

## ANALYSIS OF THE PHASE DIFFERENCE BETWEEN PARTICLE MOTION COMPONENTS OF SOUND BY TELEOSTS

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### SUMMARY

The hypothesis that fish may remove 180° ambiguities concerning the sound source direction by a timing analysis between particle motion components is addressed. Analysis of the phase difference  $\psi(w, v)$  between the horizontal ( $v$ ) and the vertical ( $w$ ) components of the particle velocity of sound signals by teleost fish is investigated by cardiac conditioning. Standing wave sound stimuli (90 Hz) are used with  $\psi(w, v) = +$  or  $-90^\circ$  and equal amplitudes of  $v$  and  $w$ . The water particles move along circular trajectories for such stimuli: however, the direction of revolution is reversed in the two stimuli. These stimuli were discriminated by one whiting, one catfish and several cod, indicating that these teleosts are able to discriminate signals on a pure timing cue. If the  $w/v$  ratio is lowered, the cod can discriminate the resulting elliptical motions on the direction of revolution down to a  $w/v$  ratio of  $-12$  dB. Moreover, down to the same  $w/v$  ratio the cod discriminates an elliptical particle motion signal from a pure translatory particle motion signal. At this level, the threshold signal-to-noise ratio for the  $w$  component is exceeded by 9 dB. Apparently phase analysis can be limited by 'cross talk' between horizontal and vertical particle motion detectors. The results are discussed with respect to models of directional hearing by fish.

### INTRODUCTION

Both bony fish (Richard, 1968; York, 1972; Maniwa, 1976; Olsen, 1976) and shark (Nelson, 1965; Richard, 1968; Myrberg, Ha, Walewski & Banbury, 1972; Nelson & Johnson, 1972) can be attracted and repulsed by sound. Although attraction to an active transducer is no proof of directional hearing (Schuijf & Buwalda, 1980), directed responses of shark at the onset of a sound source transmitting low-frequency noise, as reported by Nelson (1965) and Myrberg *et al.* (1972) at distances of 200 m and over, do provide evidence for this notion. This suggests that directional hearing is a sensory ability common to the fish attracted. Indeed, for some teleosts such as the cod *Gadus morhua* L. (Schuijf, 1975) and the ide *Leuciscus idus* L. (Schuijf, Visser, Willers & Buwalda, 1977) unambiguous directional hearing has been shown using an operant conditioning paradigm.

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Directional hearing mechanisms in fish are quite different from those in terrestrial vertebrates (Schuijf & Buwalda, 1980). Essential to unambiguous directional hearing in fish are detection of the 'direction' of the particle motion component ( $\bar{v}$ ) and its time relationship to a coherent reference wave. During a cycle,  $\bar{v}$  is directed alternately towards the sound source and in the opposite direction. Fish could eliminate this 180° ambiguity concerning the propagation direction by a timing analysis between  $\bar{v}$  and the coherent reference wave (Schuijf, 1976). It has been shown that the cod is able to use sound pressure ( $p$ ) as the coherent reference wave (Schuijf & Buwalda, 1975), the swimbladder acting as a pressure-to-motion transducer (Sand & Hawkins, 1973; Sand & Enger, 1973). For fish without a swimbladder sensitivity to sound pressure is not self-evident. It has been proposed that shark might remove the 180° ambiguity by analysing the temporal relationship between vertical and horizontal components of the compound displacement pattern of the direct- and surface-reflected wave (Schuijf, 1981). Common to both mechanisms of ambiguity removal is the proposed ability to perform a timing analysis between the constituent parts of an interference pattern resulting from the interaction of *direct input* and the motions induced by the reference wave (i.e. the *indirect input*). In general, the resulting motional pattern will be a Lissajous figure, which degenerates into a translatory motion only if direct and indirect input are in phase, in antiphase or parallel to one another. These conditions are generally not met. A change in the time difference of the direct and the indirect wave will most often affect the 'shape' of the interference pattern. Behavioural experiments, in which it has been shown that the cod and the ide discriminate two sine waves on the basis of the phase difference between  $p$  and  $\bar{v}$  (Schuijf & Buwalda, 1975; Buwalda, Schuijf & Hawkins, 1983), therefore provide no sufficient proof of discrimination based on a timing cue.

A simple motional pattern that lets one investigate the capabilities of the fish hearing system with respect to timing cues and intensity cues separately is a pattern in which the water particles follow an elliptical trajectory. Reversal of the direction of revolution consists of a pure change in the time difference between the motions which constitute the ellipse without a difference in the 'shape' of the interference pattern or in the overall intensity. Such stimuli have been used successfully by P. Teunis, M. Streefkerk, A. Schuijf & R. J. A. Buwalda (in preparation) to condition the shark *Chiloscyllium griseum*. I have used these stimuli to investigate the ability of fish with a swimbladder to carry out a timing analysis between particle motion components. If 'p,v' phase discrimination is based on a timing analysis between p-induced and v-induced motions, then we may expect these species to be able to perform a timing analysis between two particle motion components. More specifically, this study addresses the following questions. (1) Are fish with a swimbladder (e.g. the Gadoids) able to discriminate two sound signals that differ only in the time relationship between two orthogonal particle motion components? (2) Can this be done by fish (e.g. the Ostariophysians) that possess specialized connections between the swimbladder and the otolith organs?

In directional hearing studies, the cod is often used as a representative of the fish that lack a special connection between the swimbladder and the ears. For reasons of availability, during the earlier part of this study the whiting (*Odontogadus merlangus* L.) rather than the cod was used. Virtually no data are available with respect to the

bioacoustics of the whiting. Two such anatomically closely related fish species as the cod and the whiting probably do not differ very much in this respect. The catfish (*Ictalurus nebulosus*) is used to investigate phase analysis between particle motion components by an Ostariophysian.

In the second part of this study, the effect of masking of the reference wave on timing analysis is investigated. One reason for studying this is that considerable vertical displacement noise levels are almost always present 'near' the water surface. Another reason is that vertical particle motion noise alters the time relationship between the direct and the reference wave at signal-to-noise (S/N) ratios above the detection threshold of the reference wave. A threshold level for timing analysis could be defined as the S/N ratio of the reference wave at which responses to a pure change in timing between the direct and the reference wave become indistinguishable from chance performance. This threshold may differ from the masked detection threshold of the reference component in isolation and from the threshold for the detection of addition of the reference wave to the direct wave. Therefore, all three thresholds are determined. To allow comparison between the last threshold mentioned and the 'threshold of timing analysis' the phase relationship between the direct and the reference wave was kept equal in both cases. During the second part of this study, cod became available and were used instead of whiting. It was felt unnecessary to repeat the experiments done on the whiting because the initial level of the stimuli of the threshold experiment of timing analysis equalled the stimuli used for the whiting. If cod behaved differently this would appear at the initial level of the threshold experiment of timing analysis. No difference was found. I had no time to repeat these experiments with the catfish.

#### MATERIALS AND METHODS

Experiments were done using one whiting (*Odontogadus merlangus* L.; length 20 cm), one catfish (*Ictalurus nebulosus*; length 18 cm) and several cod (*Gadus morhua*; length 30 cm). All animals were kept in aquaria at the laboratory for several weeks before use. During the experiments with the whiting and the catfish the experimental tank contained fresh water. Prior to the experiments with the cod the water was replaced by sea water. Fish were in all cases confined to a cylindrical cage, suspended from ropes in a standing wave tank. Care was taken to position the head of the fish at the tank centre (see Fig. 1). The cage used for the catfish was provided with a wire mesh for electrical shielding. This prevented possible stimulation of the electroreceptors by a small electrical leakage field that might arise from one of the sound projectors.

The whiting was confined to a cage consisting of a cylindrical polyethylene sheet fitted to two disk-shaped endplates of PVC with a watertight connection. The endplates were mounted on an alloy bar. A constant flow of sea water of  $2\text{ l min}^{-1}$  was maintained through two openings in the endplates. The cage was connected to a pump and a reservoir of sea water by flexible tubes. The flow increased the noise spectrum level by about 3 dB below 80 Hz, but negligibly at higher frequencies. The sound field parameters,  $p$ ,  $u$ ,  $v$ ,  $w$ , measured at the tank centre in the absence of the cage, differed somewhat from those measured with the cage put into position. Differences for the

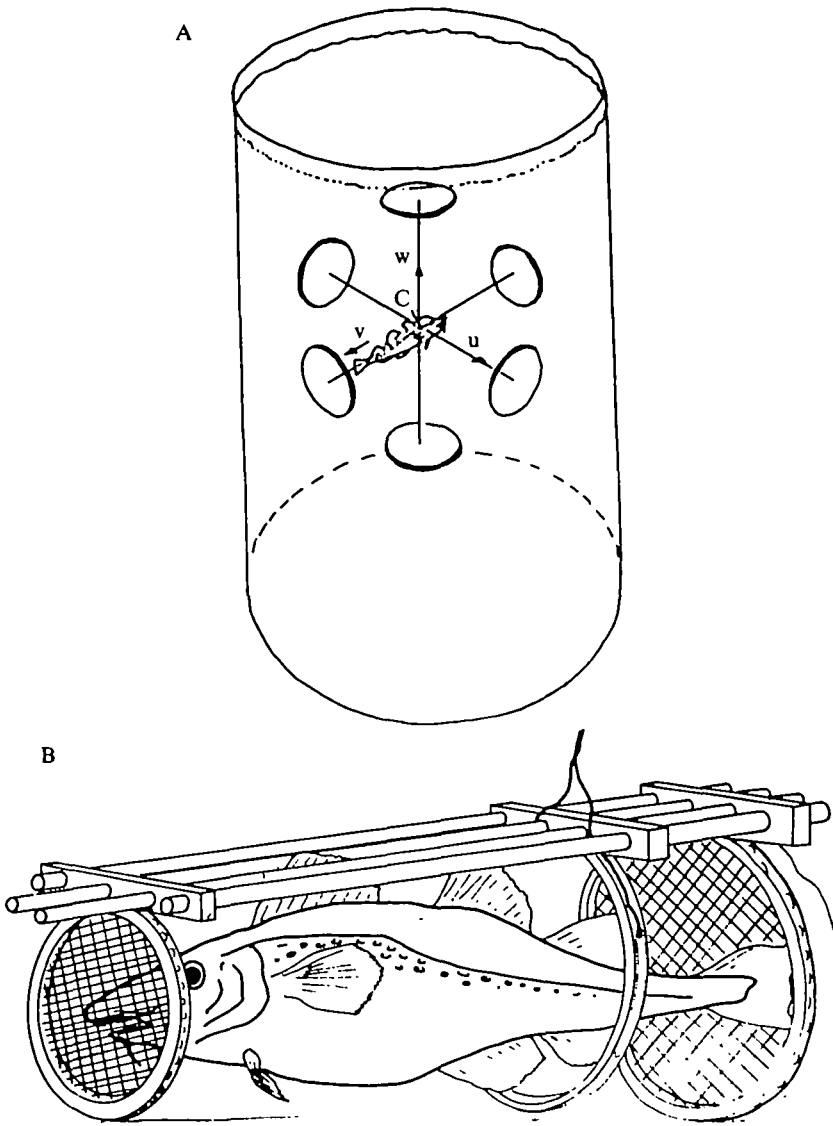


Fig. 1. (A) The experimental tank. The frame of reference of the sound field parameters ( $u, v, w$ ) is indicated. At the origin of this reference frame (which corresponds to the tank centre,  $C$ ) the head of the fish is positioned. The six disk-shaped sound projectors are aligned with the axes of the reference frame. (B) The fish restrainer used for the cod. It consists of three PVC rings mounted on a frame of four parallel tubes. The diameter of the rings decreases towards the head of the fish to constrain it maximally. The positions of the rings are adjusted to the length of the fish. Two silver stimulating electrodes (one is indicated by the dotted line) are attached to the inner wall of the ring in the middle. A polythene sheet is folded around the rings and attached to the two inner tubes of the supporting frame.

maximized variable in the standing wave were below 1 dB and  $1^\circ$ . The minimized and the uncontrolled variable varied up to 15 dB. However, in all cases the components were 20 dB lower in amplitude than the maximized variable. If the maximized and

perpendicular displacement component are added, the resulting displacement differs by less than 1% in amplitude and  $5.7^\circ$  in direction from the maximized component. Directional discrimination thresholds reported for the cod are  $12^\circ$  or over. Therefore the differences were neglected.

The standing wave tank and its acoustics have been described elsewhere (Buwalda, 1981). Briefly, the tank contains six custom-built sound projectors that are suspended from ropes. The disk-shaped sound projectors are suspended in three pairs. The sound projectors of each pair are positioned opposite each other and are carefully aligned. The three pairs are aligned along (imaginary) lines that are perpendicular to one another. At the tank centre (see Fig. 1), it is possible to have approximately independent control of sound pressure ( $p$ ) and particle velocity components ( $u$ ,  $v$ ,  $w$ ). The driving of the speakers is under the control of a microprocessor as described in van den Berg & Schuijf (1983).

A classical conditioning paradigm was used to investigate the ability of the fish to discriminate sound signals on the basis of timing cues alone. During an experimental session, a pulsed signal (rate 1 Hz; duty cycle 50%) was offered continuously (the neutral signal; NS). Fish habituated to NS within 1 min. The rising and falling sides of the pulse were of sinusoidal shape and lasted 130 ms each. The amplitude of the pulse envelope varied randomly by 1 dB. The phase relationship between  $u$  and  $w$  was constant throughout the pulse (see Fig. 2).

At the end of periods of random duration (range 2–4 min) a trial was offered. A trial consisted of one presentation of the conditioned stimulus (CS) followed by a brief shock (approx  $25 \text{ mA cm}^{-2}$ ) which acted as the unconditioned stimulus (UCS). Five alterations with a second signal type (differing from the stimulus during NS) constituted CS. Bradycardia occurred during CS when conditioning had succeeded.

#### *Measurement of responses and statistical treatment*

Two ECG electrodes were implanted under MS222 anaesthesia to measure cardiac activity. The electrodes were made of insulated stainless steel wire (diameter  $50 \mu\text{m}$ )

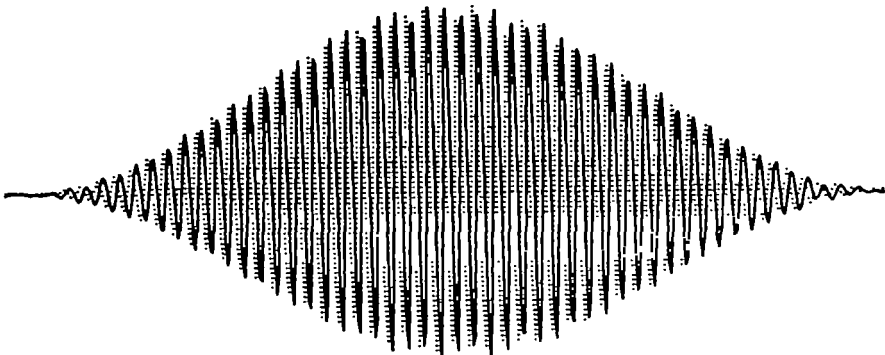


Fig. 2. A pulse of NS(1). Horizontal ( $u$ ) and vertical ( $w$ ) particle velocity were recorded simultaneously on an UHER CR204 cassette recorder. The recorded signals were fed through an A/D converter into a PDP-11/10 mini computer (sampling rate 2000 Hz) and plotted. Peak particle velocity is approximately  $200 \mu\text{var}$ . The pulse lasts 0.5 s. The dotted line indicates  $u$  and the continuous line  $w$ . Note that the phase difference between  $u$  and  $w$  equals  $90^\circ$  throughout the pulse. The flattened peaks of some periods of the sine wave resulted from sampling errors.

with a bare tip. The amplified and low-pass filtered pulses reset and triggered a ramp generator. The period of the resulting saw-tooth wave form equalled the time interval between two heartbeats. This signal was sampled by an Apple II+ microcomputer at a rate of 34 Hz through an a.d.-converter. Nine cardiac intervals directly preceding a trial (constituting the pretrial period) together with those during the trial (usually 5–10 heartbeats) were used for statistical analysis. Part of the analysis was done in real time – directly following a trial – and was identical for all stimuli to be described below. The real time computations comprised the following: (1) computation of the parameter  $\rho$  of the Mann-Whitney paired sample test (Conover, 1971); (2) computation of the ratio of the maximum cardiac interval of the trial period and the maximum cardiac interval during the pretrial (X). The cardiac intervals of each pretrial and trial period, together with their X- and  $\rho$ -values, were stored on floppy disk.

The staircase method of Dixon & Massey (1969) was used to determine thresholds in the experiments investigating the effect of masking on timing analysis. Positive responses were followed by a level decrease of the stimulus parameters under investigation (described below). Negative responses resulted in a level increase. Responses were considered positive when  $\rho$  exceeded 0.75 or X exceeded 1.1. The criterion of  $\rho$  may be met if a substantial part of the cardiac intervals of the trial exceed the mean duration of the intervals of the pretrial, although none of the intervals of the trial may exceed the longest pretrial interval by 1.1. Conversely a single bradycardium during the trial may lead to an X value above 1.1, while  $\rho$  is clearly below 0.75. In my opinion both criteria are useful for screening of a possible effect.

The experiments using the whiting and the catfish were meant to explore the possible existence of the capability of fish to perform a timing analysis. Such a decision cannot be made on the basis of a single trial for each animal. Rather a series of trials with the same signal types was offered (typically 40 trials). The responses of each animal to the series were pooled, allowing a statistical decision about whether an effect is absent or not with estimates for probabilities or erroneous decisions. Statistical analysis was done as described by Buwalda *et al.* (1983). In this analysis only the statistic X is used. Positive reactions of both the whiting and the catfish consisted predominantly of a single prolonged cardiac interval during a trial. To normalize the distribution of X the quantity Y was computed from each X value, where  $Y = \ln X$ .

Student's *t*-statistic and a special purpose test (see to Buwalda *et al.* 1983 for details) were used to compute *P*-level and  $\beta$  (i.e. probability of an error of the second kind). A *P*-level exceeding 0.05 and  $\beta$  tending to 0 indicate absence of discrimination. A *P*-level below 0.05 and  $\beta$  tending to 1 indicate discrimination.

### *Stimuli*

Altogether six pairs of NS and CS were used [NS(i),CS(i);  $i = 1, 2 \dots 6$ ]. Intertrial periods with NS(i) stimulation were followed by trials with CS(i) (see Table 1). Stimulus frequency was 90 Hz in all cases. [NS(1),CS(1)] and [NS(2),CS(2)] were the only stimulus pairs used in the experiments with the catfish and the whiting. These stimulus pairs consisted of a maximized vertical particle velocity component (*w*) and a horizontal velocity (*u* or *v*) and minimized sound pressure. The horizontal and the vertical component were of equal amplitude. Because of the phase difference of 90° between *u* (or *v*) and *w* the instantaneous particle velocity vector describes a

circle: 'anticlockwise' during NS(1) or NS(2) and 'clockwise' during CS(1) or CS(2). Both stimulus-pairs were intended to investigate the abilities of the catfish or the whiting to discriminate circular motional patterns on the direction of revolution alone, a pure timing cue. [NS(1),CS(1)] and [NS(2),CS(2)] are tests of the ability for a motion in the median plane and the transverse plane respectively. To test for possible stimulus artifacts correlated with the switching between the two stimulus types during CS(1) or CS(2), blank trials were offered followed by shock. A blank consists of a switching to the same signal type as during NS(i).

In subsequent threshold experiments, involving four cod, the last four stimulus pairs mentioned in Table 1 were used. Note that the initial conditions of  $w$  are specified in Table 1 with respect to these four stimulus pairs. In these experiments narrow band (70–120 Hz) vertical particle motion noise was present as specified in Table 1. Thresholds of the  $w$ -component were determined with the staircase method as mentioned. The level of  $w$  was adjusted in steps of 2 dB.

The  $w$ -level of CS only was varied in experiments using [NS(3),CS(3)] or [NS(4),CS(4)]. No sound signal was offered but the masking noise in NS(3). Hence the detection threshold of  $w$  was determined by the stimulus pair [NS(3),CS(3)]. A horizontal particle motion component ( $v$ ) was offered intermittently during NS(4). During trials this signal was interchanged by a signal comprising the same  $v$  as during NS(4) and  $w$  added with a phase difference of  $90^\circ$ . During this CS(4) the water particles follow an elliptical trajectory with a ratio of the short and the long axis equal to the  $w/v$  ratio. Hence [NS(4),CS(4)] was used to determine the threshold of discrimination between horizontal translatory displacements [as during NS(4)] and displacements following an elliptical trajectory.

Particle motion stimuli that follow identical elliptical trajectories with the rotation sense reversed are appropriate stimuli for discrimination experiments on timing analysis. This holds whatever the ratio of the long and the short axis of the elliptical pathway. Note that the initial conditions of [NS(5),CS(5)] are identical to those of [NS(2),CS(2)]. Hence CS(5) and NS(5) differ in the rotation sense of a circular trajectory of the particle motion. After a positive response, the  $w$ -level of both NS(5) and CS(5) was lowered equally by 2 dB.  $\psi(w, v)$  is invariably  $+90^\circ$  or  $-90^\circ$  (see Table 1). Hence the trajectories of these stimulus types are still identical (differing only in the rotation sense) but no longer circular. The threshold of timing analysis equals the  $w$ -level at which the reversal of the rotation sense is no longer discriminated.

To my knowledge, adjustment of both NS and CS stimulus parameters after a response has never been used in connection with the staircase method. The [NS(6),CS(6)] stimulus pair was intended as a control of the above described unusual method. Applying the latter procedure of stimulus adjustment on NS(6) and CS(6), a just noticeable difference of angular discrimination was determined. The phase difference,  $\psi(w, v)$ , was  $0^\circ$  in NS(6) and  $180^\circ$  in CS(6) independent of the  $w$ -level. Hence, during NS(6) a translatory particle velocity signal is offered at an elevation  $\theta$  from the horizontal. The angle  $\theta$  is determined by:

$$\theta = \arctan \{ \cos[\psi(w,v)] \cdot w/v \}. \quad (1)$$

It follows from equation (1) that an alternation with a particle motion signal of elevation  $-\theta$  is offered during CS(6). Positive responses decrease the  $w$ -levels of both NS(6)

Table 1. Values of the sound field parameters at the tank centre for the experiments on phase analysis by the whiting and the catfish [NS(1), CS(1)] and [NS(2), CS(2)]

Stimulus category	Signal				Amplitude				Phase relationship (degrees)	Displacement pattern
	p	u	v	w	p: dB re 1 $\mu$ bar					
					u, v, w: dB re 1 $\mu$ var	p	w	(w,y)		
NS (1)	4.8	—	44.6	44.6	—	—	—	—	-90	
CS (1)	4.8	—	44.6	44.6	—	—	—	—	+85	
NS (2)	4.8	43.5	—	43.8	—	—	—	—	-90	
CS (2)	4.8	43.5	—	44.1	—	—	—	—	+90	
NS (3)	—	—	—	—	—	10	29	—	—	
CS (3)	0	—	—	47.6	—	10	29	—	—	
NS (4)	0	—	46.0	—	—	10	29	—	—	
CS (4)	0.5	—	46.0	47.4	—	10	29	+90	—	
NS (5)	4.8	—	46.0	47.4	—	10	29	-92	—	
CS (5)	0.5	—	46.0	47.4	—	10	29	+90	—	
NS (6)	2.9	—	46.0	47.4	—	10	29	1	—	
CS (6)	2.9	—	45.7	47.1	—	10	29	178	—	

Stimulus pairs [NS(1), CS(1)] and [NS(2), CS(2)] were used to investigate phase analysis in the whiting and the catfish. The latter four stimulus pairs specify the starting conditions of the sound field parameters at the tank centre for the threshold experiments using cod. The w-level of the signal was adjusted by 2 dB depending on the response of the subject (see text). In the right-hand column the trajectory of the particle motion of the signal is drawn schematically. The direction of the particle motion components (u, v, w) is indicated in the lower corner of each figure. A solid line indicates the trajectory of the starting condition. To illustrate the stimulus adjustment during the threshold determination the trajectories after the first positive response are drawn by interrupted lines.



Table 2. Responses of the catfish and the whiting to stimuli as specified in Table 1

Species	Stimulus	<i>N</i>	<i>T</i>	<i>P</i>	$\beta$
Catfish	NS(1),CS(1)	38	8.45	$<2.5 \times 10^{-5}$	1
	blank	11	0.82	0.21	0.07
Catfish	NS(2),CS(2)	43	11.34	$<2.5 \times 10^{-5}$	1
	blank	9	0.02	0.49	0.02
Whiting	NS(1),CS(1)	53	11.65	$<2.5 \times 10^{-5}$	1
	blank	18	1.66	$5.7 \times 10^{-2}$	0.12
Whiting	NS(2),CS(2)	51	9.51	$<2.5 \times 10^{-5}$	1
	blank	9	0	0.5	0.02

The number of trials is denoted by *N*. The *T*-statistic is based on the mean (*m*) and the standard deviation (*s*) of the pooled response parameter *Y* (see text) as follows:  $T = \frac{m\sqrt{N}}{s}$ .

High *P*-values and low values of  $\beta$  indicate absence of discrimination for the blanks. Low *P*-values and  $\beta \rightarrow 1$  indicate discrimination of the test signals.

Table 3. Results of the *w*-threshold experiment involving four cod

Stimulus (i)	<i>w</i> -threshold (dB re 1 $\mu$ bar)					Mean $\pm$ s.d.
NS(3),CS(3)	23.6	25.6	27.6	27.3	25.7	26 $\pm$ 1.6
NS(4),CS(4)	32.1	34	37	36	35.8	35.2 $\pm$ 1.6
NS(5),CS(5)	36	33.5	34.9	34.6		34.8 $\pm$ 1
NS(6),CS(6)	30					

The stimulus category (i) refers to the specifications given in Table 1. Mean thresholds of *w* as well as individual thresholds are shown.

and CS(6). Note that the particle motion signal changes over an angle  $2\theta$  during CS(6). The threshold *w*-level ( $w^*$ ) is related to the just noticeable difference of angular discrimination ( $\theta^*$ ) by:

$$\theta^* = 2 \arctan (w^*/v). \quad (2)$$

#### RESULTS

Both catfish and whiting were able to discriminate circular motional patterns which differed in their direction of revolution (Table 2:  $P < 0.05$ ,  $\beta = 1$ ). Discrimination occurred in the median as well as in the transverse plane. Blanks were never detected ( $P > 0.05$ ,  $\beta \rightarrow 0$ ).

Table 3 shows 15 *w*-thresholds obtained from four cod. Each stimulus category was offered in two sessions of 40 trials separated by a 2-h rest period. Therefore, two thresholds were usually obtained per animal for each stimulus category offered. Results of three sessions are omitted since the subject required a long training period ( $> 20$  trials), reducing the number of trials for the threshold determination to only 10–20 trials. For a reliable threshold, 20–30 trials are necessary. When, during a regular inspection, the cod appeared to be moving restlessly within the cage, the experiment was interrupted for 30–60 min and the sound stimulus switched off. If the cod did not settle within this period the subject was taken out. This procedure ensured a well-fixed position of the animal within the sound field.

Sound fields were regularly checked between experiments. Maximum deviations of the maximized variable (i.e.  $v$  or  $w$ ) amounted to 0.5 dB while  $(w, v)$  ranged from  $-86^\circ$  to  $-94^\circ$  for NS(1) or NS(2) and for CS(1) or CS(2). The maximum values for the minimized sound field variable  $p$  are indicated in Table 1. Values were usually approximately 6 dB lower. The difference of the minimized components of NS and CS varied by no more than 1 dB in all cases.

#### DISCUSSION

The present results indicate that the whiting and the catfish can discriminate sound signals on the basis of a  $180^\circ$  shift of the phase difference  $\psi(w, v)$  between two mutually perpendicular particle velocity components that are  $90^\circ$  out-of-phase. The water particles move along a circular trajectory during such stimuli. A  $180^\circ$  change in  $\psi(w, v)$  reverses the direction of revolution of the water particles without changing the trajectory. Assuming that the fish did not react to concomitant stimulus artifacts, the catfish and the whiting are apparently able to discriminate particle velocity signals on a pure timing cue. This conclusion may seem premature because it is based on a single experiment with a single animal for each species. However, the experiment was designed to investigate the existence of the capacity for timing analysis by fish with a swimbladder. To answer such a question a single demonstration suffices. Strictly these results do not allow conclusions on the extent to which this capacity is shared by other representatives of the same species. However, both animals required quite a normal training period (10–20 trials) although the stimuli used are 'unnatural' in many respects (simulation of the 'direct' and the 'indirect' wave by standing waves, sinusoid signals, equal amplitudes of the 'direct' and the 'indirect' wave). This does not suggest that the study describes a hearing capacity that is exceptional for these species.

The phase difference between the vertical and the horizontal component of the particle displacements that results from the interaction of a harmonic wave and its surface reflection could act as a cue for discrimination between two sound sources that are opposite with respect to a vertical plane through the fish (Schuijf, 1981). The rotation sense of the water particles is reversed for opposite sources at the position of the fish. The direction of revolution also reverses if the path length difference exceeds half the wavelength ( $\lambda$ ) of the sound signal. Hence, an unambiguous relationship between  $\psi(w, v)$  and the half-plane containing the sound source exists only for path length differences smaller than  $\lambda/2$ . The ability to discriminate signals on  $\psi(w, v)$  is considered of importance for acoustic localization by fish that lack sensitivity to sound pressure. P. Teunis, M. Streefkerk, A. Schuijf & R. J. A. Buwalda (in preparation) showed that the shark *Chiloscyllium griseum* is able to perform a phase analysis between  $u$  or  $v$  and  $w$ . Recently it has been shown that subjects of the same species can discriminate sound signals on the phase difference between the sound pressure component and  $v$  [ $\psi(p, v)$ ]; see van den Berg & Schuijf, 1983). Hence, this shark species may use both proposed mechanisms of  $180^\circ$  ambiguity removal. The present results indicate that this also holds for the cod. Phase analysis between  $p$  and  $v$  was shown by Schuijf & Buwalda (1975) and  $w, v$ -phase analysis is shown in this article.

Also for representatives of the Ostariophysians both  $\psi(p, v)$  analysis (the ide *Leuciscus idus* L.; in Schuijf & Buwalda, 1980) and  $\psi(w, v)$  analysis (the catfish; this article) has been demonstrated. It appears then that, quite independent of the possession of accessory hearing structures such as the swimbladder or special connections between the swimbladder and the ear, fish are able to use  $w$  or  $p$  as a phase reference.

In a free-field the use of a surface reflection as a phase reference breaks down because the contributions of reflected waves are by definition negligible in such a field. Free-fields occur in deep water with distant shore lines. Under these conditions  $180^\circ$  ambiguity removal by  $\psi(p, v)$  analysis holds (Schuijf, 1976, 1981). The surface reflection is no longer negligible, however, if the fish is swimming near the water surface. Moreover, the water surface acts as a pressure release boundary and pressure may be subliminal for  $\psi(p, v)$  analysis. On the other hand, near the water surface path length differences between the direct wave and the reflected wave are sufficiently small to satisfy the above constraint with respect to the wavelength of the sound. Both proposed mechanisms for ambiguity removal thus appear complementary with respect to the acoustic conditions for which they are valid. Perhaps the ability to perform a phase analysis between mutually perpendicular velocity components or between  $p$  and a velocity component found among fish with such different accessory hearing structures reflects an ability of fish to localize unambiguously a sound source under acoustic conditions ranging from free-fields to sound fields dominated by reflections (such as a shallow water layer).

#### *Stimulus control*

An important drawback of the classical conditioning method employed is the general nature of the response. Bradycardia – as any part of the orienting reflex – will be elicited by any detectable change in the stimulus conditions to which the fish has not been habituated. Blank stimuli were offered to investigate the occurrence of responses to stimulus artifacts arising from the switching of the two signal types during CS. Blanks were not detected. Random variation of the intertrial period and the amplitude of the pulse envelope (see Methods) precludes discrimination based on a pattern of stimulus presentation or changes in the amplitude during presentation of CS.

Successive measurements of  $\psi(w, v)$  between the experiments showed minor deviations ( $\Delta\psi_c$ ) from the intended  $\psi(w, v)$  of  $90^\circ$ . Such deviations create elliptical motions with a slight inclination ( $\Delta\psi/2$ ) from the horizontal plane.  $|\Delta\psi|$  did not exceed  $5^\circ$ .

Our standing wave tank allows control of sound pressure and particle velocity components ( $u, v, w$ ) in a limited volume around the tank centre only. Consequently, discriminations resulting from insufficient stimulus control can be conceived of in three ways: (1) bad positioning of the fish; (2) a steep sound pressure gradient may result in pressure stimulation at the position of the swimbladder despite  $p/v$  ratios below  $-40$  dB with respect to far field at the tank centre; (3) lateral line stimulation due to the divergence of the sound field parameters near the sound projectors.

The limited freedom of movement within the cage allowed displacements of the head of the subject of 3 cm at most. At these distances the sound field parameters deviate by less than 5 dB from the corresponding values at the tank centre.

Using the same set-up, Buwalda & van den Steen (1979) found at  $p/v$  ratios of  $-50$  dB (with respect to far field) a nearly perfect cosine dependence of the

microphonic potentials recorded in the sacculus of the cod on the angle of incidence of the particle velocity. This indicates that despite the higher  $p/v$  ratio at the position of the swimbladder (due to the gradient of  $p$ ) the swimbladder-mediated input to the sacculus is very small. This input proved to be smaller for the posterior parts of the sacculus, which suggests that the lagenae (caudad from the sacculus in the cod) are even less sensitive to sound pressure. Although we may not conclude from such data that  $p$  was subthreshold during the experiments, it does not support the notion that discrimination was due to pressure stimulation. This possibility becomes even more unlikely if we realize that the stimulus was 'constructed' by addition of a vertical and a horizontal standing wave. The horizontal standing wave contributes 10–20 dB more to the pressure gradient than does the vertical standing wave (van den Berg & Schuijf, 1985). Because the horizontal standing wave was kept the same throughout the experiments, an unintended pressure stimulus caused by limited spatial control of sound pressure would be nearly the same in NS and CS and would probably not contribute to discrimination. Moreover, the successful determination of the threshold of phase analysis [NS(5),CS(5)] shows that discrimination of stimuli identical to [NS(2),CS(2)] still occurs in the presence of narrow band pressure noise of 10 dB re 1  $\mu$ bar (see Table 1). The noise may be expected to mask spurious pressure components resulting from limited spatial control of the sound pressure component.

Discrimination based on different stimulation of the lateral line by the two signal types during CS seems unlikely. The lateral line is stimulated by particle motion relative to the fish. Because the difference between the density of the fish body and water is small, the fish is carried along with the water column moving between the two opposite sound projectors. However, the longitudinally rigid fish body (cf. Denton & Gray, 1982) cannot move along its full length with zero relative velocity to the water column because a velocity gradient exists in the tank. Velocity gradients of  $v$  or  $w$  were found to be negligible along the body length of the fish up to a distance of 10 cm from the tank centre (van den Berg & Schuijf, 1985). Hence the head lateral line is probably not involved in the discrimination task. The trunk lateral line might be stimulated as a result of the gradient of the horizontal velocity ( $v$ ) parallel to the canal. The vertical standing wave contributes approximately 15 dB less to  $v$  along the fish body length than the horizontal standing wave. Hence the gradient of  $v$  was practically the same for NS and CS pulses. This makes discrimination by differential stimulation of the lateral line during CS unlikely.

#### *Threshold experiments*

The threshold experiments of  $w, v$ -phase analysis were intended to explore the limits of timing analysis between two perpendicular velocity components by the cod. The noise field not only consisted of a  $w$ -component to mask the  $w$ -signal (simulating the reference wave) but also a sound pressure component of 10 dB re 1  $\mu$ bar. The latter noise component is meant to mask unintended pressure components of the signal that might be involved in the discrimination experiment mentioned above. The threshold ratio of  $w$  at which the cod can discriminate whether  $w$  is leading or lagging  $v$  by  $90^\circ$  amounts to  $-12$  dB. The curious procedure of stimulus presentation (see Methods) necessary to obtain the threshold of  $w, v$ -phase analysis seems to be a valid method because the threshold of  $w$  of the [NS(6),CS(6)] control stimulus indicates a vertical

angular discrimination threshold of  $18^\circ$  (see equation 2 and Table 3). Angular discrimination thresholds obtained at sea for the cod range from  $16^\circ$  to  $23^\circ$  (Chapman & Johnstone, 1974; Hawkins & Sand, 1977; Schuijf, 1975). We should notice that a w-threshold of [NS(5),CS(5)] exceeds the w-detection threshold in narrow-band noise [NS(3),CS(3)] by 7 dB, but does not differ from the threshold to detect a w-component leading v by  $90^\circ$  [NS(4),CS(4)]. Apparently w, v-phase analysis is limited by the w/v ratio rather than by the S/N ratio of the w component. This result seems at variance with data of Buwalda (1981) who could not find evidence for v-noise masking a w-signal or *vice versa* at S/N ratios down to  $-10$  dB and in one case even  $-30$  dB. Such directional masking results led Buwalda to suggest that the cod hearing system consists of a set of separate input channels, each for a particular velocity direction, and one independent channel for sound pressure. The channel especially sensitive to w (henceforth the w-channel) would be stimulated by v if the hair cells which constitute necessarily the most peripheral part of such a channel were not perfectly aligned. For such a system a w-component added to a v-component is detected only if the intensity discrimination threshold of the w-channel is exceeded. Intensity discrimination thresholds for the cod are 5–7 dB (Chapman & Johnstone, 1974). A doubling of the w-amplitude leads to discrimination. Two sine waves of the same frequency but with a phase difference,  $\phi$ , and an amplitude ratio, n [i.e. a power ratio of  $20\log(n)$  dB], add up to a sine wave of the same frequency with an amplitude of  $[1 + n^2 + 2n \cos(\phi)]^{1/2}$ . The input to the w-channel will increase by 6 dB if to the crosstalk component a w-component of equal phase and equal magnitude is added. If w leads the crosstalk component by  $90^\circ$  a 6 dB increase of the stimulus strength of the w-channel occurs if the w-component exceeds the crosstalk component by 4.8 dB. We may expect, therefore, a 4.8 dB drop in w-threshold if the phase difference between w and the crosstalk component shifts from  $90^\circ$  to  $0^\circ$ . Mean w-thresholds of the [NS(5),CS(5)] experiment are about 5 dB higher than the w-threshold of the [NS(6),CS(6)] experiment (see Table 3). These data are apparently compatible with the notion of crosstalk of a v-stimulus on the w-channel.

It could be argued that thresholds are influenced by the limited perpendicularity of the stimulus-generating system. However, the horizontal standing wave generated a w-component 36 dB below the v-component at the tank centre. Measurements of the sound field parameters at the threshold level indicated deviations of  $\psi(w,v)$  from the intended values below  $5^\circ$ . Also the amplitude of w of NS(5) differed by less than 1 dB from the w-amplitude of CS(5) at the threshold level.

We may conclude then, that detection of a particle velocity component may be limited by the presence of a perpendicular velocity component and that at the w/v ratio at which a cod detects a w-component it is also able to perform a phase analysis between v and w. The latter conclusion corresponds with a finding of Fay & Coombs (1983) that masked detection thresholds of the goldfish correlate with the S/N ratio at which the spike rate of the primary afferents markedly rises. At this S/N ratio, the synchronization index is large ( $R = 0.5$ ), indicating a high level of phase locking. Thus at the detection threshold the waveform is relatively well coded in the output of the primary afferents, a prerequisite for performing a timing analysis at a higher level of integration. Similar data are lacking so far for the cod. However, Horner, Hawkins & Fraser (1981) described slow spontaneous units of nerve VIII with a

marked synchronization at the level where the spike rate intensity curve shows a steep incline.

The w,v-phase analysis model cannot account for the detection of the elevation of a sound source because the vertical particle velocity component cannot act both as a phase reference and as a cue for the elevation of the source which requires phase equality with the horizontal components. If both source and receiver are near the water surface this may be of little importance because the acoustic localization task is almost restricted to two dimensions. The azimuth of the sound source then equals the azimuth of the vertical plane through the direct wave and its surface reflection. The w,v-phase analysis could be used by the fish to remove the remaining 180° ambiguity concerning the sound source direction.

If the source is located far below the water surface the fish could determine the elevation by sounding to a deeper water layer where surface reflections are of minor importance. Behavioural data on directional hearing of fish near the water surface are lacking. For the time being, I conclude that the capacity to perform a timing analysis between perpendicular velocity components may be of relevance for fish in locating a sound source if the fish swims near the water surface.

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