Wall structure and material properties cause viscous damping of swimbladder sounds in the oyster toadfish *Opsanus tau*

Michael L. Fine¹, Terrence L. King¹, Heba Ali¹, Nehan Sidker¹ and Timothy M. Cameron²

¹Department of Biology, Virginia Commonwealth University, Richmond, VA 23284-2012, USA
²Department of Mechanical and Manufacturing Engineering, Miami University, Oxford, OH, USA

Despite rapid damping, fish swimbladders have been modelled as underwater resonant bubbles. Recent data suggest that swimbladders of sound-producing fishes use a forced rather than a resonant response to produce sound. The reason for this discrepancy has not been formally addressed, and we demonstrate, for the first time, that the structure of the swimbladder wall will affect vibratory behaviour. Using the oyster toadfish *Opsanus tau*, we find regional differences in bladder thickness, directionality of collagen layers (anisotropic bladder wall structure), material properties that differ between circular and longitudinal directions (stress, strain and Young’s modulus), high water content (80%) of the bladder wall and a 300-fold increase in the modulus of dried tissue. Therefore, the swimbladder wall is a viscoelastic structure that serves to damp vibrations and impart directionality, preventing the expression of resonance.

1. Introduction

The swimbladder, an organ responsible for much of the success and diversity of fishes, functions as an oxygen reservoir, an organ for buoyancy and in some cases, as an acoustic organ for sound production and hearing [1–3]. The bladder counteracts heavier bone and muscle tissue and can increase buoyancy to the point that fishes are weightless in water (neutrally buoyant) or in the case of the oyster toadfish weigh less in water than with the bladder removed, i.e. the fish is negatively buoyant but still gains lift from the bladder [4]. In addition to buoyancy functions controlled by gas secretion and reabsorption [5], the swimbladder can be converted to a sound-producing organ by the attachment of muscles [6–9] or an auditory organ if diverticula or ossicles connect the swimbladder to the ear [10–13].

Acoustically, the swimbladder has been modelled as an underwater resonant bubble [14,15], which radiates sound as an omnidirectional monopole. More recent work indicates that the swimbladder emits sound as a forced rather than a resonant response [16–18]. Swimbladder vibrations damp rapidly, each half cycle of a sound wave has a longer period than the preceding one, and there is a modest, if any, decrease in peak frequency with fish size [19]. The resonant bubble model, however, predicts a decrease in resonant frequency with fish size [20]. Although weakfish sounds have a lower peak frequency in larger individuals [21], the decrease is attributed to increased twitch times in bigger fish with longer muscles. There are fishes that produce resonant sounds [19], which damp slowly, maintain a relatively constant waveform period and have a steeply decreasing slope with fish size. In these cases, however, the swimbladder contacts a tendon or bone, the resonant structure. Once excited the resonant structure continues to excite the swimbladder, i.e. the resonant structure still drives a forced swimbladder response [19].

Electronic supplementary material is available online at https://dx.doi.org/10.6084/m9.figshare.c.3515196.
The swimbladder and sonic muscles of the oyster toadfish *O. tau* have been a model system for the mechanism of fish sound production. Extremely fast sonic muscles line the sides of the bladder [16,17,22–27], and their contraction rate determines the sound’s fundamental frequency. Sound and bladder movement damp rapidly, and high speed contraction is necessary to produce sound due to an inefficient quadrupole motion: the sonic muscles push the sides of the bladder inward [23] causing increased pressure that forces the bottom outward [16]. The swimbladder has an interesting heart shape that imparts a directional, as opposed to omnidirectional, sound field around the fish [28]. The reasons for the departure from predictions for the resonant bubble (rapid damping and directionality) have received little attention. Acousticians working on swimbladder scattering have tended to discount the swimbladder wall as a factor [29,30], and ascribed rapid decay to damping by surrounding fish tissue [31,32]. One complex mathematical treatment of codfish, modelled the swimbladder as an elastic rubber membrane surrounding an underwater bubble; the bladder in turn was assumed to be surrounded by viscoelastic fish tissue [33]. However, the model required parameter fitting in order to match results on live fish in a Norwegian fjord [34].

To understand the effect of the swimbladder wall on sound production, we measured its thickness in different regions, the orientation of collagen and elastin fibres, material properties in wet and dried tissue (stress, strain and Young’s modulus) and its per cent water. We find that the swimbladder is an anisotropic structure with high water content causing viscous damping, which prevents the bubble inside the bladder from expressing its natural resonance.

### 2. Material and methods

Oyster toadfish *O. tau* L. were obtained with crab pots in the York River, Virginia, USA. Fish were returned to the laboratory and maintained in 18‰ artificial seawater and fed shrimp and squid two to three times a week until sacrifice with an overdose of MS222. Swimbladders were removed to measure thickness, fibre direction, material properties and %water. Work was done under IACUC Protocol 20216.

#### (a) Regional differences in bladder thickness

In five fish the sonic muscle was carefully removed from one side of the bladder with a scalpel, and the bladder was then cut in half in the median sagittal plane. The outside of the bladder was marked with dots from a permanent marker in successive rostro-caudal transects on the dorsal, side and ventral surfaces. Thickness at each dot was measured with a Mitoyo digital micrometer with a pressure-sensitive clutch to stop measurement at a constant pressure. Measurements were repeated three times and averaged. Circular (dorsal, side and ventral) and longitudinal (four regions from rostral to caudal) directions were compared with a repeated-measures analysis of variance.

#### (b) Development of bladder thickness

Sections from a previous study [35,36] were used to measure developmental changes in bladder thickness. Sections came from the sides of the swimbladder, near the middle of the sonic muscle that were embedded in glycol methacrylate, sectioned at 2 μm and stained with haematoxylin and eosin. Bladder thickness varied within sections, and therefore, measurements from the two thickest areas were taken with the Bioquant System (R & M Biometrics, Nashville, TN, USA) and averaged. Bladder thickness was regressed against fish weight for three juveniles (6.4–19.1 g), 18 males (143–801 g) and 22 females (94 to 528 g). Data for adult males and females (juveniles deleted) were fit with linear regressions and compared with analysis of covariance.

#### (c) Fibre direction

The outer wall (tunica externa) of the swimbladder, moistened with saline, was separated into layers with a scalpel tip. Cuts perpendicular to the fibre direction allow separation of sheets of parallel fibres from those below, whereas parallel cuts sliced through but
failed to separate layers. In some cases, the scalpel tip was pressed against the wall perpendicularly until resistance was met, and ink from a felt tip pen was allowed to run between the fibres, which were observed with a dissecting microscope.

Sections from several swimbladders were cut at 11 µm with a cryostat. Collagen and elastin fibres were observed with the Verhoeff and picric–ponceau stains [37], which stains collagen red and elastin purple.

(d) Material properties

Pieces of swimbladder from various regions were cut using a dog bone stamp (18 mm long, 5.8 mm wide at the expanded tips, and a taper to 2.5 mm in the middle). The swimbladder tissue was spread out on a cutting board, and pressure was applied to the back of the stamp. Swimbladder walls were difficult to cut all the way through, and we cut around the stamp with a scalpel, which sometimes caused nicks in the tissue. Sections were cut to a taper to 2.5 mm in the middle). The swimbladder tissue was difficult to cut all the way through, and we cut around the stamp with a scalpel, which sometimes caused nicks in the tissue. Sections were cut to a taper to 2.5 mm in the middle). The swimbladder tissue was difficult to cut all the way through, and we cut around the stamp with a scalpel, which sometimes caused nicks in the tissue. Sections were cut to a taper to 2.5 mm in the middle)

Figure 2. Thickness of the dorsal, side and ventral swimbladder of the oyster toadfish (a) and of transects from the same bladders from rostral to caudal (b). Different letters indicate means that are different based on repeated-measures analysis of variance. Sec refers to §§1–4 from anterior to posterior.

Figure 3. Relationship of bladder wall thickness to fish weight for juvenile, male and female oyster toadfish.

Figure 4. Drawing showing the direction of collagen fibres on the ventral surface of the swimbladder (a) and under the sonic muscles (b). The sonic muscle has been removed from the left side of the bladder. Anterior is to the top. Arrowheads indicate crosshatching showing the ventral edge of the collagen pattern beneath the sonic muscle on the side of the bladder. In (b), crosshatching indicates the bottom third of the bladder, and parallel fibres on the dorsal two-thirds of the side show fibres heading obliquely backward. A, anterior; D, dorsal; SM, sonic muscle; C, circular fibres.

Material properties for the seven regions (anterior: dorsal, side and ventral; mid-ventral; and posterior: dorsal, side and ventral) were compared separately with analysis of variance at 0° and at 90° followed by Tukey’s test. Because of extensive interactions between orthogonal directions, we did not run the data in a single analysis. Rather, we made paired comparisons of individual regions between 0° and 90° (e.g. anterior-side pulls at 0° versus anterior side at 90°) using t-tests.

3. Results

(a) Bladder shape and thickness

The oyster toadfish swimbladder has a complex heart shape (figure 1) with an indentation on the rostral midline that forms a thick pillar at the central confluence of the right and left halves. Because the sonic muscles on the bladder sides are displaced upward, the ventral surface appears broader (more bladder is exposed) than the dorsal one. The bladder is covered by a heavy white tunica externa, which surrounds an inner bladder that is relatively clear.

Bladder thickness (figure 2) did not vary between the dorsal, ventral and side aspects (repeated-measures ANOVA: $F_{2,8} = 0.060$, $p = 0.942$). However, it decreased continuously from anterior to posterior, respectively, 495, 459, 351 and 302 µm (repeated-measures ANOVA: $F_{3,12} = 89.4$, $p < 0.0001$).
Bladders from the smallest juveniles were thin ranging from 363 to 561 μm (figure 3). Thickness in mature males and females was variable and ranged from 390 to 1259 μm. Linear regressions from males and females (juveniles deleted) were not significantly different (slope: $F_{1,36} = 2.198, p = 0.147$, intercept: $F_{1,37} = 0.417, p = 0.523$), and data were combined into one nonlinear equation $\[ Y = 117.5 + 98.4(1 - e^{-0.003298}) \]$, $r^2 = 0.0267$, indicating that bladder thickness increased in fish to about 200 g before levelling off (hence the low $r^2$) although with considerable variability.

(b) Fibre direction

The tunica externa consists of dense connective tissue layers of collagen and elastin fibres that run in different directions (figure 4). The dorsal and ventral surfaces have similar three-layer patterns (figure 5a). From the midline, fibres radiate laterally in the inner and outer layers. About two-thirds of the way between the anterior–posterior borders, there is a central focus (near the entrance of the coeliac artery on the ventral surface) from which fibres radiate caudally towards the posterior margin of the bladder (figure 4b). Fibres in the middle layer run obliquely forward in the anterior lateral direction and interdigitate for short distances on the midline (figure 5a).

On the sides of the bladder under the sonic muscles (figures 4b and 5b), fibres can be divided into two regions: a dorsal one that covers approximately two-thirds of the vertical extent and a lower one with a different orientation. In the dorsal, two-thirds fibres in the outer layer travel obliquely, approximately 50° from the vertical in the caudal direction. In the ventral part of the caudal region, fibres are canted obliquely forward also at about 50° forming a pennate pattern with the dorsal region (figure 4b). Further anteriorly, these lower fibres tend to travel horizontally forward toward the front of the bladder. There is a second deeper layer of inner fibres that course obliquely rostrocaudally (figure 5b).

The bladder wall is composed primarily of circular and longitudinal red-stained collagen fibres (figure 5a–c). The OCT embedding for cryostat sections was not rigid enough to maintain layers in their natural positions, and sometimes layers pulled apart. This is evident in figure 5a, c in which the separation between layers is exaggerated. The staining of collagen fibres tended to mask the thinner elastin fibres, which were exposed by leaving out the collagen stain. The thinner fibres travel parallel to the collagen fibres and additionally form a reticular network that connects these fibres (figure 5d).

(c) Material properties

Material properties exhibited wide variation, which potentially masked differences between regions and orthogonal
directions within the swimbladder. RS, RE and Young’s modulus (figures 6 and 7) were similar between regions in circular pulls at 0° (RS: F_{6,38} = 0.9446, p = 0.4751, RE: F_{6,38} = 2.016, p = 0.0874, modulus: F_{6,38} = 0.8577, p = 0.5345). There were significant differences in all variables from longitudinal pulls at 90° (RS: F_{6,39} = 5.131, p = 0.0006, RE: F_{6,38} = 5.377, p = 0.0004, Young’s modulus: F_{6,37} = 7.137, p < 0.0001). RS was greater for pulls of the anterior side (0.66 ± 0.19 MPa) than for other regions, and RE was greatest for mid-ventral and posterior ventral regions (figure 7). The modulus was similar for all regions except for the anterior side (3.49 ± 1.15 MPa), which was considerably greater reflecting higher RS at break (figure 7).

Comparisons between individual positions at 0° and 90° (figure 7) indicated that for RS, six of seven means were greater at 0°, although the anterior side at 90° (0.66 ± 0.19 MPa) was greater than at 0° (0.16 ± 0.19 MPa). RE was greater at six of seven sites at 90°, and the exceptional site (anterior side) was relatively similar (0.45 ± 0.06 at 0° and 0.41 ± 0.07 at 90°). Excluding one value of 3.65, RE at 0° ranged from 0.16 to 0.73. With the exception of the anterior side at 90° (3.49 ± 1.15 MPa at 90° and 0.62 ± 0.14 MPa at 0°), the other six sites at 0° had a greater modulus.

Values were combined to allow an overall comparison between 0° and 90° (figure 8). Excluding the significantly higher anterior side readings at 90°, RS was greater at 0° than for the other six pulls at 90° (RS: 0° = 0.28 ± 0.03 MPa, RS: 90° = 0.17 ± 0.02, T_{82} = 3.475, p = 0.008). RE was four-times higher at 90° than at 0° (0° = 0.386 ± 0.023, 90° = 1.632 ± 0.194, T_{88} = 6.366, p < 0.0001). Again excluding the significantly larger modulus at anterior side 90° due to higher RS, modulus was higher at 0° than at 90° because of higher RS and lower RE (modulus 0° = 1.11 ± 0.14 MPa, modulus 90° = 0.32 ± 0.06, T_{81} = 4.772, p < 0.0001).

In dog bone samples from 0° in five fish that were allowed to dry before pulling (figure 6b), RS increased to 36.9 ± 12.8 MPa, RE decreased to 0.104 ± 0.023 and the modulus increased 300-fold to 311.5 ± 7.5 MPa. Five samples that were allowed to dry indicated they originally contained 79.9 ± 1.6% water.

4. Discussion

Physiology and sound properties [16,17,28] fail to support classic notions that the oyster toadfish swimbladder behaves as either an underwater resonant bubble [14,15] or as a bubble contained by an isotropic, homogeneous, elastic rubber membrane [33], both of which would produce an

![Figure 6. Representative stress–strain curves from a fresh (a) and a dry (b) dog bone strip from an oyster toadfish bladder. The slope of the orange line on the curve represents Young’s modulus.](http://rspb.royalsocietypublishing.org/)
omnidirectional sound field. This finding has been supported in the red piranha [18], weakfish [21] and black drum [38,39]. The question of why the swimbladder might not behave as a resonant bubble has not been formally considered except to posit that the swimbladder is damped by the surrounding fish tissue [31,32]. In fact, apart from gas secretion and buoyancy, many aspects of swimbladder structure have not been addressed in terms of functional morphology. Swimbladders are typically long cylindrical structures composed of collagen I [40,41] although they have been modified into a heart shape in oyster toadfish [42–44], and Morris & Albright [44] also note the presence of elastin in the oyster toadfish swimbladder.

Although the oyster toadfish is negatively buoyant, an adaptation to living on the bottom, its swimbladder still provides lift: buoyancy is higher in intact fish than in ones with the bladder removed [4]. The function in buoyancy is an exaptation [19] that has been converted to a sound-producing organ by adding muscles that migrate from the neck and secondarily attach to the bladder [45]. Recordings from different positions around calling fish at a distance of 1 m in the field indicate a minor decrease (approx. 1 dB) in amplitude at 30° to 45° and then a consistent increase to a maximum behind the fish [28]. Bladder shape has, therefore, been interpreted as an adaptation to shield the ears from the intense sound generated at a few centimeters distance. The rostro-medial surface of the bladder forms a stiff column, and there are no muscles in this area, both of which would decrease the amplitude of swimbladder vibrations in the anterior direction. In support, Yan et al. found that removing the gas from the bladder lumen with a hypodermic needle had no effect on hearing thresholds at various frequencies or on the waveforms of auditory evoked potentials [46]. Heart- or unusually shaped swimbladders or even separation of the two halves of the swimbladder (partially or completely) that depart from the typical cylindrical form occur in several groups including catfishes, gurnards, sea robins and other toadfishes [6,47–50], and it is likely that these unusual shapes are acoustic adaptations for directing the direction of vibration for hearing and sound production.

The heart shape and the presence of muscles that line the lateral and caudal surfaces of the bladder in the oyster

---

**Figure 7.** Rupture stress, rupture strain and Young’s modulus from pulls at 0° (circular, normal to the long axis of the fish) and 90° (longitudinal pulls parallel to the long axis of the fish). AD, AS, AV refer to the anterior swimbladder (dorsal, side and ventral), MV ventral part of mid-swimbladder, and PD, PS and PV posterior swimbladder (dorsal, side and ventral). Different letters above bars indicate means that are significantly different.
toadfish are associated with modifications of the directions of collagen and elastin fibres. The muscles are circular and push the sides and back of the bladder inward [16,23], increasing internal pressure that forces the bottom of the bladder outward (the quadrupole motion). Thus, the motion of the bladder is primarily in the lateral or circular plane (our 0° measurements, and less in the longitudinal or 90° axis parallel to the long axis of the fish). Bladder movement will be increased caudally by the thinner bladder wall and the material properties. RS and modulus are higher in the circular direction of greatest movement. Fibres in the longitudinal direction have higher RE and are able to stretch further before breaking, likely because of elastin fibres that link collagen bundles. Data suggest that the directional pattern of the fibres determines the material properties rather than them being related to those of pure collagen and elastin.

Sounds of the oyster toadfish sounds and of many fishes damp rapidly [8]. Typically following cessation of muscle contraction, oyster toadfish sounds rapidly lose amplitude and waveform period increases with each half cycle [16,17]. The frequency spectrum of sounds evoked by exciting an oyster toadfish bladder with a miniature modal analysis hammer is not sharply tuned (low Q), and peak frequency does not decrease with bladder size [17]. The damping coefficient in air is equivalent to that of an automobile shock absorber [16]. Decreased loading in air does affect these findings, but it is not dramatic as demonstrated in individual Atlantic croaker recorded in both media [51]. Findings from the current study indicate that the bladder wall is responsible for differences between findings and predictions of the underwater resonant bubble. Further, water content appears important for normal acoustic function [52]. The high water content, decreased RE and increased RS and modulus in dry bladder tissue strongly implicate viscous damping.

An important question is whether these results apply generally to swimbladders of various fishes. The most serious challenge to our thesis comes from a classic paper on the bicolour damselfish in which peak frequency declines markedly with fish size, potentially implicating swimbladder resonance [53]. Subsequent work indicated that damselfish sounds are initiated by a stretched ceratombindular ligament that causes a rapid mouth closure and collision of front teeth [54]. Colleye et al. then demonstrated that striking the ribs, which are intimately associated with the swimbladder, produces sounds with a similar frequency spectrum as sounds of the intact fish [55]. Thus, energy from teeth collisions is transferred to the ribs, and rib vibration drives the swimbladder. Furthermore, known swimbladder sounds indicative of resonance are associated with a bone or tendon attached to the swimbladder [19]. The peak frequency of such sounds decreases rapidly with fish size, whereas sounds evoked by just a sonic muscle and swimbladder change gradually or not at all with fish size [19,56]. Minor changes in frequency can be mediated by larger muscles that take longer to contract [21] or by larger swimbladders that will be more effective than smaller ones at coupling low frequency sounds into water [57,58]. Therefore, they are not explicit evidence of swimbladder resonance. Thus, we suggest the results of this paper will likely generalize to swimbladders in other fishes.

5. Conclusion
The pattern of excitation by sonic muscles and the arrangement of structural elements and high water content of the swimbladder wall are responsible for directionality and rapid damping of oyster toadfish sounds.

Acknowledgements.
We thank Gary Bowlin for use of his material tester, Jennifer Wayne for plans for the dog-bone stamp and Maynard and Ricky Bonneville for obtaining toadfish.


