4 Cupular influence on fluid flow in the supraorbital lateral line canal of the ruffe (*Acerina cernua* L.)

Introduction

Fishes and amphibians have a lateral line system for the detection of water motion within the close vicinity of the animal (Dijkgraaf, 1963). The cephalic part of the lateral line system is situated on the head and in certain species of fish it consists of recessed bony canals with a series of protective bony plates arching over the canal, called bony bridges. Situated under the bony bridges are the sensory units, or neuromasts. A neuromast consists of sensory hair cells grouped together with their stereocilia anchored in a dome shaped extracellular matrix (Kelly and van Netten, 1991), called cupula.

Lateral line canal organs share a common working principle. Lateral line canal fluid couples the water motion outside of the fish to the cupula, thus driving the sensory hair cells, which code the mechanical information passed on to them into action potentials which are sent to the brain (Dijkgraaf, 1963; Kroese and van Netten, 1989). To understand the process from water disturbance to the transduction of the stimulus by the hair cells, the stimulus that is conveyed to the cupula must be known. Specifically, the interaction of the boundary layer close to the cupula and that in the vicinity of the canal wall is of interest.

Morphological differences in cephalic lateral line organs can be found between the many species of fish (Coombs *et al.*, 1988). These variations include differences in canal size, ranging from approximately 0.1 mm up to 7 mm (Denton and Gray, 1988; Münz, 1989), canal shape and bony bridge covering. In addition to this, the shape of a neuromast can vary greatly between species (Pumphrey, 1950). It is expected that these variations in the lateral line organs lead to differences in the flow of lateral line fluid and the stimulation of the neuromast (Denton and Gray, 1983; 1988; Coombs and Montgomery, 1992; van Netten, 1991; Wiersinga-Post and van Netten, in preparation).

It has been proposed that the velocity profiles within the lateral line canal are similar to the flow profiles in pipes and thus are controlled by the boundary layers of the wall (Denton and Gray, 1988; van Netten, 1991). In circular pipes, an oscillatory flow profile is characterised by the (linear) Reynolds number for periodic flow, $R_{Ac} = r^2 \omega \rho / \mu$ (Batchelor, 1967; Schlichting, 1987), where $r$ is the radius of the pipe, $\omega$ the angular frequency of the oscillatory flow, $\rho$ the fluid...
density and \( \mu \) the fluid viscosity. At low frequencies \( (R_{AC} \ll 1) \), at which the flow is determined by viscous fluid forces, the flow has a parabolic profile across the diameter of the pipe. At high frequencies \( (R_{AC} \gg 1) \), the flow profile takes on an annular form (Sexl, 1930; Womersley, 1955).

It has been experimentally demonstrated (Tsang and van Netten, 1997) that there are indeed many similarities between oscillatory flow profiles in the supraorbital lateral line canal of the ruffe \((Acerina cernua \, \text{L.})\) and those in circular pipes. In those experiments, the cupula was removed to investigate the influence of the canal and bony bridge on the flow in the canal. The flow in the vicinity of the bony bridge was found to be comparable to the flow in a pipe, in showing a gradual change from a parabolic like profile to an annular profile with increasing frequency. The frequency characteristics of the flow were clearly found to be controlled by the canal wall, similar to the flow behaviour found in pipes.

As expected, there are also differences between the measured flow in the lateral line canal and the flow in a pipe. These differences were most pronounced at the top of the canal, away from the bony bridge region, where under intact conditions no overlying bone is present and only skin covers the canal. It has been suggested that these boneless regions of the canal wall, termed hydrodynamic windows, permit the transmission of fluid motion outside the fish into canal fluid motion (van Netten and van Maarseveen, 1994). Obviously, the absence of rigid bone in the canal wall causes the velocity profile close to the top of the canal to differ from the flow profiles found near the wall of a completely closed pipe. In addition, in the experimental situation used for those flow measurements, the skin covering the windows was removed, to facilitate the insertion of a sense probe. From previous work (van Netten and van Maarseveen, 1994) it is known that the skin overlying the hydrodynamic windows is very compliant and that removal is not likely to affect the velocity distribution dramatically. The results obtained under the experimental conditions may therefore be expected to give a reasonable representation of the normal flow profiles.

As a continuation of the flow measurements of the lateral line fluid in the (empty) supraorbital lateral line organ of the ruffe \((Acerina cernua \, \text{L.})\), this chapter deals with the situation in which the cupula is present. This provides information on the interaction between the boundary layers controlled by both the cupula and the canal wall. For instance, it shows to what extent the stimulus received by a cupula is affected by the presence of neighbouring cupulae. It thus gives information on whether an array of cupulae in the lateral line canal work in a dependent mode, controlled by the hydrodynamics of the canal and other sensory units, or merely as individual fluid flow detectors.
Methods

Preparation

Thirty-nine ruffes (*Acerina cernua* L.) were used for studying radial and longitudinal canal fluid flow profiles in the supraorbital lateral line canal. The ruffe has been the subject of various studies in which the mechanosensitivity of the lateral line system has been investigated in relation to its peripheral hydrodynamics and hair cell mechanics (e.g. van Netten, 1997). Fish were anaesthetised with an intraperitoneal injection of Saffan (Pitman Moore, 25 mg/kg body weight) and placed in a fish tank with tap water, where they were artificially respired and held rigidly in place with head and body clamps.

The right supraorbital canal was opened by removing a small piece of skin covering the canal in between cupula I and III (numbering follows Jakubowski, 1963), see Fig. 1, to enable the placement of a stimulus sphere and the sense probe for detecting fluid velocity.

![Figure 1: A simplified diagram of the supraorbital lateral line canals of the ruffe showing the locations of the cupulae (according to Jakubowski, 1963). For these experiments, the flow profiles are all made in the region caudal to cupula II as shown by the dotted line. The bony bridges are not shown.](image)

During a measurement the artificial respiration to the ruffe was stopped (≈ 2 minutes) to reduce unwanted vibrations. It was turned on in between the measurements (≈ 4 minutes). To avoid the water from degassing and to reduce fluctuations in the lateral line canal fluid viscosity (Tsang *et al.*, chapter 2), the
temperature of the surroundings was kept constant at 15°C. Excess slime around the head was removed, preventing the slime from flowing into the lateral line canal during the measurements, which otherwise would engulf the sense probe.

**Mechanical stimulation**

A small piezo-electrically driven stimulus sphere (Ø 0.68 mm), placed at about 3.5 mm rostral from the caudal edge of bony bridge II was used to produce oscillatory fluid flow in the lateral line canal. The stimulus sphere was moved with a constant velocity amplitude (22 mm/s for radial profiles and 30 mm/s for longitudinal profiles) in the direction of the longitudinal axis of the canal with frequencies ranging from 10 to 500 Hz. The effect of the boundary layer of the sphere was corrected for in the measured velocity profiles.

**Sense probe for fluid flow measurement in the lateral line canal**

Measuring fluid flow with a spatial resolution down to the order of tens of microns requires a sense probe of comparable dimensions. Additional requirements of the sense probe include sufficient sensitivity to oscillating flow, down to the order of 1 μm/s in the relevant physiological frequency range of the ruffe. For *in vivo* measurements further restrictions are imposed by the relatively small dimensions and varying geometry of the lateral line canal.

These conditions can be met by using a measurement technique specially adapted to this problem, as described by Tsang and van Netten (1997). This technique makes use of a sense probe which consists of a micro-pendulum suspended from a very flexible glass fibre shank (see Fig. 2). This arrangement is designed to simulate a seeding particle that maintains a stable equilibrium position, and yet is allowed to move as freely as possible with the fluid. It also offers the possibility to measure at selected points within the lateral line canal.
Figure 2: Diagram of the sense probe used for measuring the fluid flow inside the supraorbital lateral line canal. The resin sphere diameter ($\Phi \approx 50 \mu m$), glass fibre thickness ($\Phi \approx 3 \mu m$) and length ($l \approx 2 \text{ mm}$) are not drawn to scale.

To determine the motion of the small sphere at the tip of the sense probe, a laser interferometer was used (see Fig. 3). The operation of the laser interferometer is described in more detail in chapter 2. Basically, the fluid flow is followed by the sense probe’s sphere which scatters the laser light as it moves through the (moving) fringe pattern ($\Theta 20 \mu m$) formed by the two laser beams at their point of focus. The sense probe was attached to an x, y, z-manipulator fixed to the support of the objective lens. Using the manipulator, the surface of the sense probe’s sphere was positioned exactly in the fringe pattern. This ensured continuous back-scattering of
the laser light at any depth of focus. The scattered Doppler-shifted light was
detected and converted to an instantaneous velocity signal using a modified
frequency demodulator (Polytec).

Response velocity signals were low-pass filtered using an 8 pole Butterworth
filter (Frequency Devices) set at 8x the stimulus frequency before being digitised
with a 16 bit A/D converter (Ariel, DSP-16) at a sampling frequency of 64 times
the stimulus frequency. Responses consisting of 32 periods were usually averaged
15 times, from which amplitude and phase of the harmonic component of the fluid
velocity at the stimulus frequency were calculated based on a FFT.

**Radial and longitudinal mapping of fluid flow**

The sense probe and the stimulus sphere were lowered into position as depicted in
Fig. 4. Care was taken not to come into contact with the cupula or canal wall to
avoid damaging the sense probe and to avoid picking up particles.

![Diagram of the supraorbital lateral line canal of the ruffe and experimental set-up.](image)

*Figure 4:* Diagram of the supraorbital lateral line canal of the ruffe and experimental set-up. The lateral canal fluid is driven by the stimulus sphere and oscillates in the longitudinal direction. Radial velocity profiles consist of measurements of the longitudinal velocity of the lateral line fluid flow at different depths above the canal floor. Longitudinal velocity profiles are conducted as a function of distance away from the cupula at a height of 0.2 mm above the canal floor.

Radial profiles were obtained by moving the sense probe up and down with
respect to the fish and measuring the longitudinal velocity of the lateral line fluid. Measurements started at a distance of 75 μm above the canal floor and were
repeated with steps of 0.1 mm to the top of the canal at about 0.8 mm above the
canal floor.

Longitudinal velocity profiles were produced by moving the fish in the
horizontal plane and started at approximately 0.3 mm from the cupula and were
repeated with a step size of 0.05 mm within the close vicinity of the cupula and 0.1 mm further away up to a distance of 1.3 mm. At each measurement position a frequency response was determined usually using 20 stimulus frequencies ranging between 10 and 100 Hz or 70 and 500 Hz.

Radial and longitudinal velocity profile measurements were conducted in separate fishes for each set of profiles. Due to the time needed for mapping the geometry of the canal and carrying out each set of measurements it was not possible to obtain a complete set of radial and longitudinal velocity profiles in the same fish before the canal was filled with slime.

Sense probe fabrication

The sense probe consists of a glass fibre made from the tapered tip of a micropipette pulled from a borosilicate glass capillary with an outer diameter of 1.5 mm and inner diameter of 0.86 mm using a micropipette puller (Flaming Brown P97). Micropipettes were produced to have tips which taper down from \( \approx 20 \mu m \) to \( \approx 3 \mu m \) over a distance of 20 mm. The last 2 mm of the tapered tip (\( \varnothing < 5 \mu m \)), which is extremely flexible, is used for the sense probe.

Measuring the flow with the glass fibre alone would give a weighted average velocity of the flow experienced along the total length of the glass fibre. In order to localise the measurement region to the tip, a small sphere (\( \varnothing 50 \mu m \)) is added to the glass fibre’s tip. This sphere is formed by dipping the glass fibre’s tip into a drop of Epoxy resin (Bison). The size of the resin sphere that is formed at the glass tip is determined by the length of the tip that is inside the resin, which was chosen to be approximately 50 \( \mu m \). Once the resin sphere has been hardened the other end of the glass fibre is glued onto a supporting glass shank.

Sense probe calibration

An ideal probe should move exactly with the flow to be measured, thus having a velocity sensitivity of unity over the frequency range of interest. The probe’s frequency response, however, is high-pass filtered, as a consequence of its hydrodynamic interaction with the fluid. At low frequencies the motion of the probe is attenuated as the probe is viscously driven. Then, the viscous fluid forces on the probe mainly balance the elastic bending force, resulting in the probe having a velocity proportional to the frequency of the oscillatory flow. At relative high
frequencies the probe will move in unison with the fluid, since it has a density which is similar to that of the fluid and is predominantly driven by inertial fluid forces. An estimate of the cut-off frequency of the high pass behaviour can be obtained from an expression derived for the motion of an elastically suspended spherical pendulum driven by an oscillatory viscous fluid flow. The 3 dB cut-off frequency ($f_{co}$) of such a pendulum is proportional to the stiffness $S$ of the glass fibre according to: 

$$f_{co} = \frac{S}{12\pi \mu a} \quad (\text{van Netten, 1991})$$

where $a$ is the radius of the sphere and $\mu$ the dynamic viscosity of the driving fluid. It can be seen that $f_{co}$ can be lowered by decreasing $S$ or increasing $a$. In practice, $a$ is kept small to maintain the required spatial resolution ($\approx 25 \mu m$) while a minimum value of the stiffness, of the order of $10^{-4}$ N/m, is imposed by the construction of the glass fibre but also by avoiding detection of thermal noise (see below). This yields cut-off frequencies in the range between 10 and 100 Hz in water, which covers the lowest frequencies used in this study. In practice, it means that for each individual sense probe a calibration of its frequency response is needed.

The calibration of the sense probe’s frequency response involves measuring the sense probe’s velocity while submerged in a tank of water, in response to a stimulus sphere (Ø 0.68 mm) oscillating at various frequencies with a constant velocity (22 mm/s), placed at a distance of approximately 2.5 mm from the probe. All velocity profiles of the lateral line canal are presented including a correction for the frequency characteristics of the sense probe used.

A typical example of a calibration measurement is shown in Fig. 5. The points show the amplitude and phase of the velocity of the sense probe to a constant velocity flow and confirm the expected high-pass behaviour of the probe. The solid lines depict the calculated frequency response (van Netten, 1991) of the sense probe based on a stiffness $S = 3.6 \cdot 10^{-4}$ N/m and an effective radius $a = 33 \mu m$ ($\mu = 1$ mPa s) and density $\rho = 1000$ kg/m$^3$. The related cut-off frequency ($\approx 90$ Hz) is close to the lowest frequency used in this study (70 Hz). It is clear that correction of the measured data is especially required with respect to the phase at low frequencies.

**Noise of the sense probe**

A physical restriction of the sense probe’s sensitivity to detect low fluid flow velocities is related to the probe’s low stiffness and the thermal motion of the fluid.
An estimate of the probe's rms displacement, \( x \), due to Brownian motion can be obtained using \( x = \sqrt{\frac{kT}{S}} \), where \( k \) is Boltzmann’s constant and \( T \) is the absolute temperature. At room temperature this yields \( x \approx 3.5 \) nm. Using the fluctuation-dissipation theorem and assuming a friction coefficient equal to \( 6\pi a \mu \), the related power density of the sense probe’s velocity can be estimated to be about \( 10^{-15} \) \((m/s)^2/\text{Hz}\) (logarithmically symmetrically) distributed around 250 Hz with a full frequency width at half maximum power of about 700 Hz. This yields an rms velocity of the order of 1 \( \mu \)m/s. This figure is close to the equivalent (rms) noise velocity (\( \approx 1 \) \( \mu \)m/s) of the frequency demodulator used and is also similar to the practically found threshold value of the measuring system.

Figure 5: Measured frequency response of a sense probe (circles) in response to an oscillating sphere producing a constant fluid velocity. A fit to the measured data of an equation describing an elastically coupled spherical pendulum (van Netten, 1991) is depicted by the solid line. Parameters used: glass fibre's stiffness \( S = 3.6 \times 10^{-4} \) N/m; sense probe radius \( a = 33 \) mm; fluid viscosity \( \mu = 1 \) mPa s; fluid density \( \rho = 1000 \) kg/m\(^3\) \( (P_c = 0.64; f_t = 142 \text{ Hz}) \). The related cut-off frequency \( (f_{co} = P_c \cdot f_t) \) is 91 Hz.
Results

Radial flow profile

Radial velocity profiles as a function of height above the canal floor and at several frequencies are shown in Fig. 6 and are representative for the measurement set obtained (N=25). The approximate positions of the stimulus, bony bridge, cupula and projection of measurement points with respect to each other in the particular fish used for the measurement are shown in Fig. 7.

Figure 6: Amplitude and phase of the radial flow velocity and phase profiles measured in the lateral line canal at a distance of \( \approx 0.3 \) mm away from the edge of the cupula for a range of stimulus frequencies.
Close to the canal floor, the flow can be seen to be highly frequency dependent. At low frequencies (70 and 115 Hz), the flow is significantly less than at higher frequencies (> 200 Hz). At a stimulus frequency of 200 Hz there is a flow maximum at a height of approximately 150 µm above the canal floor. The frequency at which a maximum occurred was variable among the fish used and ranged from 180 to 300 Hz. Towards the top (800 µm) of the canal, nearing the open water outside of the fish (free field), all of the profiles converge to a velocity amplitude of approximately 15 to 20 µm/s.

The phase profiles show that the flow leads the stimulus at all frequencies, most significantly at low frequencies. At a frequency of 70 Hz there is a phase lead of 40° for the lower section of the canal. The phase lead increases rapidly with increasing stimulus frequency above 70 Hz. The largest phase leads, up to 90°, were usually observed at intermediate frequencies. At the highest frequency measured (300 Hz) the phase lead is small and the lateral line canal fluid thus flows almost in phase with the stimulus. Moving to positions near the top of the canal (heading towards free field) causes the phase lead to be confined within the range of zero to 20° at all frequencies, showing that at those positions the flow is almost in phase with the stimulus. It can thus be concluded that close to the canal floor the flow deviates most severely from the stimulating fluid, most significantly at the lowest frequencies, while at the top of the canal the fluid flow is in phase with the stimulus fluid flow.

**Frequency response of fluid flow close to the cupula**

Fig. 8 shows more detailed information on the frequency response of fluid velocity close to the cupula and the canal floor. Measurements were made at a distance of 0.3 mm away from the cupular edge, at a height of 0.2 mm above the canal floor in two different fishes. The flow profiles have a plateau of fairly constant velocity
between 70 to 150 Hz. Results at frequencies below 70 Hz were found to be similar to those found at 70 Hz. Amplitudes reach maxima at around 250 Hz. At higher frequencies the velocity amplitude slowly decreases, approaching another constant velocity plateau. There is a significant phase lead at frequencies ranging from 100 to 250 Hz. At higher frequencies (300 Hz) the phase lead becomes small, indicating that the flow is nearly in phase with the stimulus.

To directly illustrate the influence of the cupula on the flow in the lateral line canal, the flow measured in an empty lateral line canal, at approximately the same distance from the stimulus sphere as in the case with the cupula present (Fig. 8), is shown in Fig. 9. The velocity measured is approximately constant (50 μm/s) over the frequency range of 70 to 500 Hz. The flow is in phase with the stimulus from 70-200 Hz; from 200-500 Hz there is a slight phase lead of up to 10°. The constant amplitude and (almost) zero phase are expected in free field, but are also in line with the equations of the Sexl/Womersley profile (Sexl, 1930; Womersley, 1955) in a closed pipe with the dimensions of the canal (⌀ = 1 mm). The cut-off frequency above which the fluid flow in a pipe is in phase with the evoking stimulus flow is given by $f_{co} = \frac{2\mu}{\pi \rho d^2}$, with fluid viscosity $\mu$, fluid density $\rho$ and pipe diameter $d$, which yields about 1 Hz, if applied to the lateral line canal. Therefore, in an empty canal no frequency selectivity is expected within the measurement frequency range.

The comparison with Fig. 8 shows that, especially at low frequencies, the fluid flow in the canal is significantly attenuated by the presence of the cupula. These are the frequencies at which the cupula is known to be only excited slightly and moves less than the evoking fluid flow. The attenuation of the flow at low frequencies can therefore be attributed to the blocking effect of the cupula.

To further investigate the blocking effect of the cupula on the fluid flow, a simplified model was used to describe the frequency responses measured. In the model it is assumed that the flow is the sum of the imposed stimulating fluid flow and the disturbance of a sphere driven by it (see Discussion). The sphere is assumed to mimic the cupula having a frequency dependent velocity as calculated with a model of cupular excitation (van Netten, 1991). The effects of the walls on the flow are neglected in the model.
Figure 8: Amplitude and phase of fluid velocity measured in the supraorbital lateral line canals of two ruffes at a distance of approximately 0.3 mm away from the edge of the cupula at a height of 0.2 mm above the canal floor. Fits to the measured data made with the model described in the text are depicted by the solid lines. Parameters used for the data depicted with the open circles: cupula sliding stiffness $S = 0.163$ N/m, canal fluid viscosity $\mu = 6.66$ mPa s, cupula radius $a = 2.04 \times 10^{-4}$ m and the distance to the cupula centre $r = 2.42 \times 10^{-4}$ m, resulting in $P_c = 39.79$ and $f_t = 25.46$ Hz. Parameters used for the data depicted with the filled circles: cupula sliding stiffness $S = 0.163$ N/m, canal fluid viscosity $\mu = 11.62$ mPa s, cupula radius $a = 1.67 \times 10^{-4}$ m and the distance to the cupula centre $r = 2.15 \times 10^{-4}$ m, resulting in $P_c = 10.69$ and $f_t = 66.25$ Hz.

Figure 9: Amplitude and phase of fluid velocity measured in the supraorbital lateral line canal without cupula at a height of 0.2 mm from the canal floor and at a comparable distance from the stimulus sphere as the case presented in Fig. 8.
The solid lines in Fig. 8 show the results of the model calculations. Fitting the equation of the fluid velocity to the amplitude and phase data sets involves essentially 3 free parameters. If the elastic coupling of the cupula to the canal floor is fixed at 0.16 N/m, according to previous results from independent measurements on cupular mechanics (van Netten, 1991; Wiersinga-Post and van Netten, 1998), the free parameters are: the cupular radius \( a \), the canal fluid viscosity \( \mu \) and the distance between measurement position and the centre of the cupula \( r \). The model gives an accurate description of the two data sets depicted with a cupular radius \( a \) equal to 0.2 and 0.17 mm, with canal fluid viscosity \( \mu \) equal to 7 and 12 mPa s and the distance to the cupula, \( r \), set to 0.24 and 0.22 mm, for respectively the data set represented with open and closed symbols in Fig. 8. The variation in the parameters found are likely to be related to geometrical variations in the dimensions of the lateral line organ. The discrepancy between the values found for \( r \) and the actual distance at which was measured \((\approx 0.9 \text{ mm})\) suggest that the canal has a significant confining effect on the flow measured close to the cupula (see Discussion).

**Longitudinal flow profile**

Longitudinal profiles were measured at a height of 0.2 mm above the canal floor \((N=14)\) and all gave basically the same results. A typical example is shown in Fig. 10. In this particular fish the approximate positions of the stimulus sphere, the bony bridge, cupula and range of measurement points with respect to each other are shown in Fig. 11.

Since at high frequencies \((300 \text{ Hz})\) the cupula is not attenuating the flow, the longitudinal profile measured at this frequency indicates the decay of the stimulus along the length of the canal, if no cupulae were present. For comparison, a curve has been added that shows the theoretically expected reduction of the flow amplitude of a dipole (vibrating stimulus sphere) in free field with respect to the distance to its centre, \( r_{stim} \), which is proportional to \((r_{stim})^{-3}\). Its similarity confirms that neither the cupula nor the canal wall has a significant effect on the fluid flow along the canal at this frequency. It also predicts that with this particular geometry, cupula III, which is approximately positioned at 3 mm from cupula II, would detect about 10% of the stimulus detected by cupula II.
Figure 10: Amplitude and phase of longitudinal flow velocity profiles measured at a height of 0.2 mm above the lateral line canal floor at several frequencies. The theoretical flow velocity as a function of distance from the stimulus ball in free field is also included in the plot and is depicted by the dotted line. The edge of the cupula is located at $x = 0$ and the caudal edge of the bony bridge is at approximately $x = -0.9$ mm.

Figure 11: A diagram of the lateral line canal for the ruffe used for the longitudinal profile measurements close to the cupula. The distances between the stimulus (S), bony bridge (BB), cupula II (C) and the range of the measurement points is indicated by the thick dotted line.

At the lower frequencies it can be seen that the influence of the cupula, via its blocking action on the flow, is present along the whole trajectory measured,
although its amplitude also reduces with distance. This is in accordance with the
phase, which remains relatively close to zero for the high and low frequency
plateaus measured close to the cupula but approaches to phase leads up to about 40°
at frequencies which evoke a phase lead deviating significantly from zero at
positions close to the cupula (see also Fig. 8, frequency response close to cupula).
The reduction along the canal length of the disturbance of the flow caused by the
cupula (at low frequencies) shows that cupula III is only slightly affected by the
presence of cupula II.

**Frequency response close and further away from the cupula**

![Figure 12: Amplitude and phase of flow velocity measured at distances of 0.3 mm and 1.3 mm away from the edge of cupula II. All the measurements were conducted at a height of 0.2 mm above the canal floor.](image-url)

More detailed frequency characteristics of the flow close (0.3 mm from edge of the
cupula) and further away (1.3 mm) are compared in Fig. 12. The difference in the
low and high frequency amplitude plateaus, reminiscent of the presence of the
Discussion

The results presented show that the flow measuring method used, consisting of a flexible sense probe in combination with laser interferometry, enable the *in vivo* measurement of fluid flow in the lateral line canals of fish down to the order of 1 µm/s, with a spatial resolution of the order of tens of micrometers. This method has been utilised to map the fluid velocity along two principal directions in the supraorbital lateral line canal of ruffe.

Several observations have been made that provide information on the effect of the presence of a cupula and the canal wall on the flow of canal fluid. Direct experimental comparison between two experimental situations, in which fluid flow with and without a cupula was measured, show that the cupula influences the flow significantly in the lateral line canal at distances of the order of a cupular diameter (see Figs. 8, 10 and 12). This influence is most significant at frequencies which are below the resonance frequency of the cupula (=130 Hz; Wiersinga-Post and van Netten, 1998). The cupula is only slightly excited at those frequencies and thus blocks the flow of canal fluid. At frequencies above the resonance frequency, stiffness forces of the elastic coupling via the hair cell bundles to the canal floor are negligible, resulting in a neutrally buoyant behaviour in which a cupula mechanically behaves like the canal fluid. This means that at these frequencies the cupula is mechanically transparent to flow in the canal.

From modelling the cupula as a sphere driven by a viscous fluid, an expression was derived that describes the resulting fluid flow along the direction of vibration (longitudinal canal axis). This model is based on the frequency response of the velocity of a cupula, $V_{cup}$, in response to a spatially constant fluid stimulus with velocity $V_{stim}$.
where $S$ is the elastic coupling of the cupula to the canal floor, and $a$ is the cupular radius, while $\mu$ and $\rho$ are fluid viscosity and density (van Netten, 1991). The resulting fluid flow at a distance $r$ from the centre of the sphere is then given by the sum of the spatially constant stimulus fluid flow and the disturbance relative to this stimulus flow as caused by the presence of the sphere of this field:

\[
V(r) = V_{\text{stim}} + \frac{a^3}{r^3}(V_{\text{cup}} - V_{\text{stim}}),
\]

Equation 2 adequately describes the measured data close to the cupula, as shown in Fig. 8. The resulting fit parameters, however, deviate from the real known physical values. The values found from fitting Eq. 2 to the data yields for the viscosity, 7-12 mPa s, which is 5 to 10 times higher than the experimentally determined value (1.3 mPa s at 15 °C, see chapter 2). Also, the range found from fitting for the distance from the centre of the cupula to the point of measurement (0.17 - 0.2 mm) is about a factor 5 lower than the real distance (0.9 mm) (see Fig. 11). This discrepancy for both viscosity and distance can be explained by the effect the canal wall has on the fluid flow. It is known from low Reynolds number hydrodynamics that the drag force exerted by a viscous fluid flowing with velocity $V$ past a rigid sphere in a circular container, as given by Stokes' law, $F_{\text{drag}} = 6\pi a \mu V$, has to be corrected by a factor larger than 1, depending on the distance between sphere and container wall (Happel and Brenner, 1983). This factor can be interpreted as an increase of the viscosity and amounts to about 10 if the canal wall is located 0.3 mm from the sphere’s surface, which is a realistic estimate for the distance between cupular surface and canal wall. Also, the smaller distance found while fitting for $r$ when using a free field model reflects the confining action of the canal on the flow. It seems therefore likely that the influence of the cupula on the flow caudal to it, as
measured at low frequencies, is enhanced by the presence of the canal wall. The
effect of the canal wall on the flow further away from the cupula and bony bridge,
which is the region where the canal is not covered with bone, is less. In fact, in this
region and at high frequencies, the decay with distance is similar to the dependency
($r^{-2}$) found in free field. At these locations the filtering effect of the cupula
(blocking of low frequencies) is still present but substantially reduced. In this
respect, the presence of hydrodynamic windows between cupulae (van Netten and
van Maarseveen, 1994) make the neuromasts in the ruffe’s supraorbital lateral line
canal an array of relatively independent motion sensors. Placed in a closed tube
with the same dimensions, the neuromasts would all detect exactly the same flow
since the fluid velocity in a tube is essentially forced back into the Sexl/Womersley
profile within a distance of a cupular diameter (Meeuwissen, 1994).

The frequency responses of fluid flow, also those measured close to the cupula,
as presented in Fig. 12, differ significantly from the frequency responses as
measured for cupula II (Wiersinga-Post and van Netten, 1998). At low frequencies,
a plateau in velocity amplitude was always found, contrary to the velocity of the
cupula which approaches zero. At high frequencies, both cupula and flow will
attain the same frequency dependent velocity, since then the cupula is mechanically
the same as the fluid. The difference between the velocity plateaus of the fluid
reached at the two frequency limits can be seen from Eq. 2 to equal $V_{stim}(1-a^{2}/r^{2})$
and thus depends only on cupular size and the distance measured at.

Conclusions

It can be concluded that, at frequencies below the resonance frequency of the
cupula, the radial and longitudinal velocity profiles are predominantly determined
by the influence of cupula. At frequencies above the cupular resonance frequency,
the cupular influence diminishes and the flow profiles resemble those found in an
empty lateral line canal.

The decay of the flow velocity as a function of distance along the longitudinal
canal axis suggests that cupula II and cupula III operate relatively independently
from each other, which seems to be related to the presence of the hydrodynamic
windows.
References


