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Quantitative Aspects of the Spatial Distribution and Morphological Characteristics of the Sea Bass (*Dicentrarchus labrax* L.; Teleostei, Serranidae) Trunk Lateral Line Neuromasts

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Key Words
Fish · Teleost · Sea bass · Trunk lateral line · Neuromast · Hair cell

Abstract

The results presented herein report quantitative data relative to the distribution and morphological characteristics of both types of neuromasts encountered on the trunk lateral line of the sea bass (*Dicentrarchus labrax*, L.). These data were obtained from scanning electron micrographs. They indicate that, as expected, each modified scale of the sea bass possessed a single canal neuromast with long axis oriented parallel to the fish's long axis. In contrast to several fish species, two thirds of superficial neuromasts observed herein were oriented perpendicular to the fish's long axis. However, whatever the main orientation of superficial neuromasts, two thirds of their hair bundles were oriented parallel to the long axis of the animal with approximately half of them in the direction of the head. Similar ratios were observed for canal neuromasts whatever the area of the maculae: central or peripheral. For both types of neuromasts it was not possible to clearly distinguish a paired organization of hair bundles with opposing polarities. Superficial neuromasts on each trunk canal scale were located on either the dorsal or ventral side of the canal and appeared to be distributed along the trunk lateral line with a higher probability to be encountered closer to the operculum. The frequency of presence and the average number of superficial neuromasts per scale increased with fish size. We observed a size gradient for canal neuromasts between the operculum and caudal peduncle. This gradation was correlated with a reduction of the width of the central area of the canal segment. Canal neuromasts were always localized in the larger portions of the canal segments. Taken together, these results point out some specific features associated with the sea bass trunk lateral line. With the previous report, they establish the first full description of the trunk lateral line of sea bass and will be useful for upcoming experiments regarding the function of the two types of neuromasts.

Introduction

The lateral line system is present in all fishes and aquatic amphibians [Dijkgraff, 1962; Blaxter, 1987; Lannoo, 1987; Coombs et al., 1989; Webb, 1989a; Northcutt,

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1992]. In fishes, this sensory system detects near field water movements caused by the animal's own movements and water currents [Denton and Gray, 1983; Bleckmann, 1993; Montgomery et al., 1995; Coombs et al., 1996]. This sensitivity to hydrodynamic stimuli enables the fish to locate congeners, predators, preys and other stationary or moving objects [Dijkgraff, 1962; Partridge and Pitcher, 1980; Blaxter and Batty, 1985; Hoekstra and Janssen, 1986; Montgomery, 1989; Bleckmann, 1993; Janssen et al., 1995, 1999; Montgomery and Hamilton, 1997; Coombs, 1999]. The lateral line system also plays a major role in rheotaxis [Bleckmann, 1993; Pavlov and Tyuryukov, 1993; Montgomery et al., 1997; Northcutt, 1997; Baker and Montgomery, 1999; Coombs et al., 2001] which allows fish to travel and intercept food carried downstream, potentially reducing energetic costs of displacements, food search and capture [Montgomery et al., 1997; Baker and Montgomery, 1999]. Based on these observations, it has been shown that the involvement of a lateral line system is essential for fish living in environments where visibility is significantly reduced [Schellart and Wubbels, 1998] such as in estuaries. The sea bass, *Dicentrarchus labrax,* an economically important fish species for fisheries along the French Atlantic coast, is known to potentially use its lateral line system under turbid or turbulent conditions. This fish inhabits the littoral zone and is found near rocky coasts or sandy beaches with high wave energy in addition to estuaries, lagoons and occasionally rivers [Barnabé, 1978; Quéro, 1984]. Because the coastal or estuarine habitats of the sea bass are also known to be sites exposed to metal ion pollution [Elbaz-Poulichet and Martin, 1987; Latouche, 1988; Jouanneau et al., 1990; Lapaquellerie et al., 1996; Michel et al., 2000] which can temporarily block its lateral line system [Karlsen and Sand, 1987] and thus affect its survival [Janssen, 2000], this potential threat to the continuation of the species motivated us to make a detailed analysis of the functional anatomy of the lateral line system.

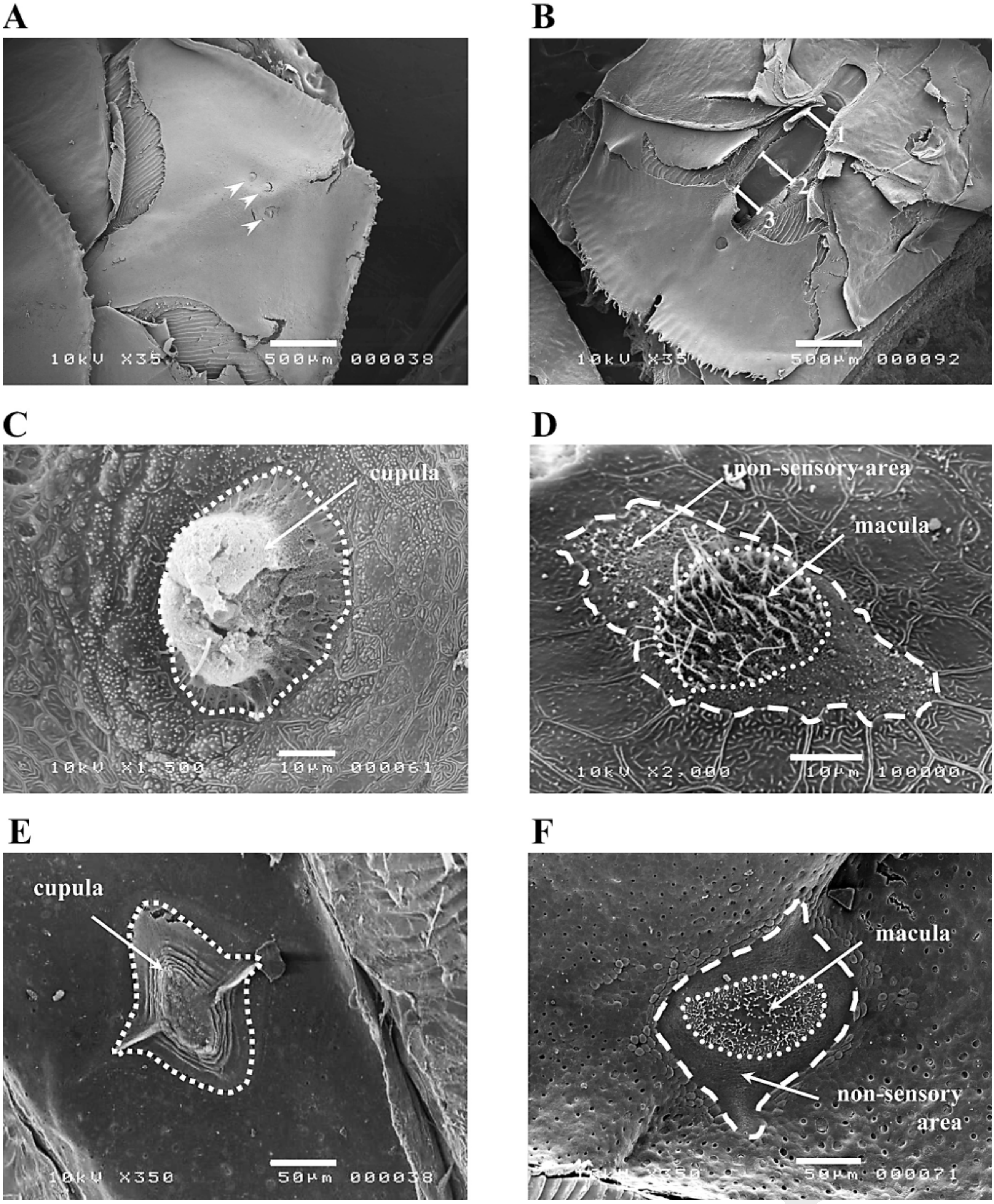
The functional units of the lateral line system are called the neuromasts. They are distributed on the head, trunk and tail [Coombs et al., 1989; Northcutt, 1992, 1997; Bleckmann, 1993]. These mechanoreceptors are divided into two types according to their location. Superficial neuromasts are present on the epidermis of modified scales and the canal neuromasts are enclosed in canals below the epidermis [Coombs et al., 1989]. Morphological data on lateral line systems have been reported for several fish species. These studies indicated that among teleosts there is a diverse set of morphological features and distribution patterns [e.g., Münz, 1979, 1989; Coombs et al., 1989; Song and Northcutt, 1991; Coombs and Montgomery, 1994]. Similar assertions could be described for non-teleost fishes [Webb and Northcutt, 1997; Maruska and Tricas, 1998; Maruska, 2001; Peach, 2001, 2003]. Few data are available for the sea bass lateral line system [Diaz et al., 2003; Faucher et al., 2003]. Most of the studies listed above are qualitative and to date little is known about the number, the size or orientation of either type of neuromasts and their associated sensory cells along the fish trunk lateral line [Münz, 1979, 1989; Coombs et al., 1989; Song and Northcutt, 1991; Coombs and Montgomery, 1994; Peach, 2003]. An accurate anatomical description of this sensory system is necessary to improve our understanding of the functioning of each type of neuromast as well as suggesting how evolutionary processes contributed to the orientation and directional sensitivity of lateral line sense organs in fish species living in turbulent environments.

Materials and Methods

Animals and Tissue Processing

For this study, modified scales isolated from the trunk lateral lines of thirty-eight specimens of sea bass, *Dicentrarchus labrax,* were examined. The fish averaged 20 cm in length and 120 g in weight. The fish were obtained from a commercial source (Ferme des Baleines, Ile de Ré, France). All specimens were housed in 240-liter tanks filled with filtered seawater and kept under natural photoperiod and constant temperature (16 °C). They were fed twice a week with live molluscs and crustaceans.

Fig. 1. Electron micrographs which illustrate the different quantitative parameters measured on neuromasts of the sea bass trunk lateral line. Superficial neuromasts were observed on the epidermis of trunk lateral line scales (**A**, scale bar = 500 μm), whereas canal neuromasts were located inside the segment canal of these scales (**B**, scale bar = 500 μm). For each neuromast, different parts were delimited: the whole neuromast (non-sensory area and macula), its macula and when possible the base of its cupula. The morphological limits taken into account for all measurements (length, width, perimeter and surface area) performed in the cupula (**C**, **E**), the macula and the whole neuromast (**D**, **F**) are indicated on the micrographs (dotted and dashed lines). The lengths and widths of the neuromasts, cupulae and maculae were measured at their largest place based on the limits previously set. The diameter of the canal segment was estimated by measuring the length of a line drawn through the width of the canal segment at the level of three specific marks: the central axis of the canal neuromast (2 in **B**) estimated from its morphological limits and the presence of its wing-like extensions, and the two extremities of the subdermal tube, the nearest possible to suprascalar (1 in **B**) and infrascalar pores (3 in **B**).


A
10kV X35 500µm 000038
B
1
2
3
10kV X35 500µm 000092
C
cupula
10kV X1,500 10µm 000061
D
non-sensory area
macula
10kV X2,000 10µm 100000
E
cupula
10kV X350 50µm 000038
F
macula
non-sensory area
10kV X350 50µm 000071

Prior to sacrifice, the fish were anaesthetized with 75 mg/l MS 222 (3-aminobenzoic acid ethyl, Sigma) and measured in standard length: from the tip of the snout up to the indentation of the caudal fin. For each fish, both entire trunk lateral lines were sampled in natural seawater. They consisted of a single row of modified scales, differing from the others by the presence of superficial neuromasts and the canal tube containing canal neuromasts, running within the mid-section of each flank from the operculum up to the tail. No superficial or canal neuromasts were observed on the trunk outside these specific areas [Faucher et al., 2003]. Tissue samples were dissected in an artificial solution (composition in m*M*: NaCl: 150; KCl: 5; $CaCl_2 \cdot 2H_2O$: 3; $MgCl_2 \cdot 6H_2O$: 1.5; HEPES: 10) for which the pH was adjusted to 8 with NaOH. Each tissue sample consisted of two to three consecutive scales. Some were left intact in order to observe superficial neuromasts. The roof of the canal segment of other scales was carefully removed from the suprascalar to infrascalar pores to allow visualization of canal neuromast (fig. 1A, B).

Tissue samples were fixed in 4% glutaraldehyde (Fisher Scientific Labosi) in sodium cacodylate buffer (0.4 *M*, pH 7.2), dehydrated in graded acetone concentrations and critical point-dried using liquid CO_2 (BALTEC CPD 030). They were then mounted on brass supports and sputter-coated with gold (Cressington Sputter Coat). Observations were performed with a JEOL JSM-5410LV scanning electron microscope. All quantitative measures were obtained using the image analysis software Biocom Visiol b 200.

Morphological Data

To allow comparisons between scales and/or fishes, an identifying number (I.D. #) was assigned for each modified scale that composed the trunk lateral line. The I.D. # 1 was given to the first scale located just after the operculum and sequentially until the limit of the caudal fin. A minimum of five observations per scale I.D. # was performed. In addition, according to the total number of modified scales, the trunk lateral lines were divided into three equal segments: anterior (S_1), middle (S_2) and posterior (S_3). This allowed us to account for the different number of modified scales (between 51 and 78) per trunk lateral line. Thus, depending on the fish examined, each segment was composed of 17 to 26 scales.

The distribution of superficial or canal neuromasts along the trunk lateral line was assessed through the determination, per scale and then per segment, of the average number of neuromasts and of the average frequency of neuromast occurrence. In order to do that, the number of modified scales examined was recorded. This led to the total number of scales observed per segment (N_{obs}). Then, the number of positive observations (x_{Si}), that is the number of scales for which one or more canal or superficial neuromasts were observed, was estimated. Finally, the total number of neuromasts observed per segment was determined and noted as n_{Si}.

These data were then used to calculate the frequency of neuromast occurrence (f_{Si}) and the average number of neuromasts per scale ($\overline{n}_{Si}$) for the different trunk segments according to equations 1 and 2.

$$f_{Si} = \Sigma\, x_{Si}/N_{obs} \qquad (1)$$

$$(\overline{n}_{Si} = \Sigma\, n_{Si}/N_{obs} \qquad (2)$$

Each neuromast can be considered either as a whole or as constituent parts, i.e., the non-sensory and sensory (macula) epithelia and the cupula. The image analysis software Biocom Visiol b 200 allowed us to outline these different areas (fig.1C–F) and quantify some related metrics such as their respective length (l), width (w), perimeter (p) and surface area (a). For a given neuromast, according to the presence or not of the cupula, we can measure at the same time either the surface area of the whole neuromast and of the cupula or the surface area of the whole neuromast and of the macula.

Then, additional information can be deduced for each parameter previously mentioned. As an example, for a given type of neuromast the average surface area ($\overline{a}$) was calculated (equation 3), according to the total number of neuromasts observed (nm). Likewise, for each type of neuromast the average surface area per segment ($\overline{a}_{Si}$) was calculated using equation 4, in which nm_{Si} corresponded to the number of neuromasts per segment from which data were obtained.

$$\overline{a} = \Sigma\, a/nm \qquad (3)$$

$$\overline{a}_{Si} = \Sigma\, a/nm_{Si} \qquad (4)$$

The values obtained for f_{Si}, $\overline{n}_{Si}$ and $\overline{a}_{Si}$ were then averaged according to the corresponding number of fish examined (between 30 and 38) and noted as $\overline{F}_{Si}$, $\overline{N}_{Si}$ and $\overline{A}_{Si}$, respectively.

Image analysis software Biocom Visiol b 200 was also used to determine, as much as possible, the width of the canal segment at the level of three specific marks: the center of the neuromast and the two extremities of the subdermal tube, the nearest possible to infrascalar and suprascalar pores (fig. 1B).

Orientation of the Neuromasts and Their Hair Bundles

When possible, the orientations of the two types of neuromasts and their hair bundles were examined. The orientation of the neuromast was determined by the angle established between the main axis of the neuromast, that is its length, and a reference axis (0–180°) which corresponded to the long axis of the animal with 0° on the head side. Results were expressed as percentages of the total number of neuromasts observed. Round superficial neuromasts (with length <1.25 times the width), for which no major axis could be determined, were not accounted for in this part of the study.

In order to study the possibility of a preferential axis of sensitivity for the neuromasts, the orientations of the hair bundles were examined. The angle, from 0 to 360°, between the axis of polarity of the hair bundles, conferred by the relative position of the kinocilium in relation to the stereocilia, and the long axis of the animal was determined. Results were expressed as percentages of the total number of hair bundles observed per angular intervals of 45°.

Finally, the percentage of hair bundles associated in pairs of opposing polarities within a given neuromast was calculated. For this, the polarity of each hair bundle was compared with the polarity of all adjacent hair bundles, on the rostral, caudal, dorsal and ventral sides. In order to be classified as opposite, the difference between the polarities of adjacent hair bundles should be equal to 180 ± 20°. Then, the percentage of observed pair-wise hair bundles was calculated relative to the number of possible pair-wise hair bundles inside the macula of each neuromast.

Statistical Analyses

All quantitative data were expressed as the mean ± SEM (standard error of the mean). The statistical analyses were performed with the statistical software XLSTAT-Pro 6.0. According to the essence of the parameters and the relationships to be tested different statistical tests were used ($\alpha = 0.05$). The value of the test was noted: H for a Kruskal-Wallis test, U for a Mann-Whitney test, r_s for a Spearman's rank correlation procedure, $F_{\upsilon 1,\upsilon 2}$ for a linear regression test and χ^2 for a χ^2 test.

Results

Neuromast Distribution along the Trunk Lateral Lines

The distribution of superficial and canal neuromasts along the trunk lateral lines was examined through the determination of their frequency of occurrence and their number within each segment S_i (table 1).

The figure 2A represents the average frequency of occurrence ($\overline{F}_{Si}$) of superficial neuromasts calculated for each segment. This distribution was heterogeneous along the trunk lateral line ($H = 7.136$, $p = 0.028$; table 1). The probability of encountering these neuromasts was higher at the beginning (S_1) than at the end (S_3) of the trunk lateral line ($U = 756.000$, $p = 0.005$). The figure 2B shows that the frequency of occurrence of superficial neuromasts in a fish body tended to increase with the animal's size ($F_{1,16} = 7.275$, $p = 0.016$, $R^2 = 0.313$). Nevertheless, the distribution of frequency of occurrence was homogeneous among the three segments (S_i) of the fish body regardless of fish size.

The average number of superficial neuromasts per scale in each segment, S_i ($\overline{N}_{Si}$), was also examined (fig. 3A). No significant difference among these three segments was observed ($H = 4.388$, $p = 0.111$; table 1). In addition, an examination of their position on the scale revealed that they were located indiscriminately on the dorsal or the ventral side of the canal segment. As for the frequency of occurrence, the average number of superficial neuromasts increased with fish size (fig. 3B; $F_{1,16} = 5.427$, $p = 0.033$, $R^2 = 0.253$). There were no significant differences among segments S_i of the fish body in the average number of superficial neuromasts for fish of all sizes.

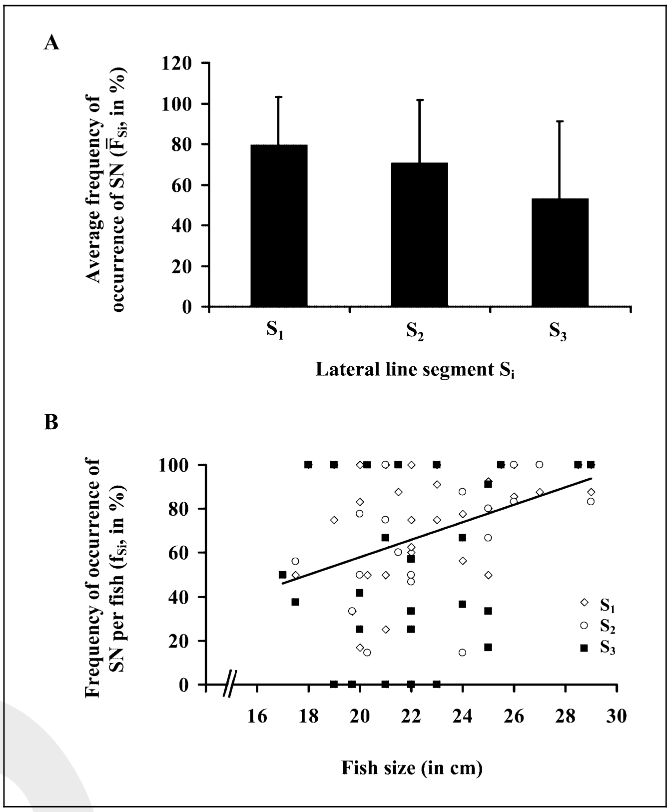


Fig. 2. Distribution of the frequency of occurrence of superficial neuromasts (SN) along the sea bass trunk lateral line and their relationship with the fish size. **A** Average frequency of occurrence of SN calculated for each segment S_i ($\overline{F}_{Si}$). Frequencies are expressed in percentage and vertical bars represent the standard error of the mean. SN were more frequently seen at the beginning of the trunk lateral line (S_1). **B** Relationship between the frequency of occurrence of SN per fish (f_{Si}) and the fish size in each segment S_i of the trunk lateral line. Frequencies are expressed in percentage. The frequency of occurrence of SN increased with fish size ($Y = 3.4898x - 8.1544$, $R^2 = 0.3126$).

Table 1. Quantitative data concerning the distribution and the morphology of both types of neuromasts

	Lateral line segments S_i			Total average ± SD	N_{obs}	Nm	Number of observed fish
	S_1	S_2	S_3				
$\overline{F}_{Si}$ of SN ± SD, %	79.54 ± 23.79	70.73 ± 30.95	52.91 ± 38.46	68.69 ± 32.67	662		38
$\overline{F}_{Si}$ of CN ± SD, %	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	263		38
$\overline{N}_{Si}$ of SN ± SD per scale	1.25 ± 0.52	1.12 ± 0.64	0.91 ± 0.72	1.10 ± 0.63	694		38
$\overline{N}_{Si}$ of CN ± SD per scale	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	263		38
$\overline{A}_{Si}$ of SN ± SD, μm^2	812.61 ± 549.28	924.18 ± 742.81	1,046.15 ± 896.67	892.35 ± 684.21		357	38
$\overline{A}_{Si}$ of CN ± SD, mm^2	0.06 ± 0.03	0.04 ± 0.03	0.02 ± 0.01	0.05 ± 0.03		141	38
Ce.D. of canal segment, μm	414.70 ± 85.72	357.16 ± 53.63	289.04 ± 42.07	355.57 ± 79.83	220		29
R.D. of canal segment, μm	315.00 ± 48.25	297.82 ± 44.07	262.90 ± 48.43	290.15 ± 50.00	119		24
Ca.D. of canal segment, μm	326.42 ± 64.20	296.56 ± 39.54	271.61 ± 43.59	295.54 ± 50.96	129		25

$\overline{F}_{Si}$ = average occurrence; $\overline{N}_{Si}$ = average number; $\overline{A}_{Si}$ = average surface area; Ce.D. = central diameter, R.D. = rostral diameter; Ca.D. = caudal diameter; SN = superficial neuromasts; CN = canal neuromasts; SD = standard deviation of the mean; N_{obs} = number of scales observed; Nm = number of neuromasts measured.

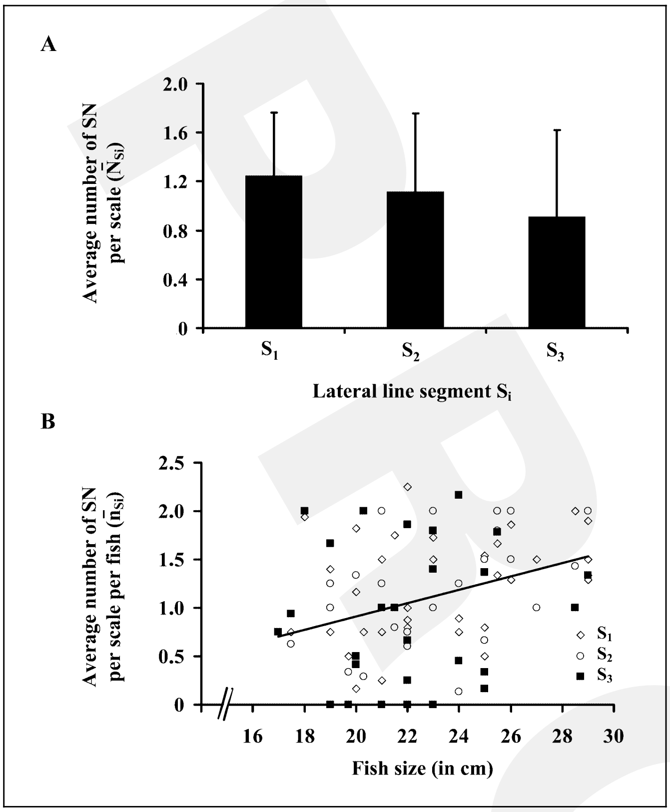


Fig. 3. Distribution of the average number of superficial neuromasts (SN) per scale along the sea bass trunk lateral line and their relationship with fish size. **A** Average number of SN per scale in each segment S_i (N_{Si}). Vertical bars represent standard error of the mean. The data did not support a specific pattern of distribution. **B** Relationship between the average number of SN per scale per fish (n_{Si}) and the fish size in each segment S_i of the trunk lateral line. The average number of SN per scale increased with fish size ($Y = 0.0609x - 0.2432$, $R^2 = 0.2533$).

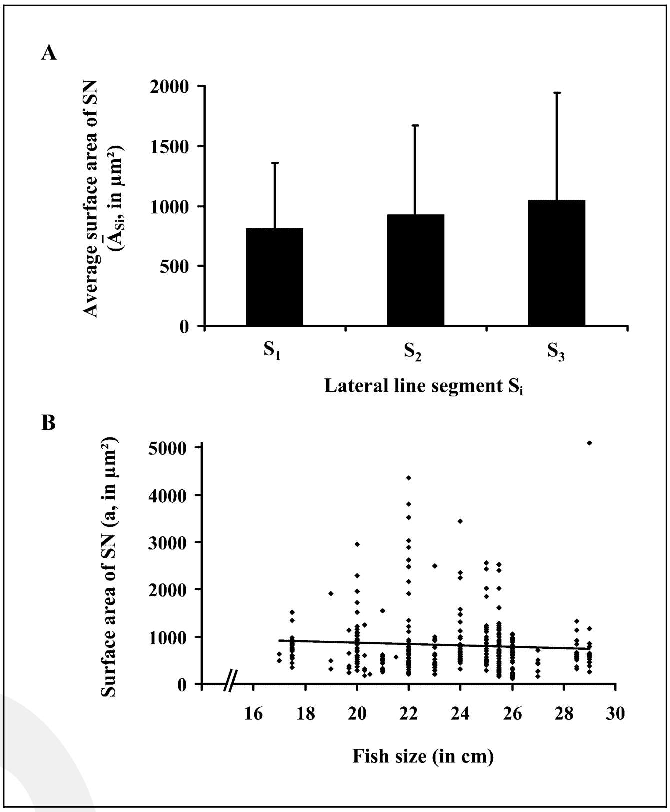


Fig. 4. Distribution of the size of superficial neuromasts (SN) along the sea bass trunk lateral line and their relationship with the fish size. **A** Average surface area (A_{Si}) by segment S_i (expressed in μm^2). Vertical bars represent standard error of the mean. No specific pattern of distribution could be established. **B** Relationship between the surface area (a) of SN and the fish size. Data show that the size of these sensory organs varied according to individuals but not with the fish size ($Y = -14.256x + 1150.3$, $R^2 = 0.0046$).

In contrast to superficial neuromasts, only one canal neuromast could be observed on each modified scale (n = 263; table 1).

Morphological Data

Among the different measures performed on neuromasts of each type and their constituent parts we have elected to consider the surface area of the whole neuromast as the parameter of reference for comparisons (table 1). This choice was based on several observations. First, this parameter incorporated aspects of all other metrics (perimeter, length and width). Second, analyses indicated that for both types of neuromasts the whole surface area was significantly correlated with the surface area at the base of the cupula ($r_s = 0.940$, $p < 0.0001$, $n = 111$ for superficial neuromasts and $r_s = 0.850$, $p < 0.0001$, $n = 53$ for canal neuromasts) and the surface area of the macula ($r_s = 0.874$, $p < 0.0001$, $n = 74$ for superficial neuromasts and $r_s = 0.978$, $p < 0.0001$, $n = 69$ for canal neuromasts).

The average surface area of superficial neuromasts within a given segment S_i ranged from 230 to 3,600 μm^2 which corresponded to an average surface area of 892.35 ± 684.21 μm^2 (n = 357). This difference in size was also observed between superficial neuromasts present on the same scale. Along the trunk lateral line, the average surface area ($\bar{A}_{Si}$) of superficial neuromasts was examined (fig. 4A). Statistical analyses indicated that there was no significant difference among the three segments ($H = 0.672$, $p = 0.715$; table 1). The relation between the sur-

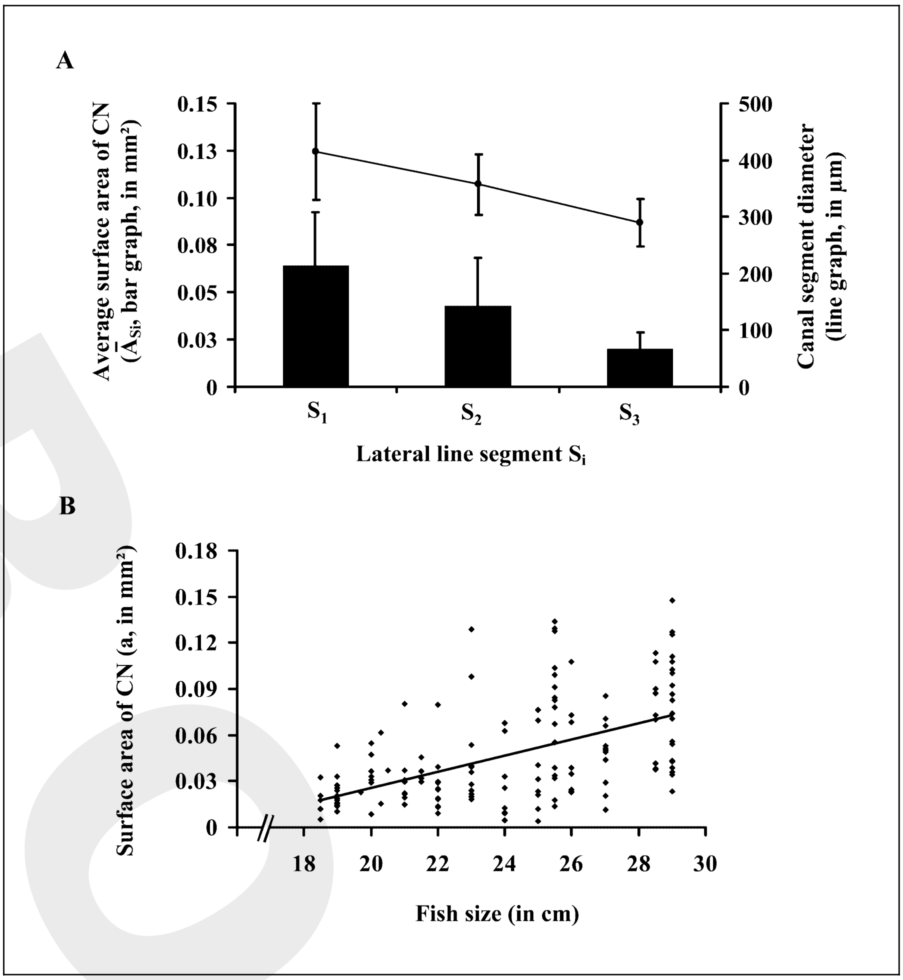


Fig. 5. Distribution of the size of canal neuromasts (CN) along the sea bass trunk lateral line and their relationship with the fish size. **A** Evolution of the average surface area ($\bar{A}_{Si}$) of the CN (expressed in µm²; bar graph) and of the average diameter of the canal segment (in µm, measured at CN location; line graph) along the trunk lateral line. Vertical bars represent standard error of the mean. The size of the CN decreased significantly with the central diameter of the canal segment between the beginning (S_1) and the end (S_3) of the trunk lateral line. **B** Relationship between the surface area (a) of CN and the fish size. The average size of these sensory organs varied according to individuals and was also positively correlated with the fish size ($Y = 0.0053x - 0.0799$, $R^2 = 0.2869$).

face area of superficial neuromasts and the fish size, illustrated in figure 4B, showed that the former varied among individuals but was not correlated with the fish size ($F_{1,333} = 1.544$, $p = 0.215$, $R^2 = 0.005$).

Similar studies applied to canal neuromasts indicated that within each segment (S_i) the average surface areas ($\bar{a}$) could also be variable: from 0.005 to 0.130 mm² for an average surface area of 0.05 ± 0.03 mm² (n = 141). Along the trunk lateral line, the average surface area of these neuromasts was significantly larger in the anterior part (S_1) of the trunk lateral line than in the posterior part (S_3; U = 446.000, $p < 0.0001$; fig. 5A; table 1). Statistical analyses also revealed that canal neuromasts were significantly larger (0.05 ± 0.03 mm², n = 141) than superficial ones (892.35 ± 684.21 µm², n = 357; U = 5,110.000; $p < 0.0001$).

The diameter of the canal segment was measured, when possible, in its rostral, central and caudal regions (table 1). Data showed that average canal segment diameters measured in its rostral (290.15 ± 50.00 µm, n = 119) and caudal (295.54 ± 50.96 µm, n = 129) ends were not significantly different (U = 8,080.500, p = 0.473). Thus, these peripheral diameters could be pooled and compared to the diameter of the canal segment in its central part (355.57 ± 79.83 µm, n = 220). A Mann-Whitney test indicated that the central diameter of the canal segment, where canal neuromasts were located, was significantly greater in diameter at their peripheral ends (U = 41,764.000, $p < 0.0001$). As observed for the size of canal neuromasts, the central diameter of the canal segment decreased significantly between S_1 (414.70 ± 85.72 µm, n = 73) and S_3 (289.04 ± 42.07 µm, n = 67; H = 31.498, $p < 0.0001$; fig. 5A; table 1). Finally, the surface area of the canal neuromasts was positively correlated with the fish size ($F_{1,146} = 58.731$, $p < 0.0001$, $R^2 = 0.287$; fig. 5B).

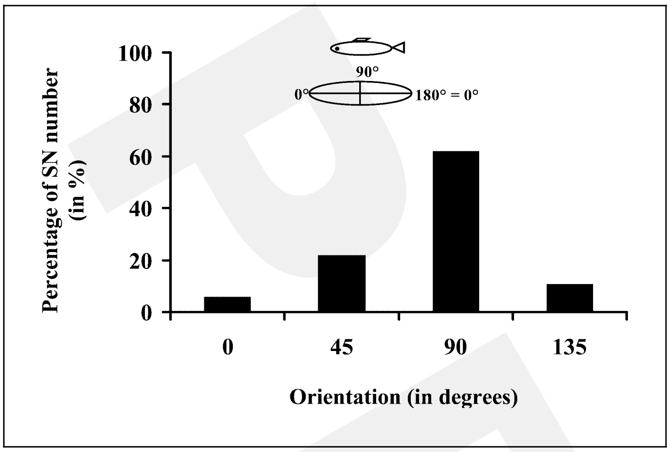


Fig. 6. Orientations of superficial elliptical neuromasts (SN) in regard to the canal segment main axis. The reference axis (0–180° line) was parallel to the canal axis. The insert shows the multiple orientations of the SN observed (0 and 180° were not distinguished). The 100 % value corresponded to the total number of SN observed (n = 378). The majority of SN was oriented perpendicularly to the canal axis.

Neuromast Orientations

The orientation of superficial neuromasts was only examined for the elliptical type. For the round ones (length inferior to 1.25 times the width), it was not possible to determine a major axis. Figure 6 shows that among the 378 elliptical superficial neuromasts observed, two thirds were oriented perpendicular to the animal's long axis, whereas 22% of the neuromasts were oriented at 45° and 10.5% at 135° ($\chi^2 = 134.075$, $p < 0.0001$). Only 5.5% were parallel to the fish's long axis.

In contrast to superficial neuromasts, the major axis of all canal neuromasts observed was oriented parallel to the animal's long axis which corresponded also to the axis of the canal segment (n = 263).

Hair Bundle Orientations

When possible, the orientation of hair bundles within the maculae of each type of neuromast was also examined. In the maculae of superficial neuromasts, elliptical and round, hair bundles presented a preferential orientation (fig. 7A). Indeed, among the 121 hair bundles observed, nearly two-thirds (65.3%) were oriented according to the fish's long axis ($\chi^2 = 11.314$, $p = 0.000$). Among these hair bundles 32.2% were more or less oriented towards the head (between 316 and 45°) and 33.1% towards the tail (between 136 and 225°). The last third could be divided into hair bundles which were dorsally oriented (10.8% between 46 and 135°) and hair bundles which were ventrally oriented (24.0% between 226 and 315°). The orientation of hair bundles in regard to the fish's long axis was also examined according to the orientation of superficial neuromasts. In this analysis, distributions of the hair bundle orientations within the maculae of superficial neuromasts oriented at 0, 45, 90 and 135° were compared (fig. 7B). Whatever the main orientation of these neuromasts, differences between the orientations of their hair bundles were not significant ($\chi^2 = 0.154$, $p = 0.985$, n = 32), they were preferentially oriented according to the 0–180° axis, which corresponded to the animal's long axis. In order to examine the orientation of hair bundles within the maculae of canal neuromasts, 79 hair bundles were observed. They were preferentially oriented along the fish's long axis (75.6%; $\chi^2 = 23.405$, $p = 0.000$, n = 79; fig. 8A). Among these hair bundles 38.1% were more or less oriented towards the head (between 316 and 45°) and 37.5% towards the tail (between 136 and 225°). The remainder of the hair bundles were dorsally (14.3% between 46 and 135°) or ventrally (10.1 % between 226 and 315°) oriented. The possibility of a different orientation pattern between the central and peripheral regions of the maculae was also considered (fig. 8B). In the central region, hair bundles were indiscriminately oriented toward the rostral (32% between 316 and 45°) or the caudal (42% between 136 and 225°) side of the fish. Analogous values were obtained for the peripheral area with 38% oriented toward the rostral (between 316 and 45°) versus 35% toward the caudal side (between 136 and 225°) of the fish. A χ^2 test showed that this distribution of orientations inside the maculae of canal neuromasts was homogeneous between the central and the peripheral areas ($\chi^2 = 0.465$, $p = 0.496$, n = 64).

Very few hair bundles organized in pairs with their kinocilia facing in opposite directions were observed in superficial (19.0%) or canal neuromasts (9.9%).

Discussion

The efficiency of the lateral line organs in response to stimulation is dependent on (1) the distribution of hair bundle polarities within the maculae together with (2) the scattering of neuromasts on the flanks of fish. Flock and Wersäll [1962] were the first to demonstrate the relationship between hair bundle polarization, given by the eccentric position of the kinocilium, and the directional sensitivity of hair cells in response to stimulation. In sea bass, hair bundles within the maculae of superficial neuromasts

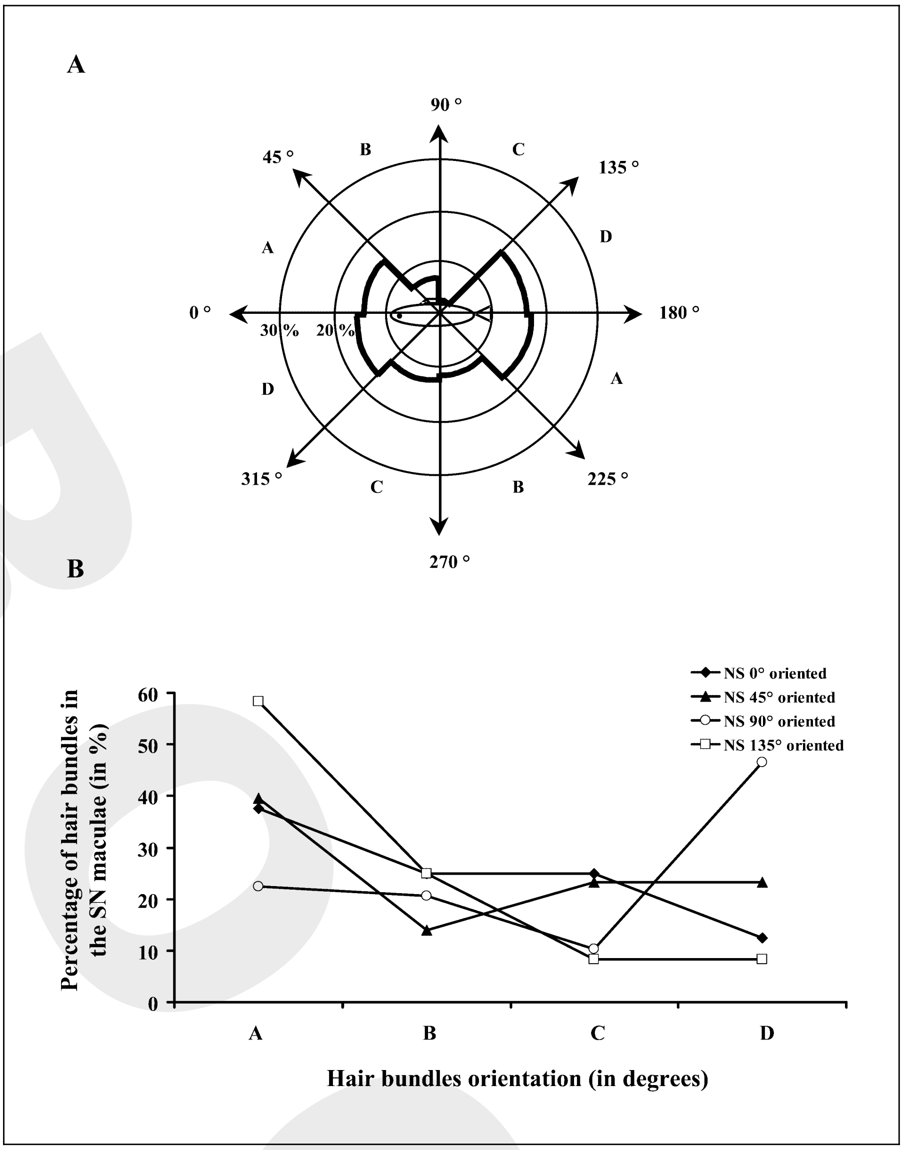


Fig. 7. Repartition of the hair bundle orientations within the maculae of superficial neuromasts (SN) and their relationship with the SN main orientation. The polarity of each hair bundle was defined by the line drawn from the smallest stereocilia towards the kinocilium. Each value was expressed in degree based on a 360-degree reference plane, 0 ° towards the rostral side and 180 ° towards the caudal side of the trunk lateral line. Results were expressed as percentages of the total number of hair bundles observed. **A** Orientation of the hair bundles within the maculae of SN in a 360-degree reference plan with respect to a lateral view of the fish. Most were oriented along the canal axis either towards the rostral or the caudal side of the trunk lateral line. **B** Relationship between hair bundle orientations (A: between 0 and 45 °, then 181 and 225 °; B: between 46 and 90 °, then 226 and 270 °; C: between 91 and 135 °, then 271 and 315 °; D: between 136 and 180 °, then 316 and 360 °) within the maculae of SN and main orientation of SN (0, 45, 90 or 135 °). Hair bundle orientations were constant regardless of the orientation of SN.

were predominantly (70%) oriented parallel to the animal's long axis, regardless of the main orientation of the neuromast. Half of the superficial neuromast hair bundles were directed towards the head. The remainder exhibited various orientations. Similar ratios were observed within canal neuromasts, whatever the hair bundles' location in the central or the peripheral area of the maculae [Faucher et al., 2003]. The observation of a major pattern of bipolar orientation parallel to the animal's long axis for both types of neuromasts agrees with most reports on teleosts [Flock, 1965; Yamada and Hama, 1972; Münz, 1979; Denton and Gray, 1983; Webb, 1989c; Rouse and Pickles, 1991; Bleckman, 1993; Coombs and Montgomery, 1994; Tsukamoto et al., 1995; Montgomery et al., 2001]. However, in contrast with these authors, we could not clearly distinguish a paired organization of hair bundles. Taken together, these observations strongly suggest that the trunk lateral line of sea bass is essentially sensitive to stimuli applied along the fish's long axis. This was expected for canal neuromasts, due to physical constraints set by the canal segment, in which these receptors were enclosed [Coombs et al., 1989; Webb, 1989b]. However, such limits should not have applied to superficial neuromasts, which were freestanding on the skin and thus might potentially be the target of stimuli coming from any direction. At first, this assumption appeared to be supported by two facts: (1) the

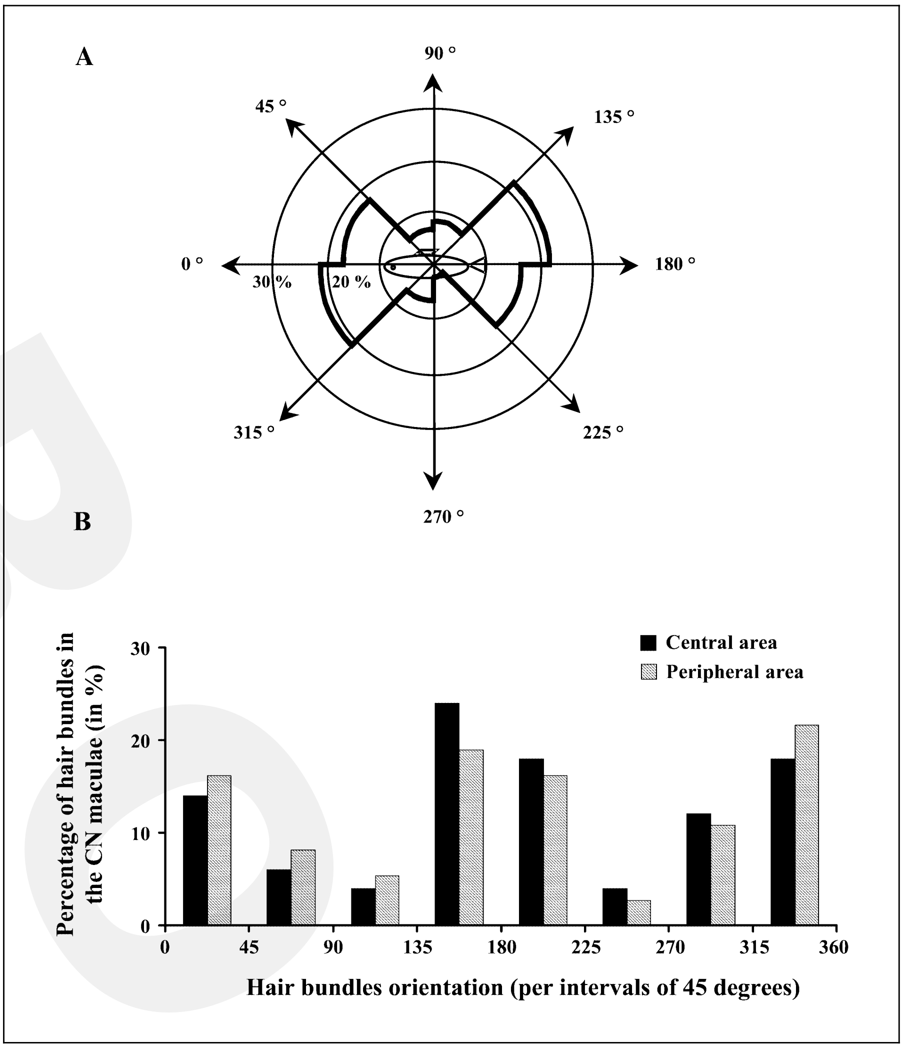


Fig. 8. Repartition of the hair bundle orientations within the maculae of canal neuromasts (CN) and their relationship with their macula localization (in peripheral or central area). The polarity of each hair bundle was defined by a line drawn from the smallest stereocilia towards the kinocilium. Each value was expressed in degree based on a 360-degree reference plane, 0° towards the rostral side and 180° towards the caudal side of the trunk lateral line. Results were expressed as percentages of the total number of hair bundles observed. **A** Orientation of the hair bundles within the CN maculae in a 360-degree reference plan with respect to a lateral view of the fish. They were mainly oriented parallel to the axis of the canal segment, indiscriminately towards the rostral or the caudal side of the trunk lateral line. **B** Relationship between hair bundle orientation and location within the CN macula. The predominant bi-directional orientation of the hair cell bundles within the maculae of CN was constant regardless of area: peripheral or central.

observation that both round and elliptical superficial neuromasts exhibited circular maculae [Faucher et al., 2003] and (2) the presence of one third of the hair bundles presenting various orientations. However, as mentioned above, the observation that most hair bundles within the maculae of superficial neuromasts were oriented along the longest axis of the animal body contradicts the possibility of a multi-directional sensitivity. Thus, at this point, the significance of the presence of elliptical superficial neuromasts oriented perpendicularly to the long axis of the fish, as previously observed in other species [Song and Northcutt, 1991; Harvey et al., 1992; Peach and Rouse, 2000], can only be a matter of speculation. To conclude, hair bundle orientations within the maculae of sea bass superficial and canal neuromasts support the assumption of a single axis of best sensitivity parallel to the long axis of the fish.

Pertaining to the receptive field, spatial distribution of both types of neuromasts along the sea bass trunk lateral line was typical of actinopterygian fishes [Münz, 1979, 1989; Coombs et al., 1989; Puzdrowski, 1989; Webb, 1989a, b, 1990; Song and Northcutt, 1991; Tsukamoto et al., 1995; Webb and Northcutt, 1997; Northcutt et al., 2000]. A closer look at the distribution of superficial neuromasts showed that their frequency of occurrence per modified scale was higher closer to the operculum. Comparatively, this segment of the trunk lateral line also corresponded to the area where canal neuromasts were the biggest. Such gradients in spatial distribution of the neuromasts were previously observed by Dijkgraaf [1962] and Schellart and Wubbels [1998]. It can therefore be suggested that there might be a gradient of sensory capacity from the beginning to the end of the trunk lateral line. It has been shown that unlike most teleost fishes [e.g., Flock, 1965; Münz, 1979; Appelbaum and Schemmel, 1983; Coombs et al., 1989; Webb, 1990; Harvey et al., 1992; Kroese and Schellart, 1992], the modified scales of sea bass trunk lateral lines did not present a direct connection between the interior of the canal segment and the external medium [Faucher et al., 2003]. Moreover, the overlap of the pores between contiguous scales appeared to result in the formation of a quasi-continuous tube starting at the operculum and ending at the caudal peduncle. Thus, it is likely that for this species the surface of contact between the canal fluid and the external medium should be greatly reduced and might essentially occur at the beginning of the trunk lateral line. Remember that canal neuromasts are sensitive to: (1) water currents relative to fish movements [Abdel-Latif et al., 1990], (2) prey and predators in the presence of unidirectional water flow [Engelmann et al., 2000] and (3), like many predator fishes, sea bass always swim against the current [Barnabé, 1978]. In this case, the fish's sensitivity will be more efficient in the anterior part of the animal where water turbulence is lower [Webb, 1978]. Thus, it was not surprising that this anterior area corresponds with the greatest canal neuromast development and the frequency of superficial neuromasts occurrence.

As observed in most species [Münz, 1979; Webb, 1989c; Song and Northcutt, 1991; Maruska and Tricas, 1998; Northcutt et al., 2000] the size of the sea bass trunk lateral line canal neuromasts and that of their maculae were significantly greater than those of their superficial counterparts. Although their sensory contribution is supposed to be different, it appears that if we consider both types of neuromasts, this size difference could be compensated for by the relevant number of superficial neuromasts combined with their higher hair cell densities [Faucher et al., 2003]. Still regarding the size of canal neuromasts, we observed that it decreased significantly and progressively between the first and the last modified scale of the trunk lateral line. This phenomenon was correlated with a reduction in the width of the canal. It is the first time that a decrease of the canal segment diameter along the trunk lateral line has been reported. This observation constitutes a preliminary finding that requires further substantiation. Previous reports [Coombs et al., 1989; Tarby and Webb, 2003] suggested that the development of canal neuromasts is subordinate to the mechanical limits set by the growth of the dermal bone. Accordingly, it was not surprising to notice that, as observed in Cottidae [Janssen et al., 1987] and Cichlidae [Münz, 1989; Tarby and Webb, 2003], the size of canal neuromasts was positively correlated with fish size. Consequently, and in agreement with Coombs et al. [1989] and Webb [1989b], the size of superficial neuromasts, which are free from morphological constraints, presents a wide range of values and, at the same time, their number increases with fish size [Blaxter et al., 1983; Blaxter, 1987; Prié-Granié; 1988; Harvey et al., 1992; Higgs and Fuiman, 1996; Appelbaum and Riehl, 1997; Poling and Fuiman, 1997; Webb and Shirey, 2003]. Noting that the size of canal neuromasts increases with animal size, whereas superficial neuromasts increase in number during the post-larval growth [Janssen et al., 1987; Münz, 1989], we can suggest, in accordance with the previous authors, that these phenomena allow the fish to maintain a constant density of receptors during development.

Finally, canal neuromasts were always located in the larger width of the spindle-shaped ducts which contrasted with most observations [Jakuboswki, 1967; Coombs et al., 1989; Webb, 1989a; Gibbs, 1999]. According to Schemmel [1977], these constrictions might amplify the canal neuromasts' sensitivity, as their presence will increase the velocity of fluid flowing in the canal ducts. In turn, Montgomery et al. [1995] showed that these constrictions were responsible for an attenuation of low frequencies and an amplification of higher frequency stimuli. In the sea bass trunk lateral line, it thus appears that these effects do not take place at the level of the neuromast, but at both ends. Might this be an adaptation for a species living in turbulent waters?

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