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FEASIBILITY STUDY OF EXPERIMENTALLY  
ESTABLISHING ECOLOGICAL CONTEXT FOR  
LATERAL LINE SYSTEM

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## **CONTENT**

<b>1. EXECUTIVE SUMMARY.....</b>	<b>3</b>
<b>2. FEASIBILITY STUDY OF EXPERIMENTALLY ESTABLISHING ECOLOGICAL CONTEXT FOR LATERAL LINE STUDY .....</b>	<b>4</b>
2.1. A REVIEW OF LITERATURE ON LATERAL LINE MEDIATED BEHAVIOURS .....	4
2.2. METHODOLOGICAL CONSIDERATIONS .....	8
2.3. BEHAVIOURAL EXPERIMENTS.....	10
2.3.1. <i>Source detection using breathing rate as a monitor.....</i>	<i>10</i>
2.3.2. <i>Source localization using approach behaviour.....</i>	<i>14</i>
2.4. SUMMARY AND CONCLUSIONS .....	17
<b>3. REFERENCES .....</b>	<b>18</b>



## 1. EXECUTIVE SUMMARY

A literature research on lateral line mediated behaviours was conducted demonstrating that the lateral line is involved in a variety of behaviours in different fish species, including orientations to water flow, communication, spatial orientation, predatory behaviour, object avoidance and discrimination, and wake following. Of particular interest for the project are behaviours that involve detection and localization of hydrodynamic stimulus sources. Here, substantial knowledge is available for source localization on the water surface whereas the ability of fish to localize subsurface waves sources is less well investigated.

Behavioural experiments were performed in the lab to investigate stimulus detection by fish. In one type of experiments, spontaneous behaviours, here a change in breathing activity in response to a sinusoidally vibrating sphere were recorded in goldfish, *Carassius auratus*, and oscar, *Astronotus ocellatus*, to determine detection thresholds in still water and in flow (velocity 5 cm/s). In 6 out of 8 goldfish and 4 out of 5 oscars, detection thresholds were not affected by the flow. Control experiments in which parts of the lateral line or the entire lateral line was deactivated indicate that the lateral line was not necessary for stimulus detection in this kind of experiment. Most likely fish relied on other information, e.g. input from the inner ear, when the lateral line was not functioning. In another type of experiment, we trained Oscars by food reward to approach a sinusoidally vibrating sphere. Detection was quantified by post-hoc analysis of video sequences recorded during training sessions. To date, video sequences from 2 out of 6 oscars are analyzed. Control experiments in which the lateral line was deactivated suggest that in these experiments, animals relied on sensory input from the lateral line. The pros and cons of the conducted types of experiments are discussed.



## 2. FEASIBILITY STUDY OF EXPERIMENTALLY ESTABLISHING ECOLOGICAL CONTEXT FOR LATERAL LINE STUDY

The objective of this study was to investigate what information is already available on the behavioural capabilities of the fish lateral line, to determine what more might be needed, and how such data might be obtained. To achieve this objective a review on the existing literature was performed and behavioural detection experiments were conducted. The results of this feasibility study outline possible experimental strategies for obtaining systems level data.

### 2.1. A REVIEW OF LITERATURE ON LATERAL LINE MEDIATED BEHAVIOURS

The behavioural relevance of the lateral line has been demonstrated for different species of fish. These studies show that in many species the lateral line is not only involved but perhaps even sufficient to mediate certain behaviours like communication and predation.

#### Orientation to water flows

Like other animals fishes can orient themselves to large-scale water motions, a behaviour that is called rheotaxis. It means that fishes actively or passively orient themselves either positively (upstream) or negatively (downstream) to water flows. In nature this can be observed for instance in fishes holding station in a stream or orienting themselves with respect to tidal currents. However, at present there are no studies investigating the involvement of the lateral line in the orientation to tidal currents.

Laboratory experiments with flow tanks suggest that the lateral line may support rheotaxis in water currents (Baker and Montgomery 1999, Montgomery et al. 1997). However, rheotaxis is not possible without a stationary reference frame that is typically established by visual or physical contact with the substrate. This means that the lateral line alone is not sufficient for rheotaxis but that instead multiple sensory modalities control this behaviour.

The role of the superficial neuromasts of the lateral line system in rheotactic behaviour has been demonstrated in a small number of species (Antarctic notothenioid, *Pagothenia borchgrevinki*, torrentfish, *Cheimarrichthys fosteri*, and blind cavefish *Astyanax fasciatus*, Montgomery et al. 1997; Port Jackson shark, *Heterodontus portusjacksoni*, Peach 2001; mottled sculpin, *Cottus bairdi*, Kanter and Coombs 2003). For instance, in *Pagothenia*, a fish that sits on the substrate and turns into the flow as current speed increases, the threshold current speed for rheotaxis increases following selective ablation of the superficial neuromasts (Montgomery et al. 1997). However, at high flow velocities these fish show normal rheotactic behaviour. Selective blockade of canal neuromasts has no effect on rheotaxis thresholds. This suggests that superficial neuromasts provide information about water flows over the surface of a fish's body and thus contribute to the control of swimming, mediating, for instance, the dynamic modulation of fin reflexes as a function of flow speed, or the active control of boundary layer flows (Anderson et al. 2001).



## Hydrodynamics of Swimming

In streams fishes will experience complex flow patterns that contain valuable information that can be interpreted by the lateral line system. Flow refuges (e.g., those generated by hollows, bottom contours, or obstructions, such as boulders) are prime sites where a fish can hold station at little metabolic cost yet remain close to the flow to pick up food carried in the drift. Hydrodynamic features, such as shear gradients or turbulence may provide information to the lateral line system that will allow a fish to select and optimize the use of these locations. When a cylindrical obstacle is placed across the flow in a flume, a trout will position itself either in front of or behind the cylinder in a way which reduces the metabolic cost of swimming. In front of the cylinder it is able to hold position against the flow with minimal effort. Behind the cylinder, the fish alters its body kinematics to synchronize with the shed vortices in a way that suggests it can capture energy from vortices generated by the environment to maintain station in downstream flow (Liao et al. 2003). This behaviour has been termed a “Karman gait” after the name of the vortex street that is shed by a cylinder in a flow. The lateral line system has been implicated in obstacle entrainment (Sutterlin and Waddy, 1975) and may involve both the superficial and canal neuromast submodalities (Montgomery et al. 2003), but the precise role of the lateral line system in organizing the Karman gait has yet to be determined.

## Communication

While sound production and acoustic communication are well documented across a wide range of fish species, the role of hydrodynamic signals in communication and the potential use of the lateral line in the interpretation of such signals is only poorly studied. Two examples for which the use of hydrodynamic signals for communication has been documented are mating interactions and schooling communication. During reproductive behaviour many fish species exhibit body vibrations which cause water flows that stimulate the mating partner (e. g. Keenleyside 1979, Matsushima et al. 1989, Satou et al. 1991a, Satou et al. 1991b). In the hime salmon, *Oncorhynchus nerka*, the lateral line plays a major role for the detection of these hydrodynamic signals during spawning (Satou et al. 1994a, Satou et al. 1994b, Satou et al. 1991b, Satou et al. 1995). The lateral line is also used by schooling fishes like herring, *Clupea harengus*, (Gray and Denton 1991) to match changes in velocity and heading of at least the closest neighbours (e. g. Partridge and Pitcher 1980, Pitcher 1979, Pitcher et al. 1976). Finally, Weeg and Bass (2002) provide evidence that acoustic vocalizations by the plain midshipman (*Porichthys notatus*) may stimulate the lateral line system in addition to the inner ear, thus allowing multimodal interpretation of these signals.

## Spatial orientation

The role of the lateral line for spatial orientation has been studied extensively in the blind cave fish (reviewed in Bleckmann 1994). Blind cave fish can detect, avoid and discriminate between stationary objects while gliding along them (e.g. Campenhausen et al. 1981, Hassan 1989, Weissert and Campenhausen 1981). For instance, blind cave fish can discriminate the distance between vertical bars with a precision of 10 % (Hassan 1986). As fishes glide forward they generate a high-pressure bow wave in front of them, and the water that is pushed out of the way flows around the fish's body into the low-pressure area behind it, thus generating a dipole-like flow field (Hassan 1985, 1989). The low-frequency water velocity and pressure alterations generated by swimming or gliding fish depend on swimming



velocity and acceleration as well as body size and shape. As a fish approaches or passes a stationary object, the flow field and the pressure distribution over the body's surface are altered, thus allowing the lateral line receptor array to detect flow distortions and provide information on the nature of that object (Campenhausen et al. 1981, Hassan 1985, 1989, 1992a,b). When gliding past an object, the water velocity between an object and a fish is increased locally and this local increase travels along the fish's body as a transient event (Hassan 1985). As the distance between the object and fish decreases, the amplitude of the transient event increases, and its spatial extent decreases. As object size increases, its amplitude also increases. By measuring the spatial extent and size of such transient events, Hassan (1985) showed that the lateral line system may provide a fish with precise information about the size and distance of the object. This allows a fish to use a sort of 'hydrodynamic imaging' process (Weissert and von Campenhausen 1981, reviewed by Montgomery et al. 2001) where the lateral line is employed in an "active mode", similar to electrolocation by weakly electric fish. More recently it was shown that blind cave fish can establish a cognitive map of their environment based on the same principles of hydrodynamic imaging with the lateral line that underlie object discrimination (de Perera 2004a, b, 2005).

### **Moving object discrimination**

Hydrodynamic stimuli that are generated by a moving source are highly complex. In terms of particle velocity, the water motions that are caused by a passing object consist of a reproducible transient component followed by an ill-defined, long-lasting wake (Mogdans and Bleckmann 1998). The associated pressure changes and the corresponding pressure gradients that are generated by a moving object are prominent only as the object passes by the receptor, but are negligible in the object's wake. It has been shown that fishes can detect a moving sphere (e.g., Janssen 1996). Using a breathing rate conditioning paradigm, Vogel and Bleckmann (2003) showed that goldfish can discriminate between the water motions that are produced by an object that is moved with different speeds or in different directions (from anterior to posterior or opposite) along the side of the fish. Fish were also able to discriminate between the water motions generated by objects of different size or shape. When the lateral line was blocked with Cobalt-chloride, animals did not discriminate object motion direction, speed, shape or size. These are the first data of its kind to demonstrate that fish can discriminate, at least under still water conditions, between complex water motions opening up the possibility that fishes may analyse complex wave patterns to obtain information about certain aspects of the wave source, and that this ability requires input from the lateral line.

### **Predatory behaviour**

Animals generate water flows passively as a by-product of ventilation, feeding, and locomotion, and actively as the result of muscle contractions, which result in body and fin movements. All of these flows are sources of stimuli for the lateral line system of piscine predators. The role of the lateral line system in feeding and predation has been studied extensively, but these studies have tended to concentrate on fishes from these habitats, especially more accessible nocturnal species. The relative importance of the lateral line system increases at night and in low light habitats (e.g., in the deep sea, in caves, or at high latitudes, where light intensities are low or day length is short).



The overall predation strategy employed by a fish depends on the nature of the target and the combination of sensory modes employed. For pure lateral line-mediated predation, stationary (but vibrating) targets allow a saltatory approach, where the predator appears to sample the available field and then moves closer to the target in a series of steps before aiming its strike (e.g., mottled sculpin, *Cottus bairdi*, Coombs et al. 2001). Ventilatory currents generated by stationary prey may result in significant hydrodynamic flows that can be detected by the lateral line system of dwarf scorpionfish (*Scorpaena papillosus*), which use them to track and strike at their source (Montgomery and Hamilton 1997). When pursuing fast swimming planktonic prey, the lateral line system of a fish must provide sufficient information to determine and intercept the prey's locomotory trajectory. When feeding on mobile prey, the predator may encounter and then follow a turbulent wake generated by the swimming motion of the prey (Hanke et al. 2000). Such hydrodynamic trail following has been demonstrated in a nocturnal catfish (Pohlmann et al. 2001, 2004). Other evidence from trout (*Onchorhynchus mykiss*) shows that prey tracking depends on both superficial and canal neuromasts and may be accompanied by chemical trails, which may be sufficient to allow predators to track moving prey (Montgomery et al. 2003).

### Wake following

During steady locomotion a subundulatory swimming fish generates a trail of vortices (Blickhan et al. 1992). The basic frequency of this vortex trail is equal to the tail beat frequency of the fish but additional high frequency components up to at least 100 Hz can be measured in such a vortex street (Bleckmann et al. 1991). The hydrodynamic trail that is left in the water by a swimming fish may last for up to several minutes (Hanke et al. 2000) and contains information about direction, velocity and swimming style of a swimming fish (Hanke and Bleckmann 2004). This raises the possibility that predatory fish can track fish-generated wakes by analyzing these trails with the lateral line system. In agreement with this idea, Pohlmann et al. (2001) showed that piscivorous cat fish (*Siluris glanis*) can detect and follow the trail generated by a prey fish (guppy, *Poecilia reticulata*) under infrared illumination.

### Source localization on the water surface

Source localization has been studied extensively in animals that feed on the water surface (review see Bleckmann et al. 1989). One of the best studied examples is the clawed frog *Xenopus*, who has a lateral line system that consists exclusively of superficial neuromasts and allows the animal to precisely localize and target prey items (Claas et al. 1989, Elepfandt 1985, 1989a, b). Similarly, the topminnow *Aplocheilichthys lineatus*, and the African butterflyfish *Pantodon buchholzi*, feed on insects that fall on the water surface. Each has a specialized head lateral line system that is able to determine the direction of a wave source from the intervals between the arrival times of a surface wave at different head neuromasts (Tittel et al. 1984; Müller and Schwartz 1982). Moreover, these fish can determine the distance to a wave source from the curvature of the concentric surface wave, its frequency composition, and the frequency modulation of the first seven to eight wave cycles that arrive at the fish (Hoin-Radkovski et al. 1984; Bleckmann 1988, 1993; Bleckmann and Topp 1981; Bleckmann and Käse 1987; Bleckmann et al. 1981). *Aplocheilichthys lineatus* can discriminate between surface wave frequencies (Mogdans et al. 2002) and is highly sensitive to frequency changes within a wave stimulus but is unable to discriminate pure sine wave stimuli from amplitude-modulated sine wave stimuli (Vogel and Bleckmann 1997).



### Subsurface source localization

Whereas the sensory cues available to the lateral line for locating wave sources on the water surface are well known (review Bleckmann et al. 1989a), much less is known on how the lateral line might be used in locating subsurface wave sources. There is evidence from several fish species that they can detect the hydrodynamic stimuli produced by planktonic organisms (e. g. Enger et al. 1989, Hoekstra and Janssen 1985, Montgomery 1989, Montgomery and Coombs 1998, Poulson 1963). There is also clear evidence that piscivorous fish can detect prey fish with the lateral line (Enger et al. 1989). More recent experiments suggest that predatory fish can detect and follow the hydrodynamic traces generated by swimming prey fish (Pohlmann et al. 2001).

Laboratory experiments with vibrating spheres have shown that fishes can detect and localise a stationary subsurface dipole source (e. g. Coombs 1994, Coombs and Janssen 1990) and discriminate between vibration frequencies (Frühbeis 1984). The lateral line system appears to be necessary if not sufficient for this task (Hoekstra and Janssen 1985, Janssen 1990). Observations of the approach paths of mottled sculpin to a vibrating sphere combined with a description of the flow field around the dipole and the description of the responses of primary lateral line afferent fibers to changing dipole locations support the hypothesis that spatial excitation patterns along the lateral line system play a role in encoding both direction and distance of a dipole source (Coombs and Conley 1997a, Coombs and Conley 1997b). This assumption is further supported by lesion studies in which distinct parts of the lateral line of mottled sculpin were unilaterally denervated (Conley and Coombs 1998). Lesioned animals were still able to approach and localise a sinusoidally vibrating sphere. However, site-specific deficits in the ability of animals to make accurate orienting responses were found. For instance, when the head lateral line was denervated animals were less accurate in orienting when the sphere was located on the denervated side of the head. These results support the notion that sculpins rely on a point-by-point spatial representation along the sensory surface of the lateral line for source localisation.

To date only one study quantitatively investigated detection and orientation towards a hydrodynamic wave source in the presence of a background water flow (Kanter and Coombs 2003). In this study, mottled sculpin were trained by food reward to approach a stationary sphere vibrating at 50 Hz. Sculpins were able to detect relatively weak signals (estimated to be approx. 0.001– 0.0001 peak–peak cm/s at the location of the fish) in the presence of strong background flows (2 -8 cm/s), and signal levels at threshold increased by less than twofold for a fourfold increase in flow velocity. Thus detection of dipole stimuli is only marginally affected by a background flow. These results are consistent with the idea that lateral line canals behave as high-pass filters to effectively reject low frequency stimuli such as those provided by slow dc currents.

## 2.2. METHODOLOGICAL CONSIDERATIONS

Methodical aspects need to be considered if one wants to conduct experiments on the behavioural capabilities of the fish lateral line in the laboratory. One important aspect is the choice of stimulation. In the past, two types of hydrodynamic stimulus source have been used, vibrating spheres (dipole sources) generating sinusoidal water motions and, moving objects generating more complex water motions.

The advantages of a stationary vibrating sphere are that water motions are easy to generate, stimuli are highly reproducible, the corresponding flow fields are well described in



mathematical terms (Kalmijn 1989) and thus can be easily modelled, and that afferent nerve fibres respond to the stimuli in a reliable and predictable way. Moreover, the pressure gradient pattern generated by a vibrating sphere provides unambiguous information about the location of the source that is reflected in the excitation patterns of the peripheral lateral line (Coombs et al. 1996, Curcic-Blake and van Netten 2006). Thus, information about the location of a sinusoidally vibrating sphere is available to the lateral line at least at the level of the afferent nerve fibres.

Vibrating spheres are intended to mimic the oscillatory water motions produced by small prey animals like crustaceans. Pure sine waves, however, are rare in nature and this is why experiments involving vibrating spheres have been criticised in the past. In contrast to dipole sources, moving objects generate more complex water motions (Mogdans and Bleckmann 1998) that resemble in many aspects those of swimming fish and therefore appear more natural. However, such stimuli are more complicated to generate, less reproducible, difficult to describe in mathematical terms and therefore difficult to model. There is no report that fish have been trained to approach a moving source, even though there is evidence that fish can discriminate between moving sources (Vogel and Bleckmann 2003) and that fish can follow the wake generated by a moving fish (Pohlmann et al. 2001).

A second aspect that needs to be considered is the choice of the species. This depends on the kind of question one wants to ask, for instance whether one wants to compare lateral line systems of different peripheral morphology, or species from different habitats. However, not all species may be suited for a particular kind of experimental procedure. For instance goldfish have a well-developed lateral line system that is well described both in terms of morphology and neurophysiology, but yet the fish can hardly be trained to approach a hydrodynamic stimulus source. Perhaps this is due to the fact that goldfish are not active predators. Other species that are hard to be trained in source localization experiments are those that swim around continuously to monitor their environment, like blind cave fish. These fish are more useful for spatial orientation tasks. Active approach behaviour is studied best in species that exhibit unconditioned orienting responses to vibratory sources, like the mottled sculpin, a North American species from Lake Michigan, that is extensively studied by S. Coombs (see literature in Annex). But even if a species can be conditioned to approach a vibrating sphere, the experiments usually are very time consuming requiring enormous sensibility and patience by the experimenter. Therefore such experiments will not produce a large amount of data in short time. If fish cannot be trained to perform active orienting responses, alternative methods need to be used. These include the recording of conditioned and/or unconditioned autonomous responses like changes in heart rate and breathing activity in response to hydrodynamic stimulation (e.g. Vogel and Bleckmann 2003, Lu et al. 1996, but see discussion below).

Another important aspect is the interpretation of behavioural data on hydrodynamic stimulus detection. Fish can detect relative movements between themselves and the surrounding water not only with the lateral line but also with the inner ear. Lateral line receptors are low-frequency sensors with an operating range from less than 1 Hz up to about 150 Hz. This overlaps at least partially with the low-frequency end of the hearing range of many fish species. Thus, in principle, localization and identification of hydrodynamic stimuli could be made with one or both of these systems. To distinguish between lateral line and inner ear mediated behaviour, standard methods have been established in the literature. To test for a participation of the lateral line, fish are kept in Calcium-free water containing cobalt-chloride.  $\text{CoCl}_2$  is known to block selectively and reversibly the lateral line receptors (Karlsen and Sand 1987). Thus, if fish that have previously responded to hydrodynamic stimuli do not



continue to respond after  $\text{CoCl}_2$  treatment, it is assumed that the lateral line is necessary for the behaviour under investigation. If, in contrast, fish continue to respond after  $\text{CoCl}_2$  treatment, then the behaviour can be mediated by the inner ear but this does not rule out that the lateral line may also play a role. However, the use of  $\text{CoCl}_2$  in high dosages has been criticised since this may cause unspecific effects and even lead to the death of the experimental animals (Janssen 2000). Thus one needs to monitor carefully the general behaviour of the treated animals, like feeding and swimming, that should not be affected by  $\text{CoCl}_2$  treatment. Finally, a method to distinguish whether a behaviour is mediated by superficial or canal neuromasts, is to treat fish with gentamycin (Song et al. 1995). This antibiotic is known to selectively and reversibly destroy hair cells of canal but not superficial neuromasts and is therefore a nice tool to distinguish between the two lateral line subsystems.

## 2.3. BEHAVIOURAL EXPERIMENTS

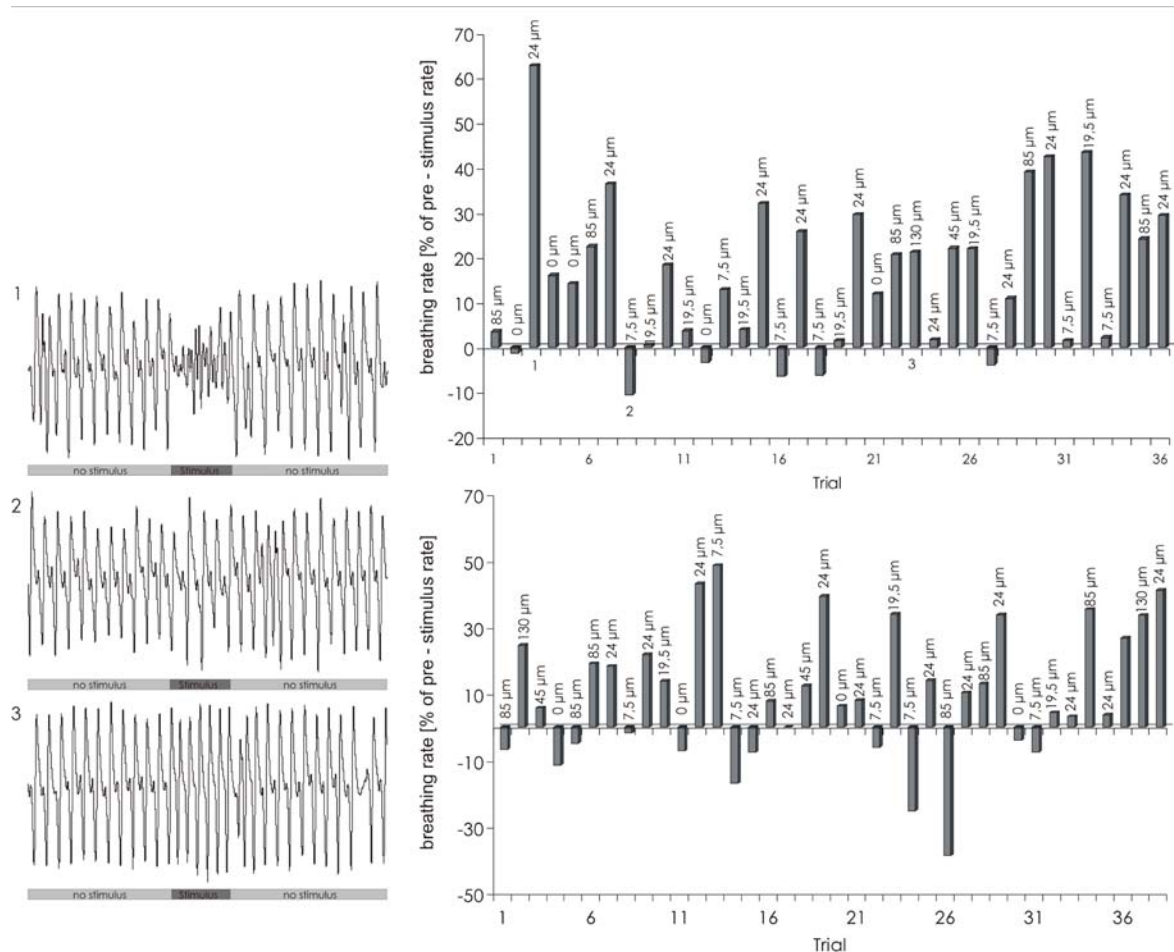
### 2.3.1. Source detection using breathing rate as a monitor

We investigated the ability of fish to detect hydrodynamic stimuli. We used goldfish, *Carassius auratus*, and oscars, *Astronotus ocellatus*, two species that differ in lateral line and inner ear morphology. The goldfish has a lateral line that consists of a well-developed canal system and an abundance of superficial neuromasts (see WP 1.1, Task 1.1.1). In addition, the goldfish is a so-called hearing specialist. Its inner ear is connected to the swimbladder via a chain of ossicles resulting in a high sensitivity of the inner ear to a broad range of frequencies. In contrast, oscars have a lateral line system that is comprised of a canal system but only few superficial neuromasts. Moreover, oscars are hearing generalists without special hearing aids and therefore their inner ear has a lower sensitivity and is restricted to a low-frequency band.

We recorded breathing activity in stationary but unrestrained fish by monitoring the movements of the opercula with a piezo element. Both goldfish and oscars responded to the presentation of a sinusoidal wave stimulus (100 Hz stimulus generated by a vibrating sphere of 10 mm diameter) with changes in breathing activity. These changes were used as an indicator that the fish had actually detected the stimulus. However, changes in breathing activity were small and, at least in goldfish, highly variable across stimulus presentations (Figure 1). In order to obtain more reliable responses we tried to establish a classical conditioning paradigm in which we paired the vibrating sphere stimulus with an electric shock. Electric shocks were assumed to elicit unconditioned changes in breathing rate and were used in previous classical conditioning studies to determine thresholds and directionality of the goldfish inner ear (e.g. Lu et al. 1996, Mann et al. 1997, Yoshida et al. 2004). In our experiments, none of the animals ever responded to an electric shock with a change in breathing activity even at shock amplitudes that caused twitches of the trunk musculature. Consequently the animals could not form an association between the vibrating sphere and the electric shock. We have no explanation why this procedure was not successful in our experiments, even though we used the same experimental procedures and the same species that were used by other scientists. However, conditioning to an electric shock supposedly is not possible with every individual of the same species (Yoshida, personal communication). We also used other stimuli (acoustic noise, vibrations to the fish tank, lights on, moving shade) but none of these stimuli caused predictable changes in breathing activity and therefore could not be used to condition our animals. We therefore decided to conduct



our experiments with spontaneously evoked changes in breathing rate. Although small and variable (Figure 1), these changes allowed us to measure detection thresholds in still water and in the presence of a 5 cm/s background water flow.



**Figure 1: Variability of the responses of a goldfish (fish F55) to a sinusoidally vibrating sphere (100Hz). Left: original recordings traces showing breathing activity prior to, during and after stimulus presentation. Note that the fish responded differently in different trials. (1) Change in breathing rate and amplitude, (2) decrease in breathing rate, (3) increase in breathing rate. Right: Percent change in breathing rate over the course of an experimental session. Upper graph: responses in still water. Lower graph: responses in running water (5 cm/s flow velocity). Numbers represent displacement amplitudes of the sphere.**

We determined detection thresholds in eight goldfish and five oscars under still- and running water conditions with respect to the displacement amplitude of the vibrating sphere. In still water thresholds of individual animals ranged between 7.5 µm and 45 µm (goldfish) and between 7.5 µm and 24 µm (oscar). In running water comparable threshold values were obtained: 7.5 µm to 85 µm for goldfish and 7.5 µm to 24 µm for oscars. In 6 out of 8 goldfish and in 4 out of 5 oscars detection thresholds under still- and running water (flow velocity 5 cm/s) conditions were not different from each other (Mann-Whitney U-test,  $p > 0.05$ ). Thus, in most animals running water did not impair detection. Two goldfish and one oscar, however,



did not respond to the vibrating sphere stimulus in running water suggesting that these animals were not detecting the vibrating sphere in running water. It is not clear why these individuals behaved different than most of our experimental animals.

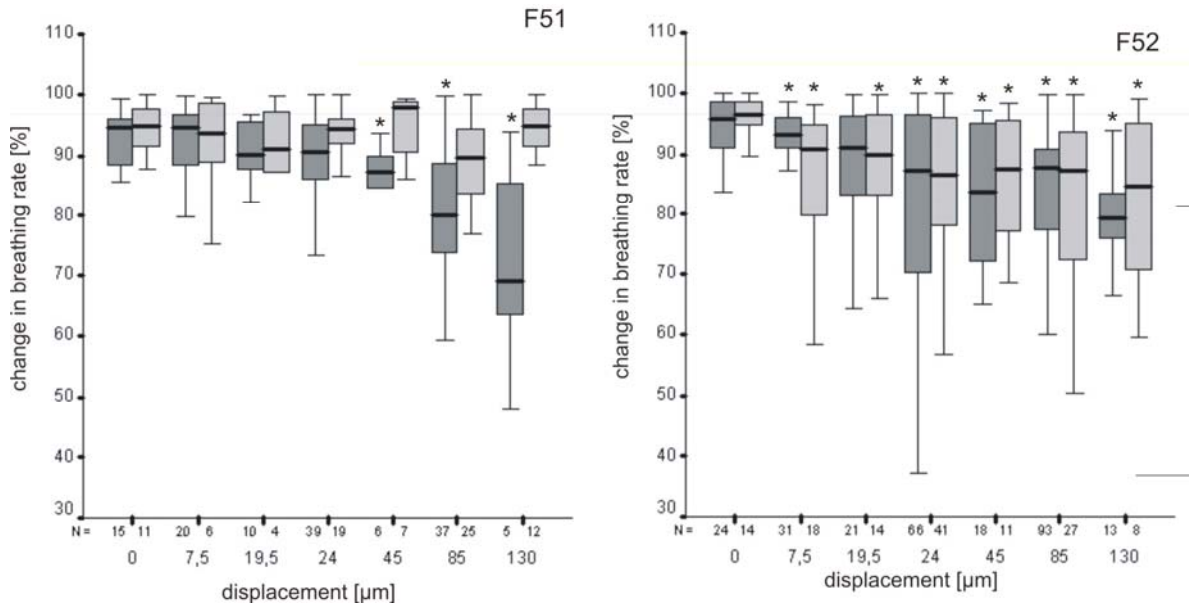


Figure 2: Responses of two goldfish to a vibrating sphere in still water (dark grey boxes) and running water (light grey boxes) (flow velocity 5 cm/s). Asterisks indicate changes in breathing rate that were significantly different from spontaneous changes without stimulus presentation (blank trials, represented by 0  $\mu\text{m}$  displacement) (Mann-Whitney U-test,  $p < 0.05$ ). Boxplots show medians and percentiles.

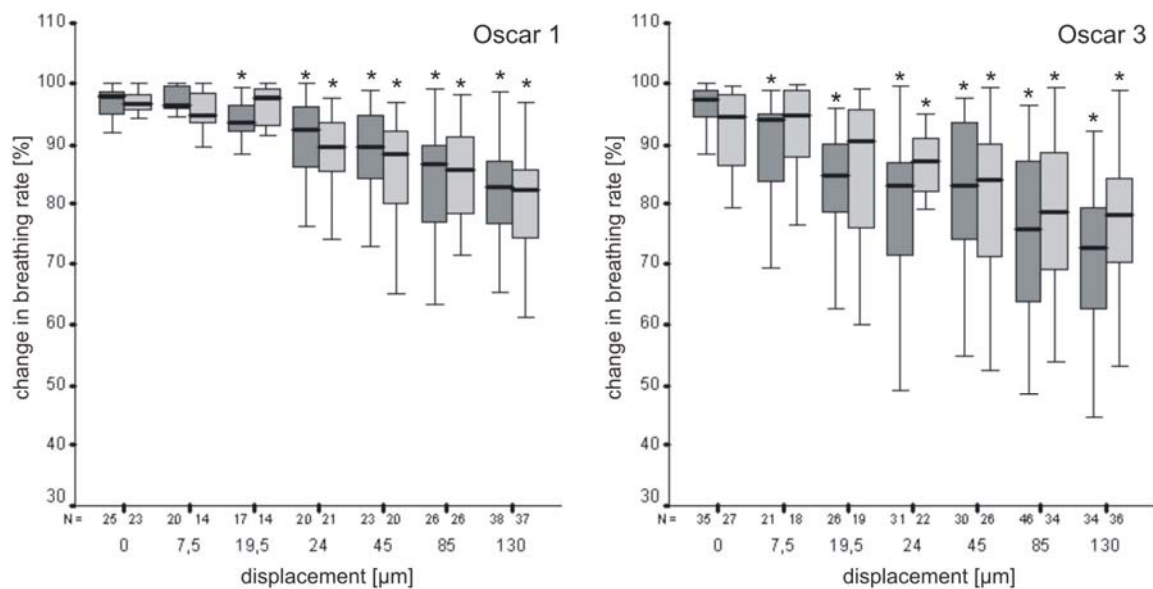
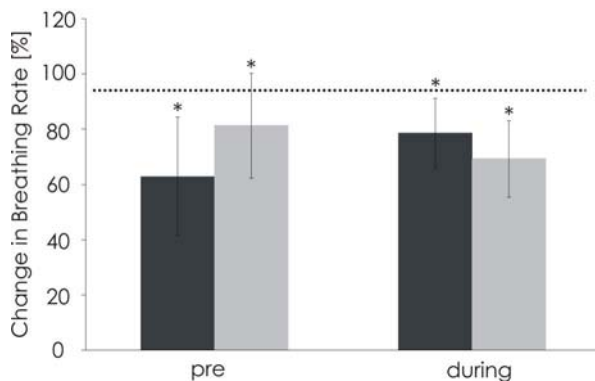


Figure 3: Responses of two oscars to a vibrating sphere in still water (dark grey boxes) and running water (light grey boxes) (flow velocity 5 cm/s). Asterisks indicate changes in breathing rate that were significantly different from spontaneous changes without stimulus presentation (blank trials, represented by 0  $\mu\text{m}$  displacement) (Mann-Whitney U-test,  $p < 0.05$ ). Boxplots show medians and percentiles.



One explanation for our results is that the background flow was not strong enough to mask hydrodynamic detection by the lateral line system. However, at threshold the displacement amplitudes of the sphere were about 20  $\mu\text{m}$ , which corresponds to as little as 0.4  $\mu\text{m}$  displacement on the fish surface. At 100 Hz this corresponds to a velocity amplitude of 0,013 cm/s. The background flow velocity exceeded this value by a factor of more than 300. Another explanation for our results is that fish detected the stimulus in running water not with the superficial neuromasts but with the lateral line canal system. To test this possibility we conducted experiments in which canal neuromasts of three goldfish and one oscar were selectively and reversibly blocked with gentamycin (for methodology see Song et al. 1995). All animals responded to the stimulus before and after treatment under both still- and running water conditions. There was no difference in the magnitude of the response before and after gentamycin treatment.

In addition to the gentamycin experiments, we reversibly blocked the entire lateral line system of one goldfish with 0.1 mmol/l cobaltchloride solution (for methodology see Karlson and Sand 1987). As the animals in the gentamycin experiments, this animal responded to the stimulus before and after treatment under both still- and running water conditions. There was no difference in the magnitude of the response before and after cobaltchloride treatment.



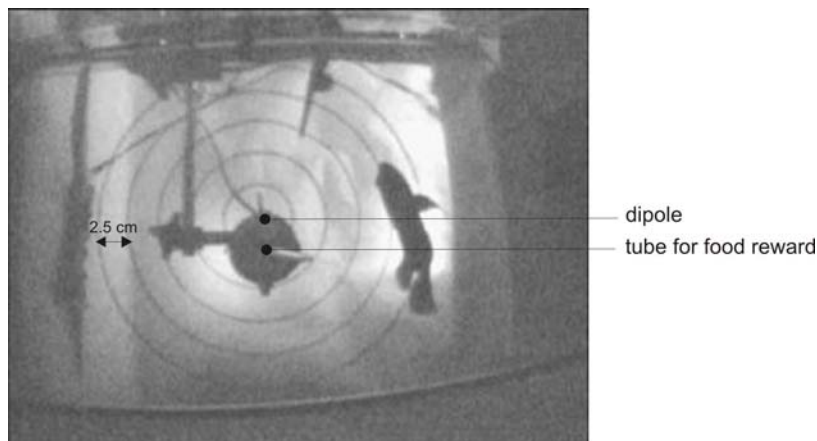
**Figure 4: Percent change in breathing rate of a goldfish before (left columns) and after (right columns) treatment with cobaltchloride. Asterisks indicate changes in breathing rate that were significantly different from spontaneous changes without stimulus presentation (blank trials) (Mann-Whitney U-test,  $p < 0.05$ ). Bars show mean and standard deviations of the responses in still water (dark grey) and running water (light grey) (flow velocity 5 cm/s).**

The fact that animals responded with a change in breathing rate to a vibrating sphere stimulus after gentamycin and cobaltchloride treatment does not rule out the possibility that the lateral line is used for stimulus detection when available. However, the results also indicate that the lateral line is not necessary at least in this kind of experiment and that the fish in addition used other sensory systems, most likely their hearing system for stimulus detection. Thus, with this experimental approach the relative contribution of the lateral line for stimulus detection apparently cannot be determined.



### 2.3.2. Source localization using approach behaviour as a monitor

In a second strand of experiments, we investigated the ability of oscars, *Astronotus ocellatus*, to localize hydrodynamic stimuli using active approach behaviour as a monitor. Six oscars were trained by food reward to approach a stationary vibrating sphere (radius 5mm). Stimulus duration was 3 seconds with a frequency of 100 Hz and sphere displacements of up to 301  $\mu\text{m}$  in a vertical axis. Fish were swimming unrestrictedly in the tank (Figure 5). Stimuli were delivered when the distance between fish and sphere was at least 2.5 cm. Positive responses were defined as a turning of the fish towards the sphere during stimulus presentation. To find out whether turning towards the sphere was elicited by the vibration and not due to spontaneous changes in fish swimming direction or to unintentional hints given by the experimenter, blank trials were interspersed between stimulus trials. During blank trials, the experimenter performed all actions as during stimulus trials except that the sphere was not set into vibration (cable to the vibrator was disconnected).



**Figure 5: Experimental setup for training experiments with Oscars, *Astronotus ocellatus*.** The picture was taken with the video camera through a mirror that was positioned at an angle of 45° below the experimental fish tank. The dark shadow in the middle is the vibrator that is mounted to a support and located well above the water surface. Since the videos were taken under infrared illumination the resolution is not good enough to show the vibrating sphere. Therefore the sphere is represented in this picture by a black dot drawn into the shadow of the vibrator and labelled “dipole”. A second black dot below the dipole represents the opening of a tube through which the food reward was delivered to the fish. Concentric circles were drawn on the bottom of the tank representing distances of 2.5, 5, 7.5, 10 and 12.5 cm to the sphere.

The experiments were conducted in the dark (infrared illumination) and filmed with a video camera. Video sequences were analysed offline to verify and quantify ad-hoc decisions by the experimenter during the training sessions. To date, video sequences of three oscars have been completely analyzed and those of three additional oscars still need to be analyzed. Figure 6 shows the turning and approach behaviour of oscar 2 towards the vibrating sphere.

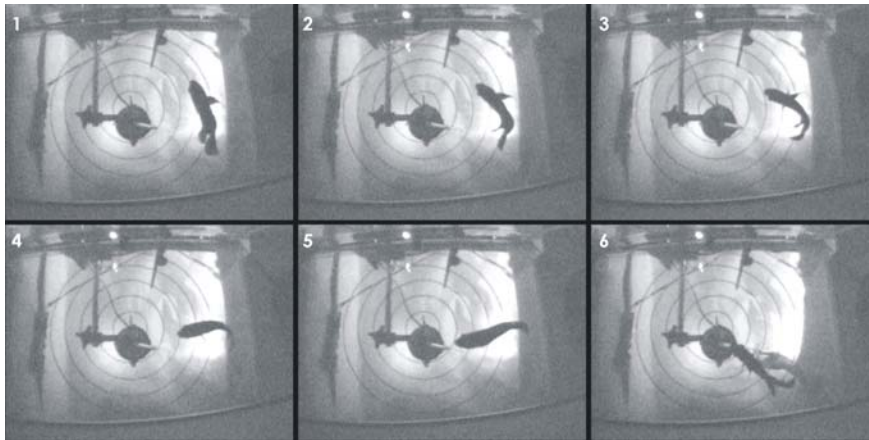


Figure 6: A video sequence (1-6) of the turning and approach behaviour of oscar 2.

From the videos fish to source distances and fish turning angles were determined. From these data displacement amplitudes of the water at the location on the fish surface that was closest to the source were calculated and grouped into classes (Table 1). Fish turning angles of 30 degrees or greater were assumed to reflect detection of source vibration. This measure was used for a post-hoc assessment of stimulus detection (Figure 7).

Class	Displacement [ $\mu\text{m}$ ]	Class	Displacement [ $\mu\text{m}$ ]	Class	Displacement [ $\mu\text{m}$ ]
1	< 0,003	10	0,022 - 0,024	19	0,055 - 0,060
2	0,003 - 0,008	11	0,024 - 0,026	20	0,060 - 0,065
3	0,008 - 0,001	12	0,026 - 0,028	21	0,065 - 0,070
4	0,001 - 0,012	13	0,028 - 0,030	22	0,070 - 0,080
5	0,012 - 0,014	14	0,030 - 0,035	23	0,080 - 0,090
6	0,014 - 0,016	15	0,035 - 0,040	24	0,090 - 0,10
7	0,016 - 0,018	16	0,040 - 0,045	25	0,10 - 0,2
8	0,018 - 0,020	17	0,045 - 0,050	26	0,2 - 0,3
9	0,020 - 0,022	18	0,050 - 0,055	27	0,3 - 0,45

Table 1: Classes of water displacements used for post-hoc analysis of behavioural data

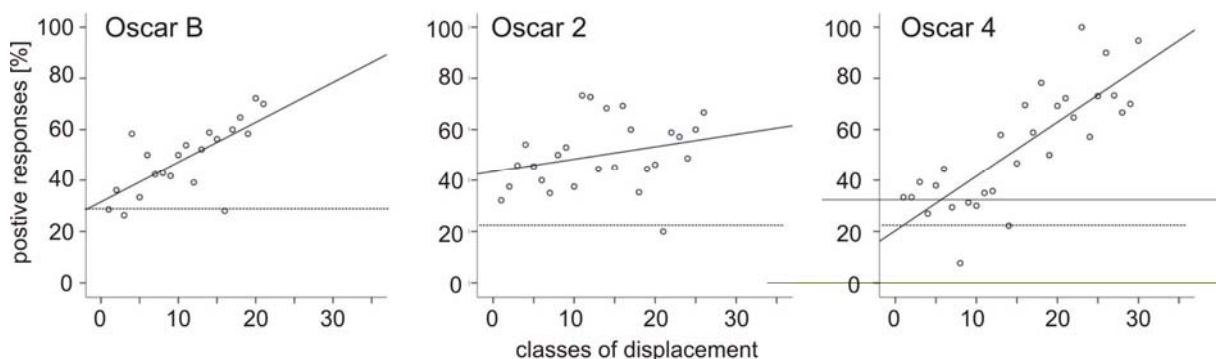
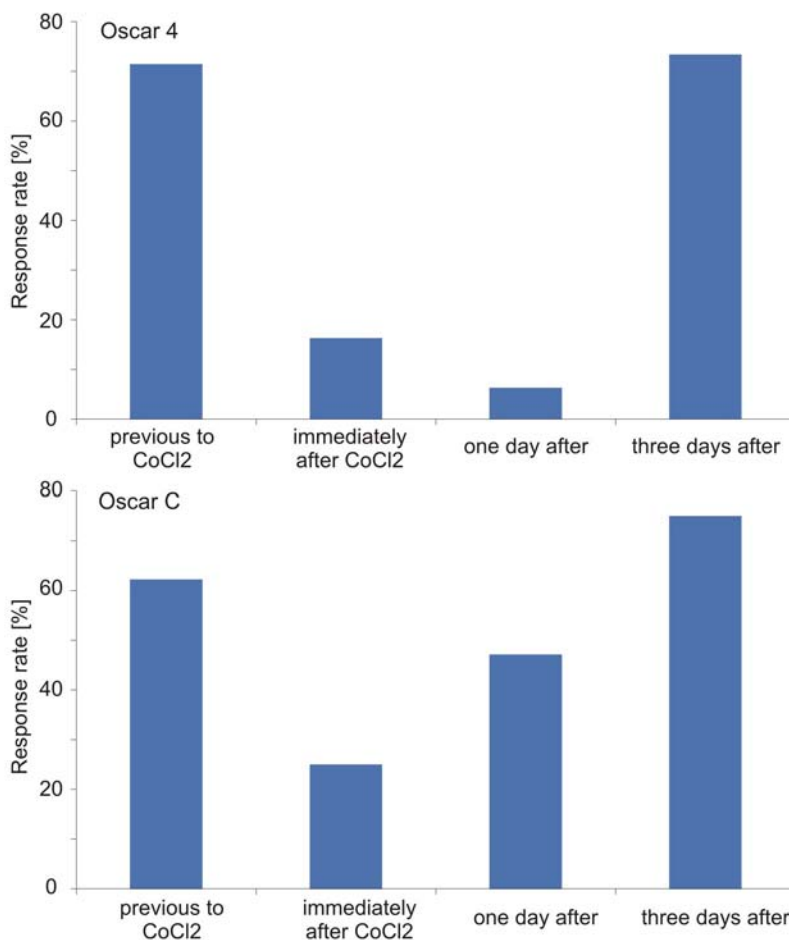


Figure 7: Behavioural assessment of the detection of a sphere vibrating at 100 Hz by three oscars, *Astronotus ocellatus*. Percent positive responses (turning angle  $\geq 30^\circ$ ) are plotted as function of classes of water displacements on the fish surface. Linear regressions (black lines) fitted to the data. Horizontal stippled lines represent percent responses to blank trials (no stimulus presented).



In order to find out whether the oscars used their lateral line to detect the vibrating sphere, we reversibly blocked the entire peripheral lateral line system of two oscars with 0.1 mmol/l cobaltchloride solution (see Karlsten and Sand 1987). In both animals the percentage of positive responses declined after treatment with  $\text{CoCl}_2$  (Figure 8). Before treatment the oscars exhibited 62% (oscar C) and 71% (oscar 4) correct responses to the vibrating sphere. After 24 hours in the  $\text{CoCl}_2$  - solution the response rate decreased to 25% and 16%, respectively. After another day (in fresh water) response rates were still low at 45% and 6.25%, respectively. Three days after treatment the percentage of correct responses had increased to 75% and 73%, values that are comparable to (for oscar C even greater than) those before treatment. These data suggest that in this kind of experiment the animals relied on lateral line input for stimulus detection. One possible explanation why  $\text{CoCl}_2$  affected the animals here but not in the breathing rate experiment is that in this experiment the animals not only had to detect but also to localize the source in order to approach it and receive a food reward. Perhaps the array of lateral line receptors provides the animal with better resolution for source localization in the near field than the inner ear.



**Figure 8: Detection of a sphere vibrating at 100 Hz by two oscars, *Astronotus ocellatus*. Percent positive responses before, immediately after and 1 and 3 days after treatment with cobaltchloride are shown.**



## 2.4. SUMMARY AND CONCLUSIONS

The research of the existing literature performed as part of this deliverable clearly indicates the ecological relevance of the lateral line by demonstrating that this sensory system is used in different fish behaviours, albeit in different species. A major objective of CILIA is to investigate source detection and separation, tasks that clearly can be performed by the lateral line. One of the most intriguing features of the fish lateral line is the fact that its peripheral morphology is highly diverse. This raises the question how lateral line design relates to ecological parameters. In other words, what we would like to know is how different types of lateral lines can detect and separate sources under different conditions.

In order to address this question, behavioural experiments are needed on the performance of the lateral line under different environmental conditions. This includes studies of detection, localization and discrimination of hydrodynamic stimuli in still water and under conditions of background noise. These studies should be performed with fish equipped with different lateral line systems. For reasons outlined above such studies are difficult to conduct. Moreover, both the hydrodynamic stimuli that are relevant for the lateral line and the environmental noise may be different for different fish species. In addition, lateral line design may not only be determined by stimuli and background noise, but may also depend on life style (active swimmer, passive fish, predator, type of predator, no predator), swimming behaviour (fast versus slow swimmer, type of swimmer), and daily activity (nocturnal versus diurnal) of a species.

The behavioural experiments that we conducted as part of this deliverable were addressing the question whether detection of a vibrating source by fish equipped with different lateral line systems is affected by running water. We used breathing rate as a monitor for stimulus detection and, although responses were small and exhibited high variability, were able to measure detection thresholds in still and running water. The drawbacks of this kind of experiments were that large data samples were required and that discrimination experiments were not possible because the animals could not be conditioned to an electric shock. Moreover, it appeared that the animals used input from the inner ear for stimulus detection. Therefore these experiments will not be continued.

We also used food-rewarded active approach behaviour to study stimulus detection and localization. These experiments were more laborious than recording of breathing activity but apparently the animals relied on input from the lateral line in this active swimming task. Therefore, it seems promising to continue these experiments in order to study stimulus discrimination with the lateral line system under still and running water conditions.

Complementary experiments to study ecological relevance of the lateral line may involve methods that do not require training of animals. One feasible approach is the study of swimming behaviour in still and running water. In such experiments, frequency, speed and type of swimming in different species equipped with different lateral line systems may be compared. In addition, ability of fish to position themselves in flows of different velocity may be determined and related to lateral line design. Finally, placing obstacles in the flow field may allow to find out whether different species prefer areas of increased or areas of decreased flow velocities.



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