Suggested running head

Sounds and sound producing system in the dory.

Two types of sounds and additional spinal nerve innervation to the sonic muscle in John dory, *Zeus faber* (Zeiformes, Teleostei).

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The John dory, *Zeus faber*, has a pair of intrinsic sonic muscles on the swimbladder wall and produces sounds by rapid contractions of the muscles. The physical properties of the sounds and the detailed innervation pattern to the sonic muscle were investigated. The dory emitted two types of the sounds: “bark” and “growl”. The bark consisted of continuous multiple pulses and lasted about 85ms on the average. The growl consisted of a group of intermittent single-pulses and lasted for 50 ms to 1.2 s. The main frequencies of both sounds were almost similar and ranged between 200 to 600 Hz. The sonic muscles were innervated by the sonic branches of the 1st–4th spinal nerves. Interestingly, the innervation from the first spinal nerve was newly revealed in the present study. Total of 1,700 myelinated axons innervated the sonic muscles on both sides. There was no sex difference in the sonic muscle size as judged by the sonic muscle-somatic index (SMSI, male: 0.675%, female: 0.670%). The sounds in the dory possibly function as a threatening means.

INTRODUCTION

The John dory (*Zeus faber* Linnaeus, Zeidae, Zeiformes) is a common solitary fish, which are usually found worldwide in the inshore waters of less than 200 m depth (Wheeler, 1985). Historically, Aristotle (B.C.384–B.C.322) noted that the “chalcis” (*Zeus faber*) made sounds in his classical “Historia Animalium” (cited in Fish & Mowbray, 1970). Dufossé (1874) described the intrinsic sonic (drum) muscles on the swimbladder wall in the dory, and reported that the sonic muscle was controlled by the 2nd–4th spinal nerves. Thereafter, Rauther (1945) histologically confirmed the spinal nerve innervation pattern for the sonic muscle.

Looking at the literatures (Tavolga, 1971; Myrberg, 1981; Ladich, 1991, 1997; Ladich & Bass, 1998; Zelick et al., 1999; Carlson & Bass, 2000; Bass & McKibben, 2003), no investigations have been performed on the physical nature of the sounds and detailed innervation pattern of the sonic muscle in the dory. The purposes of this study are (1) to analyze the characteristics of the sounds, (2) to investigate the detailed innervation pattern to the sonic muscle, (3) to perform the fibre counts in the innervating spinal nerves, and (4) to compare the size of sonic muscles between the sexes in the dory.

MATERIALS AND METHODS

Seven live specimens of *Zeus faber* with total length (TL) of 28.4–38.2 cm were collected with fisherman's set net in Katada (Shima, Mie prefecture, Japan) in November and December 1998 and January 1999. They were all females when dissected after the experiment, and no males were included. The sounds produced by the hand-held dory in air were obtained through a plaintalk microphone (unidirectional electret microphone, sensitivity: -9.5dBV ±5 dBV at 1.0kHz relative 1.0 V/Pa) and recorded with the hard disk of a personal computer (Macintosh, sampling rate=44.1 kHz). The microphone was placed within 5 cm from the fish. After sound recording, the fish were euthanized with an overdose of tricaine methanesulfonate and fixed in 10% formalin for anatomical and histological observations.

Ten live dory (TL is about 20 cm, the sex of the fish was not determined) were available for underwater recordings in June 2003 at an aquarium "Marine palace" (Takasaki, Oita prefecture, Japan). The fish were collected with fisherman's set net in Kamae (Oita prefecture, Japan) in March and April 2003. They were maintained in the aquaria filled with filtered seawater (about 3,000 litter) for display at 12 °C and fed with live Japanese anchovy, *Engraulis japonicus* Temminck & Schlegel. Sounds were recorded through a hydrophone (ST-1200, Oki Electric Industry Co., Ltd.) with a sensitivity of -180 dB re 1V/μPa and a flat frequency response up to 30 kHz. The hydrophone was placed about 10 cm away from the fish. The sounds were amplified and filtered with a amplifier (SW-1020, Oki Electric Industry Co., Ltd.) with high- and low- pass filters set at 10 Hz and 10 kHz respectively and recorded with a MD recorder (MZ-N910, Sony Co.). The sounds recorded in both ways were downsampled to 11 kHz and analyzed with SoundEdit Pro (Macromedia, Inc.). Sonograms and Frequency distributions were calculated using fast Fourier transform (FFT).

Other specimens of the dory (N=120, TL=11.2–38.2 cm) were collected through trawl fishing in the northern area of the East China Sea in August 2001. Most of them (N=105, male=66, female=39) were used to check sex difference in sonic muscle size, and their bilateral sonic muscles were removed and weighed. To correct the variation due to body size, the sonic muscle-somatic index (SMSI) was calculated as (total sonic muscle mass / total body mass) X 100 following the method of Connaughton et al. (1997). The rest of the specimens were fixed in 10% formalin for anatomical and histological observations.

The innervation pattern of the sonic muscle was examined under a dissection microscope. For tracing nerves, the weak solution of osmic acid was applied to stain nerves (Somiya, 1987). The nerve branches innervating the sonic muscle were post-fixed in 1% osmic acid and embedded in Epon. Two micron thick transverse sections were cut and stained with toluidin blue for light microscopy. Considering the deformation due to tissue shrinkage, fibre diameters were calculated by the formula: length of circumference / π. The circumferences of the nerve fibres were traced on tracing papers, digitized with image scanner and measured with NIHImage (Scion corp.). The computed values were sorted into frequency distribution.

Terminology of nerves by Parenti & Song (1996) was followed, although the term “occipital nerve” was used rather than “spino-occipital nerve” because these nerves emerged from a cranial cavity through a pair of foramens on the occipital cranium. The first spinal nerve was defined as that emerging through the foramen on the first free vertebra, and the second and following nerves were defined in the same way. To confirm the nerve passage, cranial and vertebral structures were carefully observed for a foramen in the cleared preparations stained with alizarin red S (Taylor, 1967).

RESULTS

Physical properties of Sounds

Only the sounds of female dory were recorded in air, because all seven specimens collected for this experiment were females as mentioned in MATERIALS AND METHODS. The dory emitted sounds both in air and underwater. The sounds recorded underwater contained a lot of noise. Therefore, the sounds recorded in air were mainly analyzed for the physical properties. The hand-held dory produced sounds raising their dorsal and anal fins. They emitted two types of the sounds (Figures 1and 2), which were named “bark” (Figure 1A) and “growl” (Figure 2A), respectively, because the sounds were similar to barking and growling of a dog.

A bark consisted of continuous multiple (4–14 pulses) pulses (mean ±SD: 5.8 ±0.7, N=50) as shown in Figure 1B. Duration of the bark was about 85 ms (mean ±SD: 87.0 ±10.0, N=50) and its example is shown by “bark” in Figure 1A. Individual pulse duration of the bark (IPD) was about 15 ms (mean ±SD: 15.2 ±1.1, N=288). The division into an individual pulse is indicated in Figure 1B. The main frequency component of the bark was low (less than 1 kHz), and typical dominant frequencies lay between 250 and 600 Hz (Figure 1C, D). The peak frequency of the bark was approximately 370 Hz.

On the other hand, a growl consisted of a group of intermittent single-pulses (Figure 2A). The growls were emitted by only three specimens, although the barks were recorded in all seven specimens examined. Compared with a bark, a single-pulse of the growl was faint as seen Figure 2A. The growls were observed between barks and lasted for 50 ms to 1.2 s. The single-pulse duration of the growl (SPD) was about 15–20 ms as indicated in Figure 2B. The interval duration between single-pulses of the growl (ID) was about 10–70 ms. Similar to the bark, the main frequency component of the growl was low (less than 1 kHz), and typical dominant frequencies lay in the range of 200–500 Hz (Figure 2C, D). The peak frequency of the growl was about 320 Hz.

The bark was also detected by the underwater recordings. As the hydrophone was brought close to the body, all ten specimens directed their black spots on the lateral surface of the body several times to the hydrophone raising their dorsal and anal fins and then swam away. However, only one specimen that had the clearest black spot and the surrounding bright light ring produced the sounds in this situation, and only three barks were recorded. Out of the three barks, only one bark was available for analysis. The bark consisted of 6 pulses with the duration of about 85 ms and IPD was about 14 ms. The typical dominant frequencies lay in the range 350–600 Hz, and the peak frequency was nearly 500 Hz. Growls were not recorded underwater. The above mentioned physical properties of the barks and growl are summarized in Table 1.

Sonic muscle and its associated structure

In both sexes, a pair of sonic muscles was attached anterolaterally on the anterior chamber of swimbladder (Figure 3A). The sonic muscles were regarded as intrinsic sonic muscles because their attachment sites are only on the swimbladder wall. The sonic muscle fibres were arranged dorsoventrally as indicated by the arrows in Figure 3A. The colour of the sonic muscle was pale pink in a raw specimen, but it changed into yellowish cream after fixation by 10% formalin.

Transverse section through the sonic muscles is shown in Figure 3B. The centro-lateral part of the sonic muscle faced directly the external environment through a skin of the window like structure. This window may be an apparatus enhancing transmission of the sounds by the sonic muscle to the external environment. The sonic muscle was easily observable only by removing the overlying skin (Figure 3C). Inner view of the window was shown in Figure 3D. The shape of the window was a triangle with one side facing cleithlum and other two sides facing lateral trunk muscles.

Comparison in sonic muscle size between the sexes

Sonic muscle size was examined for 66 males (TL=11.2–33.8cm) and 39 females (TL=12.4–36.2cm). The sonic muscle-somatic index (SMSI) was 0.675 ±0.100% (Mean ±SD) in the male and 0.670 ±0.097% in the female. The difference in SMSI was not significant ($t=0.24$, $df=103$), in other words, there was no sex difference in sonic muscle size. Additionally, there was no significant size difference between right and left sonic muscles, i.e., percentage for one of a pair to total sonic muscle mass was 50.53% for the right and 49.47% for the left.

Innervation pattern of the Sonic Muscle

The sonic muscle was innervated by the branches of ventral axon bundle of the 1st–4th spinal nerves (Figures 4 and 5). The each ventral bundle of the spinal nerves marked as S1–S4 in Figure 4 bifurcated into a superficial branch and a deep branch. The each superficial branch further bifurcated into the sonic branch innervating the sonic muscle and another branch which did not innervate the sonic muscle, and the bifurcation points are indicated by open arrowheads in Figures 5. No occipital nerves had innervated the sonic muscle, but they innervated mainly pectoral-fin muscles and the muscles of anteroventral portion of pectoral girdle (Figure 4). The deep branches of the 1st–4th spinal nerves, and the ventral axon bundles of the 5th–7th spinal nerves were found to run merely in the vicinity of the sonic muscle (Figure 5). In addition, it is a particularly interesting finding that the deep branch of the third spinal nerve simply passed through the sonic muscle (Figure 5, S3d, dashed line).

Fibre analysis of the Sonic Branches

Thick transverse sections of the sonic branch of the 1st–4th spinal nerve are shown in Figure 6 a–d. The myelinated axons were counted as the sonic motor neurons innervating the sonic muscles. The counts in the specimen examined (TL=31.2cm) are given in Table 2. Total number of fibres contained in right sonic branches was 909 and each branch (S1so, S2so, S3so and S4so) contained 165, 234, 252 and 256 fibres, respectively. In the left side, total number was 786, and each of the four branches contained 132, 210, 204 and 240 fibres, respectively. Therefore, it is roughly estimated that the paired sonic muscles of the dory are controlled by about 1,700 nerve axons and there is an increasing tendency in number of the nerve fibres from the anterior to posterior sonic branches in both sides.

Histograms of the fibre distributions by size are shown in Figure 7. From these figures, it was found that the fibre distribution in each sonic branch was bimodal, and the peak spectra existed at 5 μm and 12 μm when all data was combined (Figure 7F). Furthermore, it was clarified that there was also an increasing tendency in number of the thick fibres (≥10 μm) from the anterior to posterior sonic branches in both sides. The number in each sonic branch, S1so, S2so, S3so and S4so was as follows; 15, 122, 149, and 174 for the right side, and 49, 120, 145, 194 for the left side.

DISCUSSION

This is the first report on the physical properties of the sounds in the dory. All seven females emitted bark and three of them produced growl in air. Only one out of ten specimens emitted bark in underwater recordings. Comparing the characteristics of the sounds listed in Table 1, the physical characteristics of the bark recorded underwater were very similar to those of the bark recorded in air except the peak frequency. In the weakfish, the dominant frequency decreases with increasing body size (Connaughton et al., 2000). The above difference in peak frequency may possibly be due to the differences in body size, since the specimens used for aerial recordings were mostly large (TL≈30cm) and showed lower peak frequency of 370 Hz and the specimen which was successful for underwater recordings was small (TL≈20cm) and showed higher peak frequency of 500 Hz.

The ecological significance of dory's sounds is still obscure. In this study, there was no sex difference in sonic muscle size. It is well known that sexual dimorphism of sonic muscle size is concerned with their reproduction in some fish species (Fine et al., 1977). The midshipman (*Porichthys notatus* Girard) exhibits the sexual dimorphism of sonic muscle size, and only large type I male generates a mate song (hum) using the bigger sonic muscles during mating season (Bass & Marchaterre, 1989; Bass & Baker, 1990). In the present study, the dory was found to produce the sounds raising their dorsal and anal fins in air and underwater. From this observation, we assume that the sounds of the dory might have a function as a threat or warning means rather than the courting.

The sonic muscles of the dory were innervated by the 1st–4th spinal nerves but by no occipital nerves. In most of the swimbladder-associated sonic species, for example, sea catfish (Tavolga, 1962), toadfish and midshipman (Bass & Baker, 1991), squirrelfish (Carlson & Bass, 2000), rockfish (Yoshimoto et al., 1999), sea robin (Evans, 1973) and sweeper

(Takayama et al., in press), the sonic muscles are innervated by the occipital nerve, which is considered to be a homologue of the hypoglossal nerve of tetorapods (Tavolga, 1962). This difference may suggest that the sonic muscle of the dory has a different origin (possibly originated in the lateral trunk muscle) from that of most other sound producing fishes (possibly originated in the muscle concerned with cranium or pectoral fin), and had followed an independent pathway in the course of evolution.

Dufossé (1874) described that the intrinsic sonic muscle of the dory was controlled by the 2nd–4th spinal nerves, which was, thereafter, confirmed by Rauther (1945). In the present study, we have found that the first spinal nerve also innervated the sonic muscle and is concerned with the sound production. The sonic nerve fascicle of the first spinal nerve was finer than others (Figures 5 and 6a). Therefore Dufossé (1874) and Rauther (1945) might have missed the innervation of the first spinal nerve.

In a specimen of the dory (TL=31.2cm), the sonic muscles were innervated by about 1,700 myelinated fibres as a whole, and the posterior sonic branches had a large number of fibres and included a great number of large diameter fibres as compared with the anterior ones (Table 2). These large diameter fibres will bifurcate more frequently and innervate more muscle fibres than the small ones. Therefore, it is inferred that the sonic muscle may depend on the posterior sonic branches more strongly than the anterior ones. Furthermore these large diameter fibres will have higher conduction velocity than the small ones. Considering these points, the increasing innervation intensity toward the posterior part may possibly be concerned with synchronous contraction of anterior and posterior part of the sonic muscle, which is further based on the assumption that the excitation of sonic motor neurons occurs from the anterior neurons to the posterior ones, though there is, at present, no document on the contraction mechanism of the sonic muscle in the dory.

The transparent triangular window lies over the sonic muscle. The cobitid fishes have lateral trunk channel stretching laterally from the anterior swimbladder to outer body wall, and the channel forms a muscle-free acoustic window enhancing hearing sensitivity (Kratochvil & Ladich, 2000). Likewise, the membranous window of the dory possibly facilitates the transmission of the vibration by the sonic muscles to surrounding water. If the sonic muscle had been innervated by the occipital nerve in the dory, it would have been necessary to place the window more anteriorly. This is thought to be obstructed by the bones of pectoral girdle and the lateral trunk muscle. Although it is difficult to explain the reason why the sonic muscle is innervated only by the spinal nerves in the minor sonic fish group including the dory, the following idea may be presented. The membranous window facilitating transmission of sounds may be one of the key factors that necessitated the innervation of the spinal nerves to the sonic muscle.

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Figure Legends

Figure 1. The barks emitted in air by the dory. (A) Representative oscillogram of three barks. Duration of a bark is indicated by “bark”. (B) Expanded oscillogram of a bark. IPD: individual pulse duration of the bark. (C) Sonogram of the bark shown in B. (D) Frequency distribution analyzed with the bark shown in B.

Figure 2. The growl emitted in air by the dory. (A) Representative oscillogram of a growl. Asterisks indicate barks. (B) Expanded oscillogram of three single-pulses of the growl. SPD: single-pulse duration of the growl, ID: interval duration between single-pulses. (C) Sonogram of three single-pulses of the growl. (D) Frequency distribution analyzed with the middle pulse in B.

Figure 3. Sound producing system (sonic muscle and swimbladder) of the dory. (A) Lateral cutaway view. Arrows indicate running directions of sonic muscle fibres. (B) Transverse section view on the plane B indicated in A. Arrowhead indicates the position of the window. (C) Lateral view after removal of the skin in the rectangular area indicated in A. Stippled area shows the region of the window and the circle of broken line indicates

the position of the sonic muscle. (D) Inner view of the window area indicated by the rectangle in A. Arrows indicate running directions of lateral trunk muscle fibres. Scale bar: 5 mm. SM: sonic muscle, AS: anterior chamber of swimbladder, PS: posterior chamber of swimbladder, Win: window, Cle: cleithlum, Pec: pectoral fin, R: rostral side.

Figure 4. Lateral cutaway view showing innervation pattern to the sonic muscle. Dark black thick lines indicate the superficial branches that further bifurcate into a sonic branch and another branch. Only the sonic branches are shown, and another ones are omitted. The terminal closed circles show the entrance into the muscle. OC: occipital nerve. S1-S4: ventral axon bundle of 1st–4th spinal nerve. OC+S1: mixed nerve of OC and S1. SM: sonic muscle. Pec: pectoral fin. Pel: pelvic fin.

Figure 5. Lateral view of the sonic muscle and its associated nerves (Specimen: male, TL: 24.5 cm). Photograph (upper) and diagrammatic drawing (lower) show the innervation pattern of the spinal nerves to the sonic muscle. The nerves were stained black with the weak solution of osmic acid. In the drawing, the nerves indicated by dark black colour are the superficial branches of ventral axon bundle. Open arrowheads indicate

the bifurcating points of the superficial branches into a sonic branch and another branch. Dashed line indicates the nerve passage (S3d) through sonic muscle. Scale bar: 1mm. SM: sonic muscle. OC: occipital nerve. S1so–S4so: sonic branch of superficial branch of 1st–4th spinal nerve. S1d–S4d: deep branch of 1st–4th spinal nerve. S5–S7: 5th–7th spinal nerve, R: rostral, C: caudal.

Figure 6. Transverse sections (2 µm) of the sonic branches (Specimen: male, TL: 31.2cm). The transverse sections of the sonic branches of the 1st–4th spinal nerve on the right side (a–d). Scale bar: 50 µm. Schematic drawing at the upper left is a lateral view of the right sonic muscle. Arrowheads indicate rough positions of the transverse sections. SM: sonic muscle. S1so–S4so: sonic branches of 1st–4th spinal nerve. R: rostral side.

Figure 7. Frequency distributions of fibre diameter in the sonic branches. (A–D) Sonic branches in the 1st–4th spinal nerve (S1so–S4so). (E) Sum of fibre counts for the sonic branches in each side. (F) Total fibre counts for all sonic branches in both sides.

Table 1. *Sound properties of dory (bark and growl).*

	Bark (in air)	Growl (in air)	Bark (underwater)
Structure of sound	continuous multiple pulses	group of intermittent single-pulses	continuous 6 pulses
Duration of a sound	85 ms (mean)	50 ms – 1.2 s	85 ms
Duration of a pulse	15 ms (mean)	15–20 ms	14 ms
Main frequencies	250–600 Hz	200–500 Hz	350–600 Hz
(Peak frequency)	370 Hz	320 Hz	500 Hz

Table 2. *Size distribution of sonic fibres (Specimen: male, TL: 31.2 cm).*

Diameter	Right Side					Left Side				
(μm)	S1so	S2so	S3so	S4so	Total	S1so	S2so	S3so	S4so	Total
0–1	0	0	0	0	0	0	0	0	0	0
1–2	5	15	1	2	23	0	5	0	0	5
2–3	9	**19**	5	**17**	50	9	6	3	0	18
3–4	**19**	16	15	5	**55**	**13**	10	4	0	27
4–5	14	13	**16**	8	51	7	**15**	7	4	33
5–6	14	10	11	**17**	52	10	13	9	11	**43**
6–7	14	9	15	8	46	11	11	**16**	5	**43**
7–8	14	8	11	8	41	9	9	9	5	32
8–9	**38**	13	12	9	72	7	7	8	**12**	34
9–10	23	9	17	8	57	**17**	14	3	9	43
10–11	12	11	39	11	73	16	11	8	5	40
11–12	2	37	**55**	14	**108**	16	26	11	26	79
12–13	1	**44**	36	23	104	10	**30**	21	44	105
13–14	0	16	15	**31**	62	5	24	**34**	**51**	**114**
14–15	0	11	4	**31**	46	1	18	24	33	76
15–16	0	1	0	29	30	1	8	23	18	50
16–17	0	2	0	21	23	0	3	12	10	25
17–18	0	0	0	10	10	0	0	8	6	14
18–19	0	0	0	3	3	0	0	4	0	4
19–20	0	0	0	1	1	0	0	0	1	1
20–	0	0	0	0	0	0	0	0	0	0
Total	165	234	252	256	907	132	210	204	240	786
≥10μm	15	122	149	174	460	49	120	145	194	508

Bold types indicate peaks.

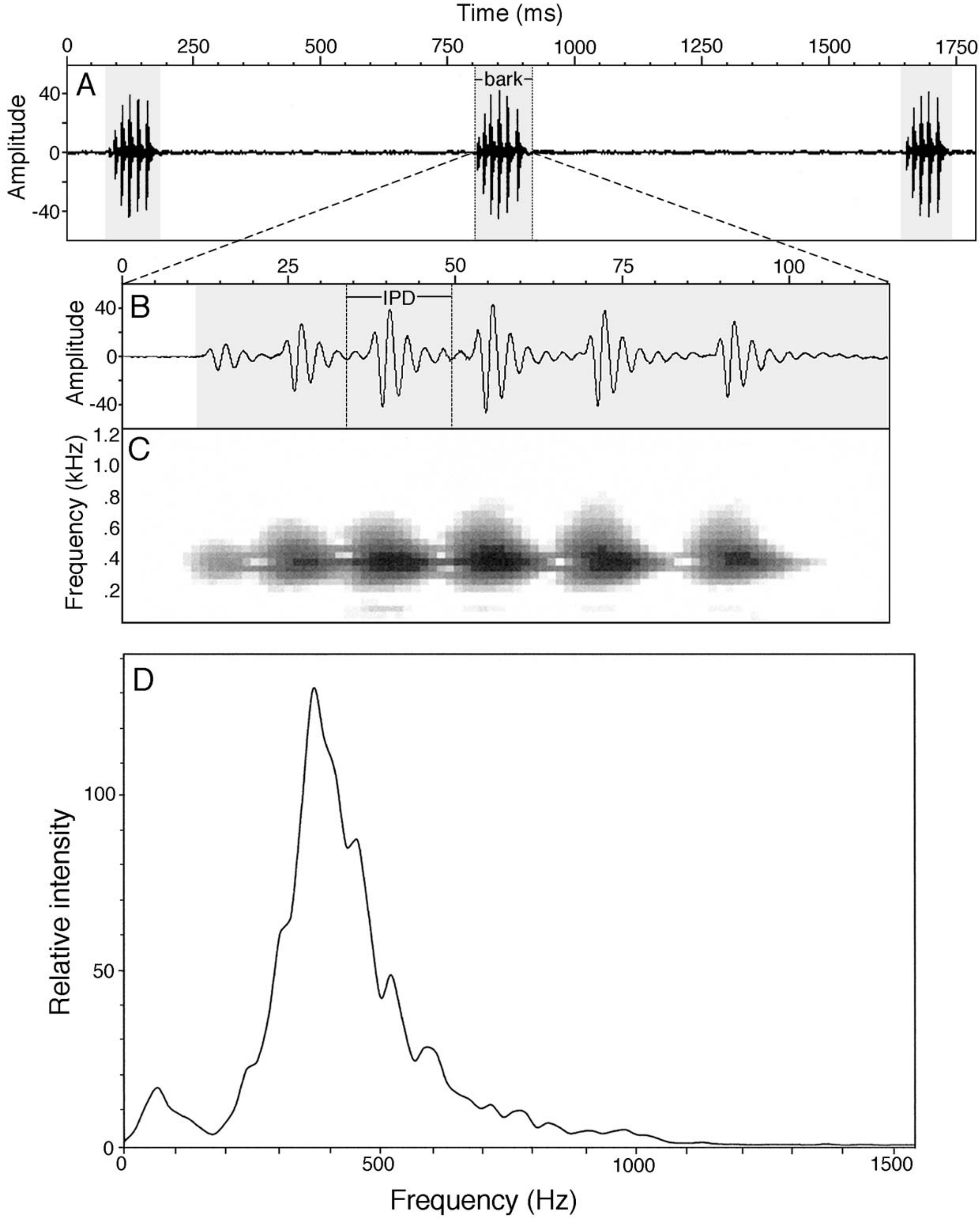
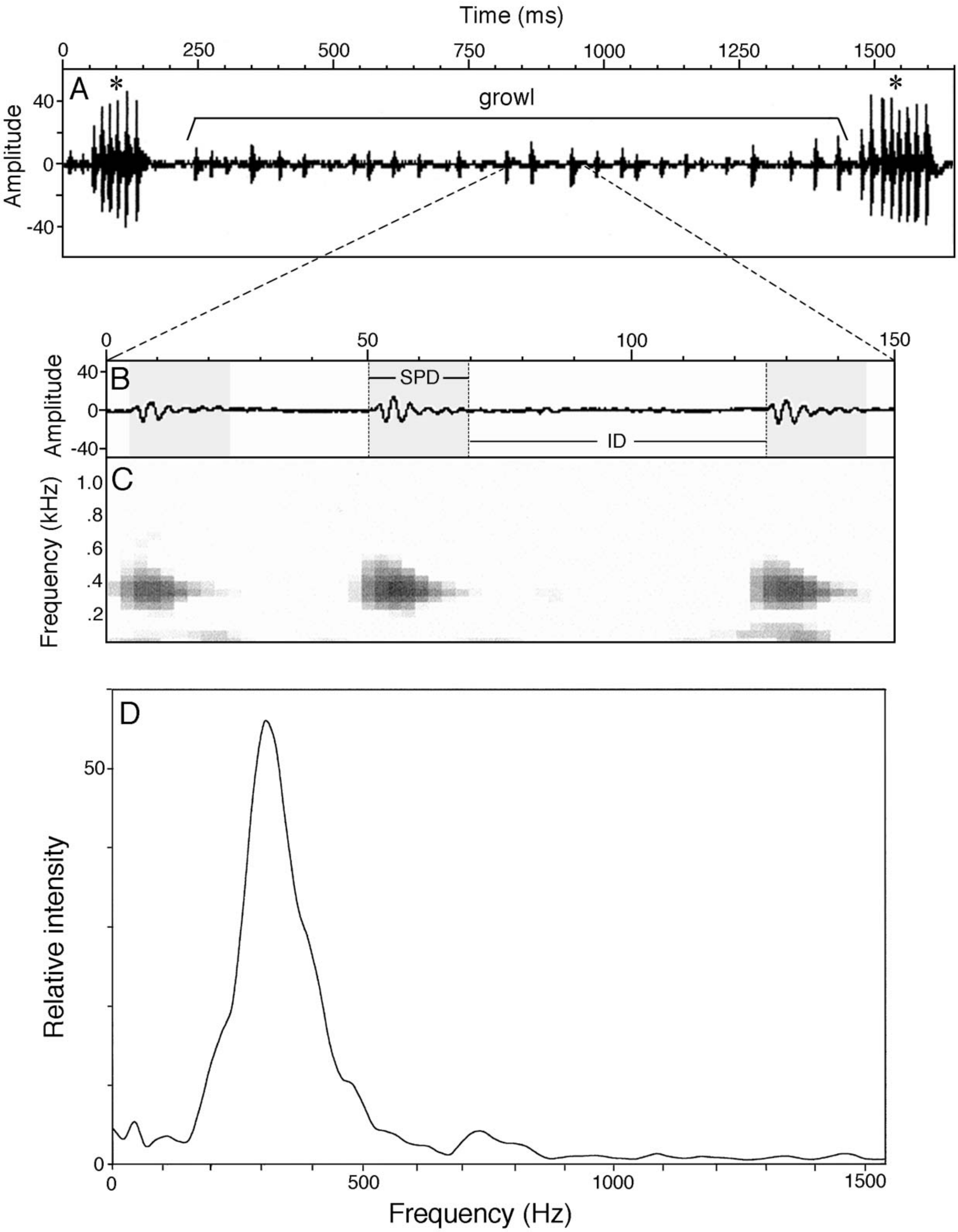

Time (ms)
0
250
500
750
1000
1250
1500
1750
A
bark
Amplitude
40
0
-40
0
25
50
75
100
B
IPD
Amplitude
40
0
-40
C
Frequency (kHz)
1.2
1.0
.8
.6
.4
.2
D
Relative intensity
100
50
0
500
1000
1500
Frequency (Hz)

Figure 1.



Time (ms)
0
250
500
750
1000
1250
1500
A
*
growl
*
Amplitude
40
0
-40
0
50
100
150
B
SPD
ID
Amplitude
40
0
-40
C
Frequency (kHz)
1.0
.8
.6
.4
.2
D
Relative intensity
50
0
0
500
1000
1500
Frequency (Hz)

Figure 2.

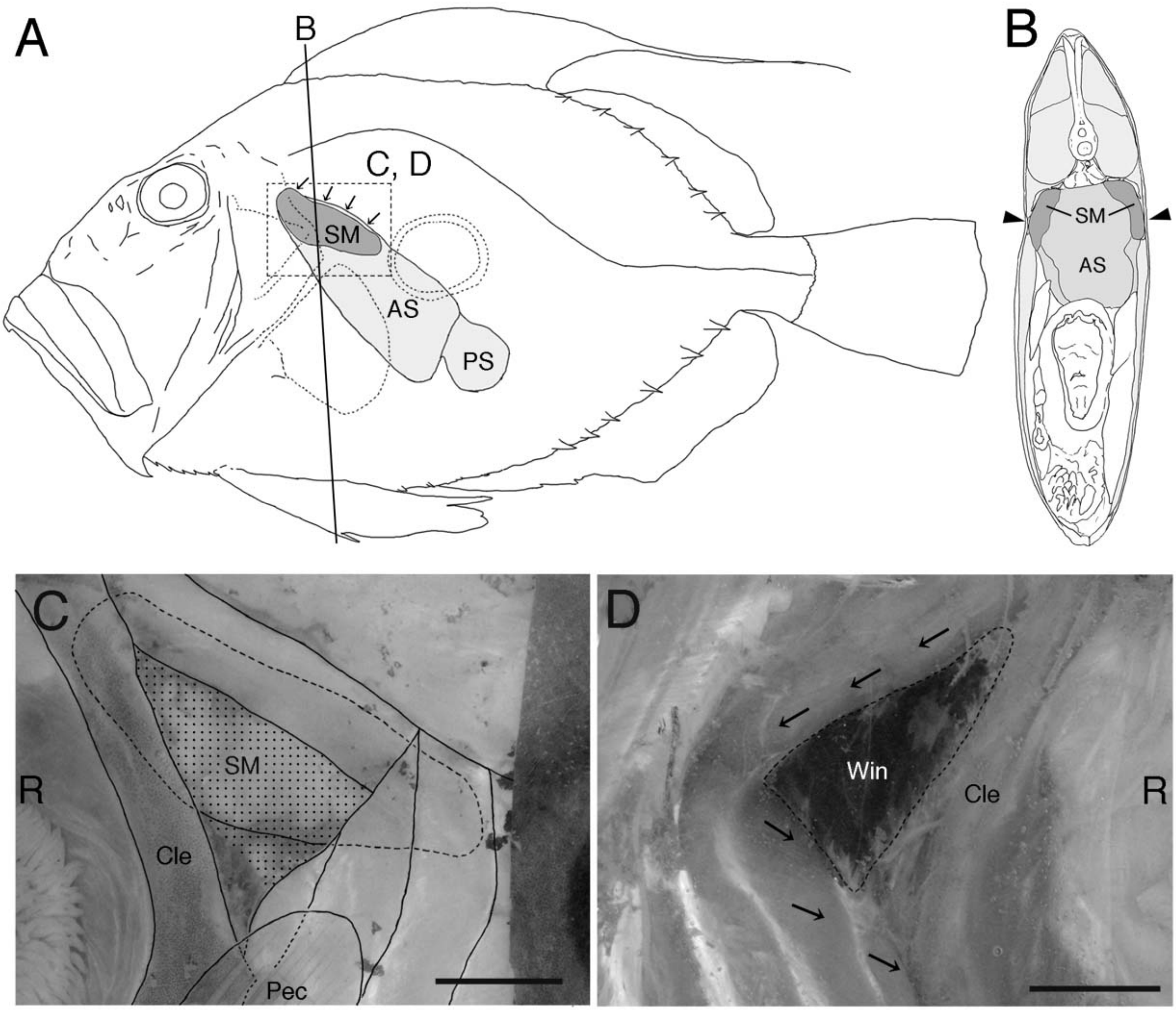


Figure 3.

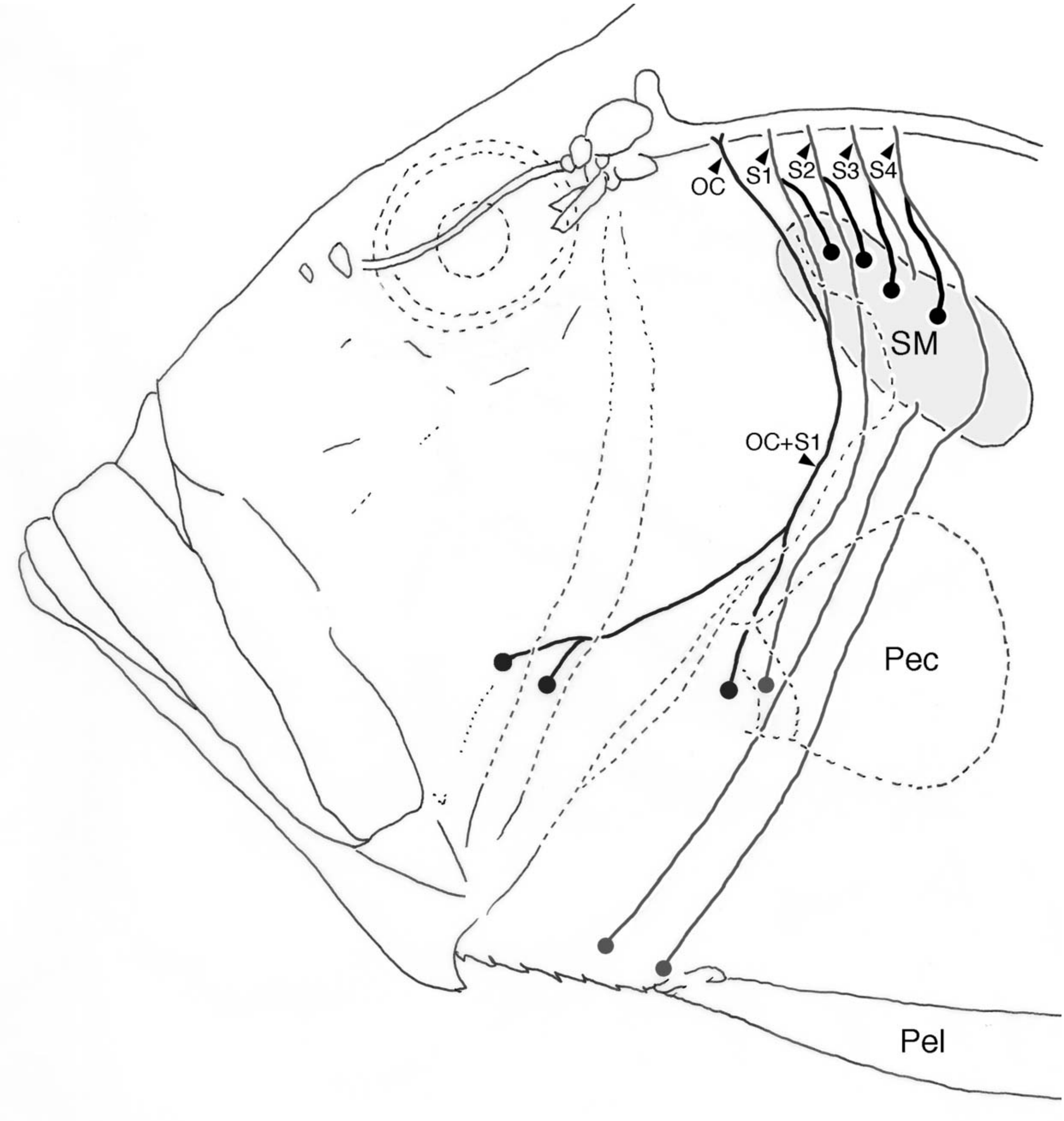


Figure 4.

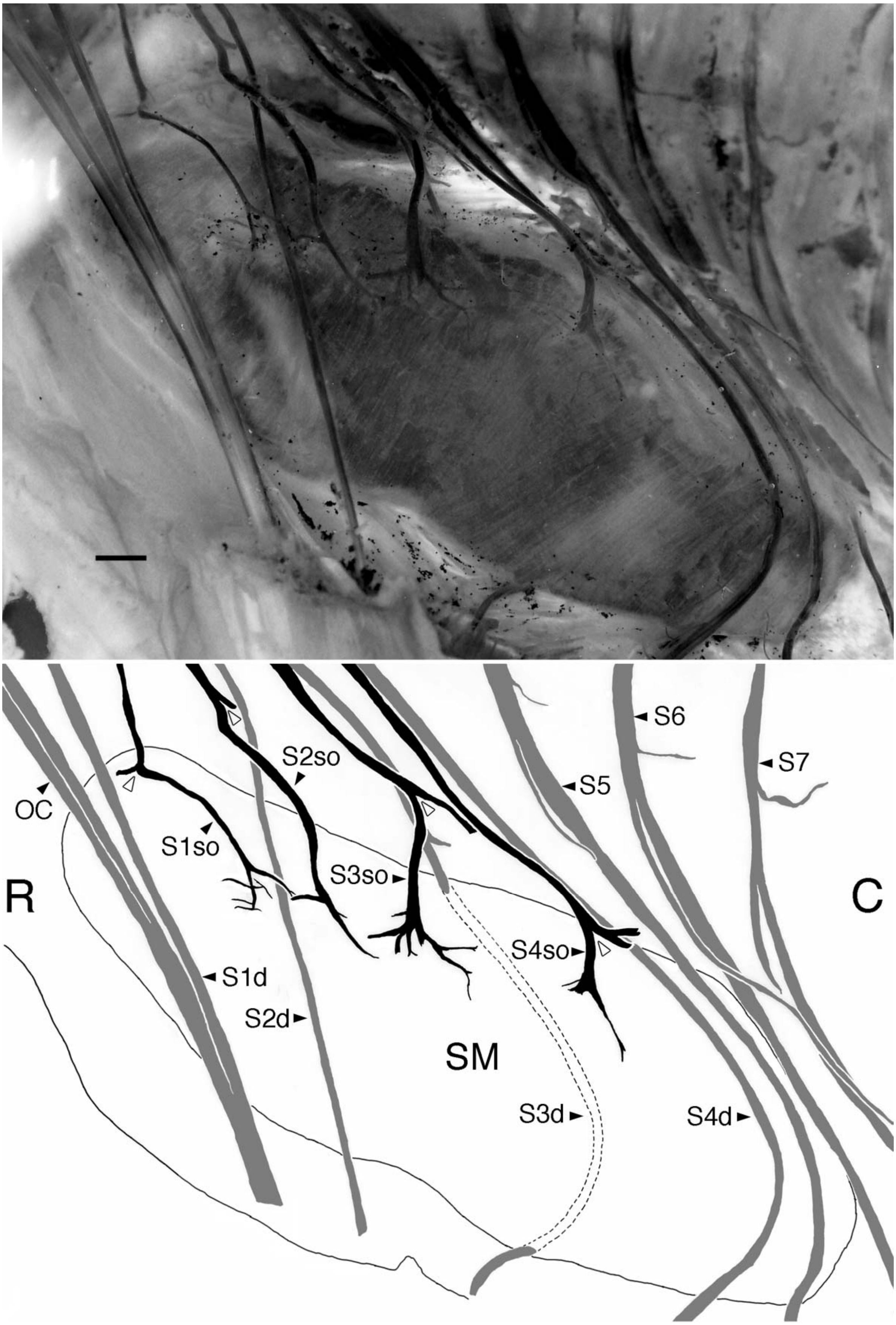


Figure 5.

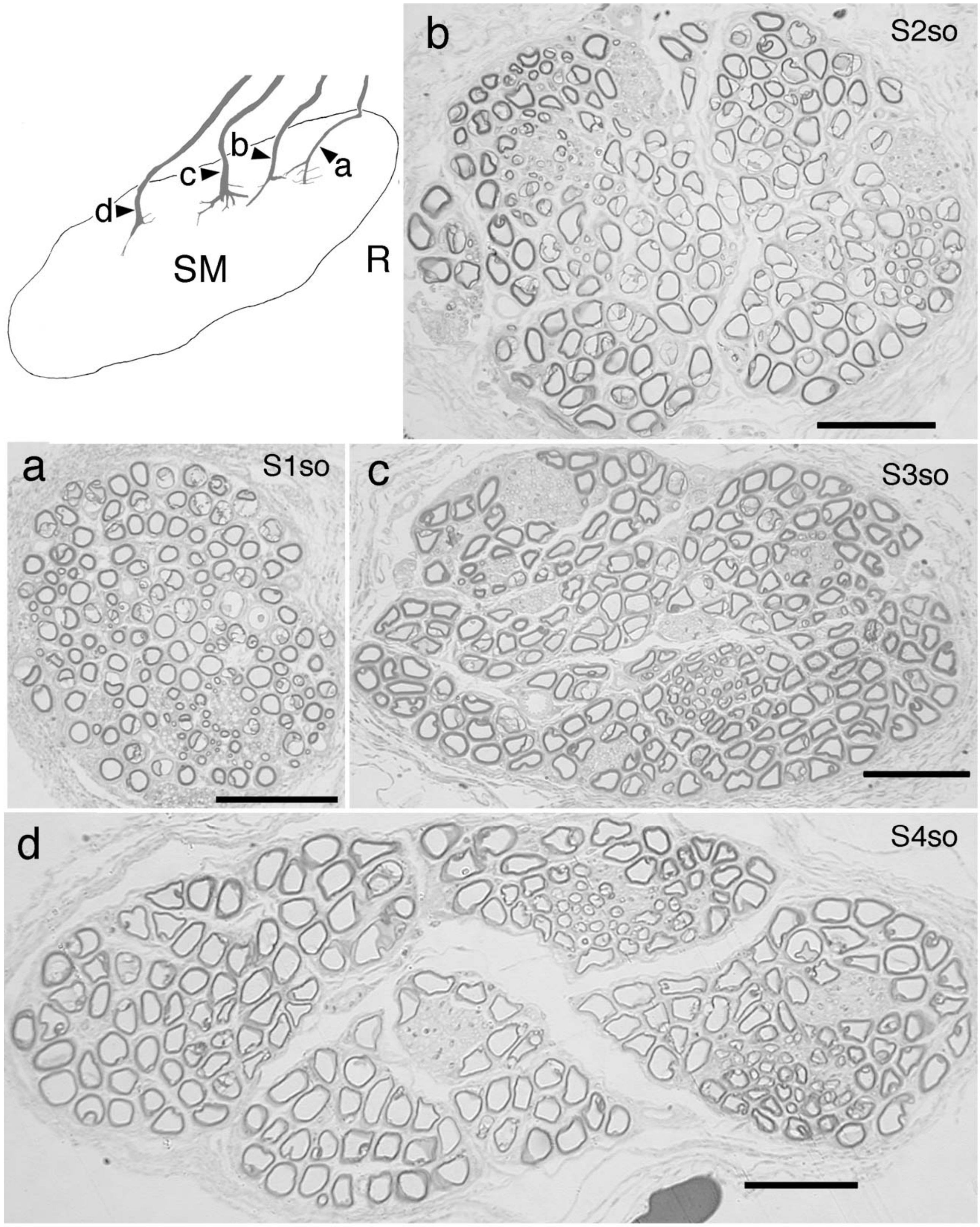


Figure 6.

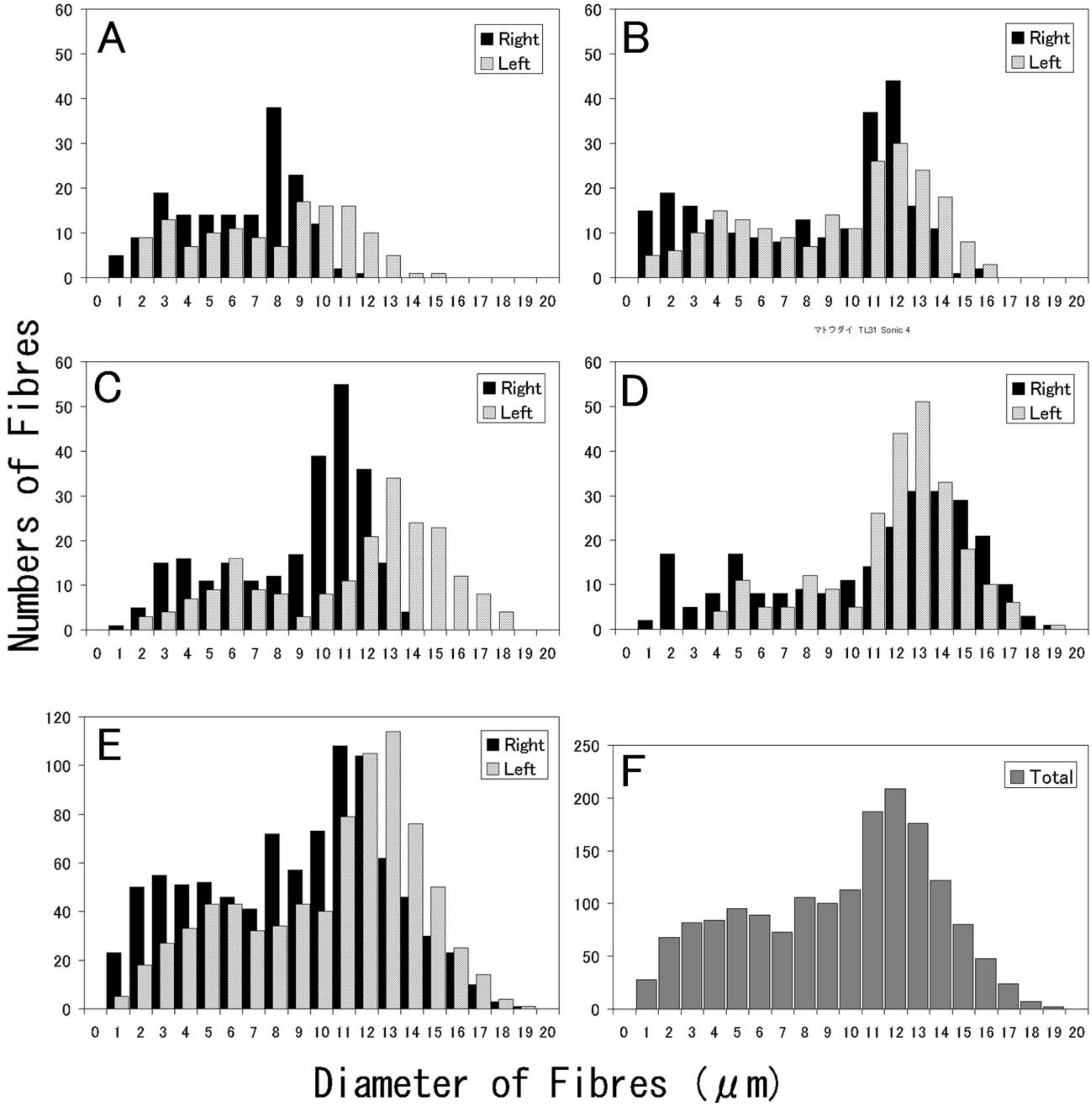

A
B
C
D
E
F
Right
Left
Total
マトウダイ TL31 Sonic 4
Numbers of Fibres
Diameter of Fibres (μm)

Figure 7.