks, 2016; Buckland-Nicks *et al.*, 2011; Stewart & Hale, 2013) and be capable of sensitive mechanosensory responses to stimuli (Aiello *et al.*, 2016; Koll *et al.*, 2020).

An endoskeletal cartilage is lacking in adipose fins of most teleost species (Sandon, 1956). In a few exceptions, cartilaginous plates develop at the base of adipose fins (Matsuoka & Iwai, 1983). Adipose fin skeletons have been described mainly from Euteleostei (Stewart *et al.*, 2014) with the catfish being the first from the Otophysi (Stewart & Hale, 2013).

In several respects, the anatomy of the adipose fin of lanternfish bears similarities with the adipose fin of the catfish, *H. brachysoma*, in that an endoskeletal rod of cartilage provides for attachment of muscles on both sides of the midline, anterior to the fin. This is only the second instance of a musculo-skeletal linkage being found in any adipose fin. In

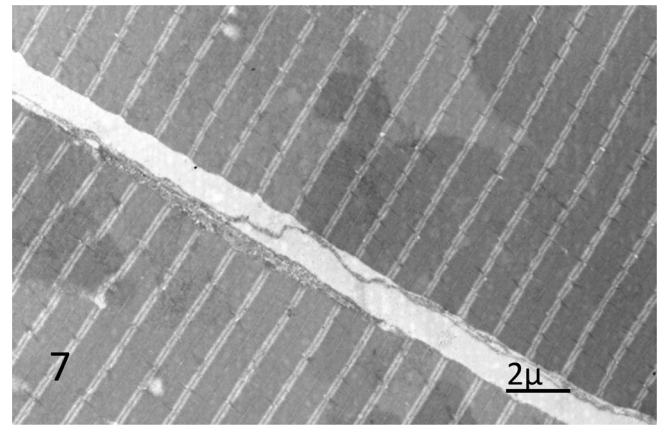


FIGURE 7 TEM revealing characteristic striations of skeletal muscle fibres. Scale bar = 2 μ

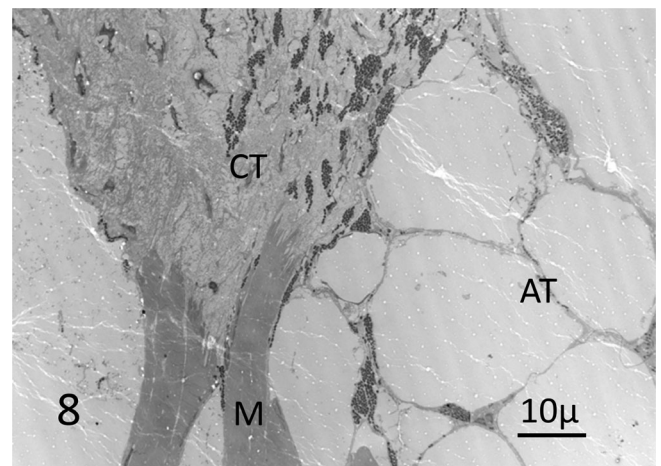


FIGURE 8 TEM of muscles (M) attaching to connective tissue (CT), next to some adipose tissue (AT). Scale bar = 10 μ

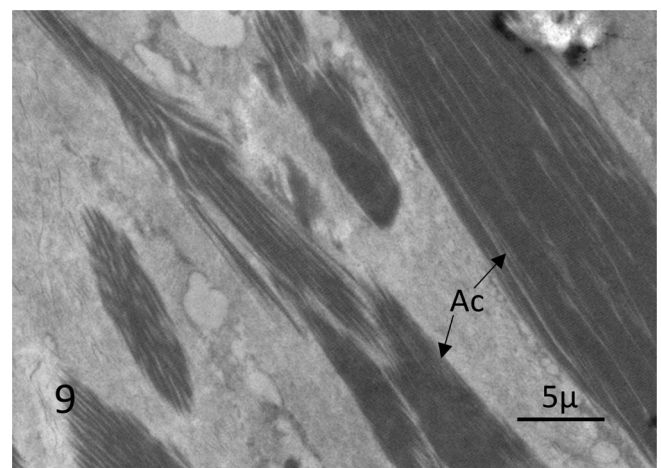


FIGURE 9 Individual actinotrichia make up bundles of striated fibres. Scale bar = 5 μ

the lanternfish, the muscles taper posteriorly and show left to right asymmetry, much as was observed in *H. brachysoma* (Stewart & Hale, 2013). The lanternfish adipose appears to have the single pair of adipose fin

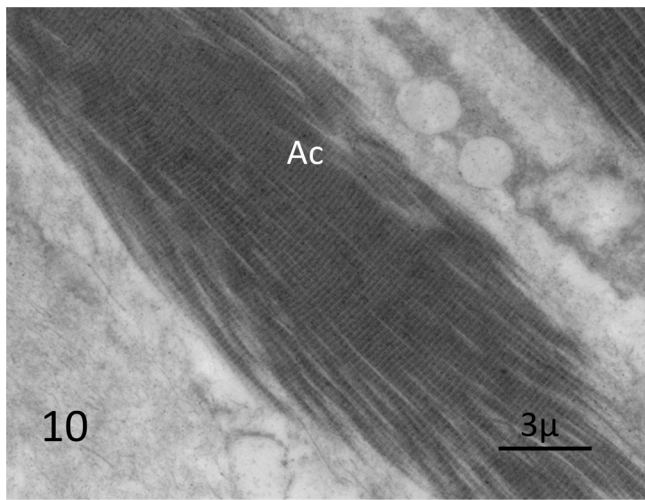


FIGURE 10 Compound actinotrichia are comprised of individual striated actinotrichia. Scale bar = 3 μ

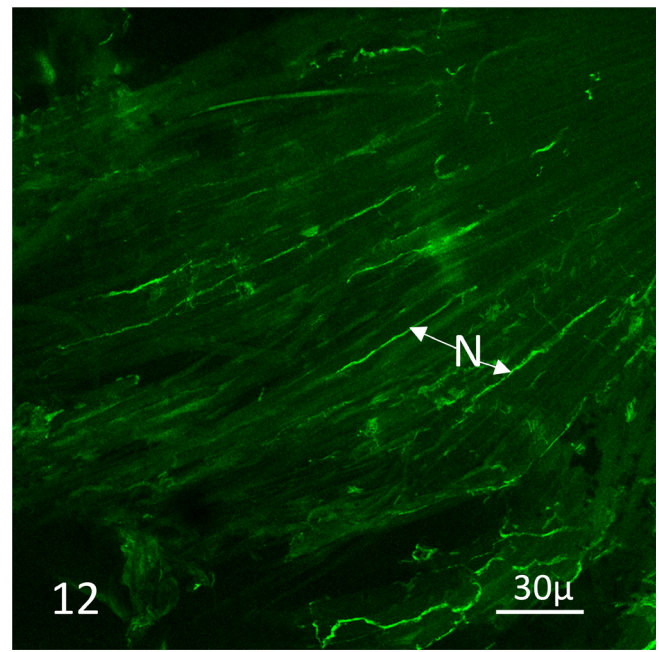


FIGURE 12 Nerves (N), revealed by Zn12 and Alexa Fluor 488, tracking actinotrichia deeper into the fin. Scale bar = 30 μ

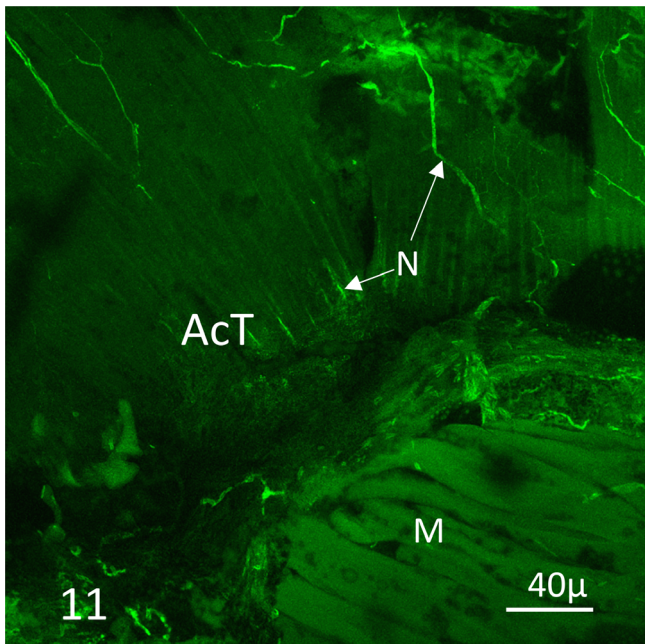


FIGURE 11 Junction between actinotrichia termination (AcT) in connective tissue area stained with Zn12 and Alexa Fluor 488, revealing nerves (N) entering adipose fin adjacent to muscle (M). Scale bar = 40 μ

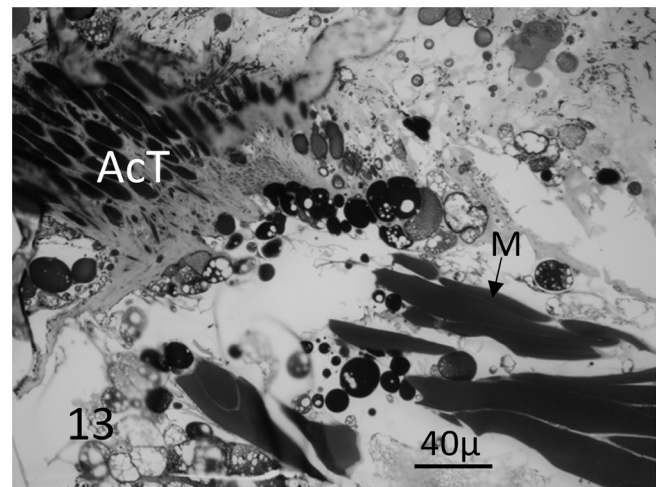


FIGURE 13 Toluidine blue stained section from a similar location to Figure 11, showing termination of actinotrichia (AcT) adjacent to muscle fibres (M) near the hinge point complex. Scale bar = 40 μ

muscles (AFM) but is lacking the SCAR-P that in catfish inserts also on the distal tip of the neural spine. This neural spine is lacking in lanternfish. Unlike the salmonids (Buckland-Nicks, 2016; Buckland-Nicks *et al.* 2011), both catfish (Stewart *et al.*, 2014) and lanternfish have adipose tissue surrounding the fin, as well as inside it.

In the lanternfish, the complex of terminating compound actinotrichia at the connective tissue junction, which creates a “hinge point” between adipose fin and the endoskeletal rod, has not been described in any adipose fin previously. This “hinge point” would

enable muscles to waggle the fin back and forth and possibly elevate or depress the fin. Movements such as these were observed in the catfish *H. brachysoma* (Stewart & Hale, 2013) but not in the lanternfish. Even though in video film sequences of lanternfish, the fin is usually seen to be raised (Rosenthal, 2017), sometimes it is observed lowered as well. By contrast the salmonid adipose fin contains no muscles and is highly flexible, likely relying on stretch receptors to convey information to nerves (Buckland-Nicks, 2016; Buckland-Nicks *et al.* 2011; Koll *et al.*, 2020). The lanternfish adipose, stiffened as it is by compound actinotrichia running

throughout, is more similar in strength and flexibility to bony fins, such as pectoral and pelvic fins of teleosts, with high aspect ratios when raised (Aiello *et al.*, 2017). High aspect ratio fins have, in general, been shown to be even more mechanosensitive than other fins (Aiello *et al.*, 2017; Hardy *et al.*, 2016), due to a larger area exposed to bending forces.

4.1 | Function of the adipose fin in myctophids

Adipose fins have been shown to have multiple origins, with long histories in some groups, suggesting functionality (Stewart *et al.*, 2014). Adipose fins have radically different structures, particularly between

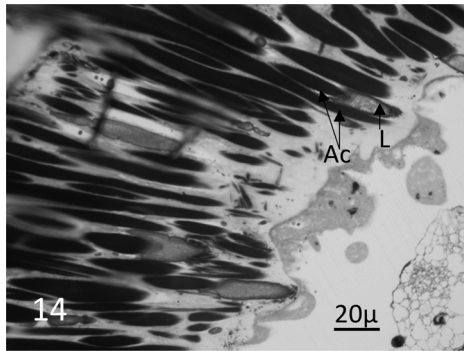


FIGURE 14 Toluidine blue stained section of actinotrichia (Ac) attaching to ligaments (L) in connective tissue near the hinge point complex. Scale bar = 20 μ

salmonids, characids and myctophids, but all investigated are highly innervated, suggesting that different solutions have been possible for repeatedly evolving a precaudal fin with mechanosensory properties (Aiello *et al.*, 2016; Reimchen & Temple, 2004).

The adipose fins of Horabragidae and Myctophidae have, in common, extensive innervation, an endoskeleton with attached muscles that can raise and lower the fin in Horabragidae (and likely also in Myctophida), and a stiff fin supported internally by bony rays or rod-like compound actinotrichia. Horabragidae also have a robust anterior spine. These fins are much stiffer than salmonid or other catfish adipose fins which have very different mechanisms of mechanosensation that do not rely on a high aspect ratio (Aiello *et al.*, 2016; Stewart & Hale, 2015).

Afferent nerves tracking fin rays, as occurring in lanternfish adipose, have been shown in a variety of other fins to respond to both bending and static positioning of the fin (Aiello *et al.*, 2017, 2018). The authors concluded that a proprioceptor response to fin ray bending is common among taxonomically distant species, suggesting that sensory feedback from these afferent nerves is important to motor function (Aiello *et al.*, 2018; Williams *et al.*, 2013). Furthermore, stiffness in fins with afferent nerves running alongside fin rays, as occurring also in lanternfish, correlates with increased sensitivity to bending forces (Aiello *et al.*, 2016). The adipose fin of the catfish *Corydoras aeneus* is sensitive to deflections as small as 0.12 mm. Afferent nerves were shown to exhibit a burst of activity at the onset of the bending stimulus and provided information about the static position and movement of the adipose fin (Aiello *et al.*, 2016).

Although the association between turbulent flow and the adipose fin as a pre-caudal sensor is evident in Salmoniformes and Siluriformes

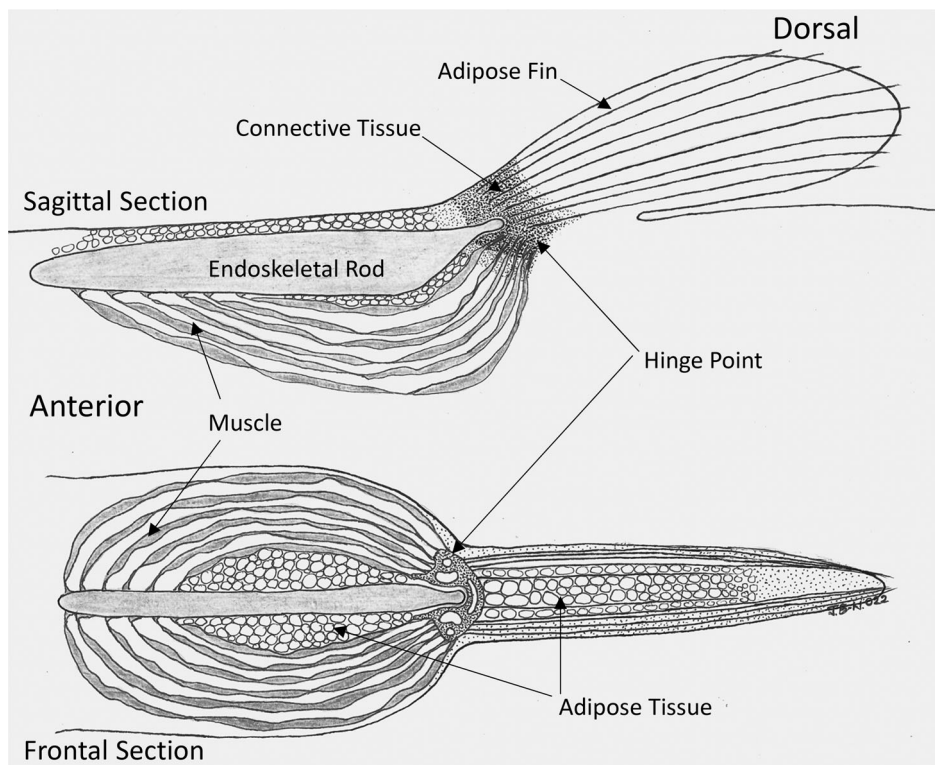


FIGURE 15 Diagrammatic representation of lanternfish adipose fin structure

(Aiello *et al.*, 2016; Reimchen & Temple, 2004; Temple & Reimchen, 2008), the mesopelagic and epipelagic habitat of the myctophids appear as clear exceptions to the complex turbulence in streams. Yet, the diel bi-directional nocturnal vertical migration of large schools of myctophids within the top 1000 m of the open ocean would create high levels of microturbulence within the school in which the adipose fin continues to operate as a pre-caudal sensor, as in stream-dwelling taxa. Close proximity of individuals within dense schools may result in lateral deflection of the adipose fin resulting from caudal fin motion of adjacent fish. It is interesting that the Osmerids (capelin, smelts, eulachon), also with an adipose fin, undertake long oceanic migrations in dense schools. Nonetheless, clupeids (herring) are also schooling but lack the adipose fin. Furthermore, adipose fin presence in myctophids may be adaptive in avoiding predators, such as squid, which cause them to make rapid changes in direction and acceleration to avoid capture (Rosenthal, 2017). Direct evidence for functionality of the myctophid adipose fin may emerge from high-resolution imaging of the fin movement during schooling and migratory behaviour.

AUTHOR CONTRIBUTIONS

J.B.-N. did the fixation, embedding, sectioning and interpretation of light and electron microscope images; and wrote the original draft of the manuscript. T.E.R. obtained fish samples from DFO; suggested, supervised and funded the study; provided intellectual input, wrote sections of the paper and revised all drafts.

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CONFLICT OF INTEREST

The authors declare that there are no competing interests. Data generated or analysed during this study are available from the corresponding author upon reasonable request.

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