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**Simultaneous suppression of tone burst-evoked otoacoustic emissions: two and three-tone burst combinations**

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**ABSTRACT**

Previous investigations have shown that components of a tone burst-evoked otoacoustic emission (TBOAE) evoked by a 1 kHz tone burst (TB<sub>1</sub>) can be suppressed by the simultaneous presence of a 2 kHz tone burst (TB<sub>2</sub>) or a pair of tone bursts at 2 and 3 kHz (TB<sub>2</sub> and TB<sub>3</sub> respectively). No previous study has measured this “simultaneous suppression of TBOAEs” for *both* TB<sub>2</sub> alone *and* TB<sub>2</sub> and TB<sub>3</sub> from the same ears, so that the effect of the additional presence of TB<sub>3</sub> on suppression caused by TB<sub>2</sub> is not known. In simple terms, three outcomes are possible; suppression increases, suppression is reduced or suppression is not affected. Comparison of previously reported simultaneous suppression data suggests TB<sub>3</sub> causes a reduction in suppression, though it is not clear if this is a genuine effect or simply reflects methodological and ear differences between studies. This issue has implications for previously proposed mechanisms of simultaneous suppression of TBOAEs and the interpretation of clinical data, and is clarified by the present study. Simultaneous suppression of TBOAEs was measured for TB<sub>1</sub> and TB<sub>2</sub> as well as TB<sub>1</sub>, TB<sub>2</sub> and TB<sub>3</sub> at 50, 60 and 70 dB p.e. SPL from nine normal human ears. Results showed no significant difference between mean suppression obtained for the two and three-tone burst combinations, indicating the reduction of suppression inferred from comparison of previous data is likely a result of methodological and ear differences rather than a genuine effect.

**Keywords:** Tone burst-evoked otoacoustic emissions, suppression, tone bursts

35 Abbreviations: Basilar membrane, BM; Fast Fourier transform, FFT; Peak-equivalent sound  
36 pressure level, p.e. SPL; Tone burst, TB; Tone burst-evoked otoacoustic emission, TBOAE;  
37 Transient-evoked otoacoustic emission, TEOAE.

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## 1. Introduction

Transient-evoked otoacoustic emissions (TEOAEs) are complex multi-component signals emitted from the healthy cochlea and recorded in the ear canal in response to short duration acoustic stimuli (e.g. Probst et al., 1991; Shera, 2004; Withnell et al., 2008). Because their presence is reliant on the normal functioning of the physiological processes that enhance hearing sensitivity and selectivity, TEOAEs are widely used in the clinical setting as a non-invasive assessment of cochlear function (e.g. Robinette and Glatke, 2007). Clicks are commonly used as the evoking stimulus, producing click-evoked otoacoustic emissions, but tone bursts can also be used, producing tone burst-evoked otoacoustic emissions (TBOAEs).

A common clinical interpretation is that TEOAEs exhibit place-specificity. The presence of a response component (i.e. a component with amplitude clear of the noise floor) at frequency  $f$  is held to indicate normal physiological functioning at the basilar membrane (BM) place tuned to  $f$ . Where response component  $f$  is absent (i.e. when its amplitude is less than the noise floor) abnormal function at BM place  $f$  is assumed. This interpretation is likely incorrect for two reasons. First, at short latencies the TEOAE response at  $f$  is thought to arise from BM places basal to  $f$  (e.g. Withnell and Yates, 1999; Withnell et al., 2008; Moleti et al., 2013). Second, previous authors have demonstrated nonlinear interactions amongst TEOAE frequency components vitiate the principle of linear superposition. Specifically, the amplitude of a TBOAE recorded in response to a 1 kHz tone burst ( $TB_1$ ) is reduced (suppressed) by the simultaneous presence of a single additional (equal level and phase) tone burst with centre frequencies at 1.5, 2 or 3 kHz ( $TB_2$ ) (Yoshikawa et al., 2000; Killan et al., 2012, 2015) or a pair of additional tone bursts at 2 and 3 kHz ( $TB_2$  and  $TB_3$ ) (Xu et al., 1994;

Killan and Kapadia, 2006). If the violation of linear superposition is significant, the conventional clinical interpretation of TEOAE place-specificity is not supported. Therefore, investigation of this simultaneous suppression phenomenon is important.

Collectively, findings from previous studies address a range of issues relating to simultaneous suppression of TBOAEs, including the effect of the frequency separation between TB<sub>1</sub> and TB<sub>2</sub> (referred to as  $\Delta f$ ) (Yoshikawa et al., 2000; Killan et al., 2012; Killan et al., 2015), tone burst level (Xu et al., 1994; Killan and Kapadia, 2006; Killan et al., 2015) and averaging techniques (Killan and Kapadia, 2006). None of these studies have measured suppression for both a single additional tone burst (e.g. TB<sub>2</sub> at 2 kHz)<sup>1</sup> and a pair of additional tone bursts (e.g. TB<sub>2</sub> and TB<sub>3</sub> at 2 and 3 kHz respectively) from the same ears. Consequently, the extent to which the additional presence of TB<sub>3</sub> affects suppression caused by TB<sub>2</sub> alone is not known. In principle, there are three possibilities. First, comparison of data from two similar studies that separately tested simultaneous suppression caused by TB<sub>2</sub> alone (Killan et al., 2015) and TB<sub>2</sub> and TB<sub>3</sub> (Killan and Kapadia, 2006) suggests TB<sub>3</sub> causes a *reduction* in the amount of suppression caused by TB<sub>2</sub>. Such behaviour is similar to the “release from masking” phenomenon described for the peripheral auditory system (e.g. Rutten and Kuper, 1982; Henry, 1987), however, it is unclear whether this is a genuine reduction, or simply reflects differences between the ears and methodologies used across studies. A reduction in suppression is also inconsistent with previously proposed mechanisms for simultaneous suppression of TBOAEs. These predict a second possible outcome where the additional

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<sup>1</sup> The convention for numbering tone bursts (i.e. TB<sub>1</sub> and TB<sub>2</sub>) was used by Killan et al. (2012). It is used here for simplicity when describing the present and previous studies, and is extended to include TB<sub>3</sub>. In the present use, the subscript number also refers to the centre frequency (in kHz) of the tone bursts.

presence of TB<sub>3</sub> causes an *increase* in suppression as a result of nonlinear interactions between response components generated at their characteristic BM place, or interference with the generation of short latency basal-source components (Yates and Withnell, 1999; Killan et al., 2012, 2015; Lewis and Goodman, 2015). Finally, the third possibility is that TB<sub>3</sub> has no effect on suppression.

To contribute to our understanding of simultaneous suppression of TBOAEs, the primary aim of this small-scale study was to explore the effect of TB<sub>3</sub> on the amount of suppression caused by TB<sub>2</sub> alone. To do this, TBOAEs were recorded from normal human ears in response to TB<sub>1</sub> presented in combination with TB<sub>2</sub>, as well as TB<sub>1</sub> with TB<sub>2</sub> and TB<sub>3</sub>, at a range of tone burst levels. In addition, observation of the effect of TB<sub>3</sub> is useful in defining the distance over which basal-source components in response to a 1 kHz tone burst arise. If TB<sub>3</sub> is shown to have no effect it can be argued that the BM region tuned to 3 kHz is not involved in the generation of components at 1 kHz (at least for the recording conditions described in this paper). Finally, the results presented within this paper could be used by future investigators to test predictions from their cochlear models.

## 2. Methods

### 2.1. Subjects

TBOAEs were recorded from a single ear (5 right, 4 left) from nine normally hearing adults (6 female, 3 male) aged between 18 and 33 years (median = 25 years). All ears tested had normal middle ear function as confirmed by tympanometry, repeatable TBOAEs at 50 dB p.e. SPL, i.e. the lowest tone burst level used in this study and did not exhibit synchronised spontaneous otoacoustic emissions as measured using the Otodynamics ILO 292 system (London, UK). Prior to testing, subjects gave informed consent in accordance with the requirements of the School of Healthcare Research Ethics Committee.

### 2.2. Instrumentation and stimuli

All TBOAE recordings were made using a custom-built system previously described by Killan et al. (2012). The synchronised input and output of a personal computer soundcard were controlled by purpose-written software. Stimuli were delivered to the ear canal via a custom-built amplifier and the earphone of an Otodynamics (London, UK) probe sealed into the ear canal with a soft plastic tip. The signal measured by the probe microphone was input to the soundcard (via a second amplifier) and was high-pass filtered (cut-off at 500 Hz with roll-off slope  $> 12$  dB/octave). The input signal was sampled at a rate of 24 kHz and time-averaged within two separate buffers. This resulted in a pair of replicate recordings, each formed from 250 averages, which were stored on disk and analysed off-line.



Tone bursts (TB<sub>1</sub>, TB<sub>2</sub> and TB<sub>3</sub>) were cosine-windowed sinusoids (rise-fall = 2.5 ms; plateau = 0 ms) with centre frequencies 1, 2 and 3 kHz respectively, identical to those used by Killan and Kapadia (2006). Tone bursts were presented sequentially and simultaneously in two combinations: (i) TB<sub>1</sub> and TB<sub>2</sub>; and (ii) TB<sub>1</sub>, TB<sub>2</sub> and TB<sub>3</sub>, which were the same combinations used separately by previous investigators. Simultaneous presentation was achieved via a complex stimulus resulting from the digital addition of the individual tone bursts. All tone bursts were presented using linear averaging at 50, 60 and 70 dB p.e. SPL (as calibrated within a passive 2 cm<sup>3</sup> cavity) and a rate of 50/s. Linear averaging was preferred to nonlinear averaging as it preserves linear and nonlinear components of the individual and complex responses. Preliminary testing indicated that stimuli at 50, 60 and 70 dB p.e. SPL corresponded to approximately 35, 45 and 55 dB sensation level respectively, and as such the response characteristic of the cochlea is assumed to be nonlinear (e.g. Kim et al., 1980; Nuttall and Dolan, 1996; Patuzzi, 1996; Rhode and Recio, 2000; Ren, 2002; Gorga et al., 2007).

### 2.3. Procedure

For each subject, TBOAE recordings were made during a single recording session lasting approximately one hour. Subjects were comfortably seated in a sound-attenuated room, and instructed to remain quiet and still throughout recordings. The probe was sealed in the ear canal with a soft plastic tip and was taped in position for the duration of testing. In order to minimise potential order effects, the presentation order of individual and complex tone bursts was randomised across tone burst level.

#### 2.4. Analysis

At each tone burst level, a mean response waveform was calculated for all individual tone bursts and the two complex stimuli. Two “composite” response waveforms were then generated by summing the mean response waveforms of TB<sub>1</sub> and TB<sub>2</sub> and the mean waveforms of TB<sub>1</sub>, TB<sub>2</sub> and TB<sub>3</sub>. Thus, for each subject and at each tone burst level, there was a two-tone burst and a three-tone burst composite (i.e. the predicted linear response) and complex (i.e. the simultaneous response) waveform. In order to minimise the influence of linearly scaling stimulus ringing components the first 8 ms (post-stimulus onset) of each composite and complex waveform was discarded from subsequent analysis. Removal of such a substantial portion of the waveform is not unusual when recording TBOAEs (e.g. Rutten, 1980; Prieve et al., 1996; Killan and Kapadia, 2006), but is done at the cost of TBOAE response components with latencies shorter than 8 ms. As the focus was on suppression of 1 kHz components, and both long and short-latency response components at 1 kHz have latencies longer than 8 ms (e.g. Notaro et al., 2007; Goodman et al., 2009), the loss of this portion of the waveform was not considered material. TBOAE frequency spectra (in dB SPL/Hz) of the composite and complex waveforms and noise spectra from the complex waveforms<sup>2</sup> were then calculated using a 512-point fast Fourier transform (FFT). These noise spectra were used as estimates of the noise floor. Any values in the composite and complex spectra below the noise floor were replaced by the value of the noise spectrum at that frequency. This ensured any differences subsequently observed between the composite and complex TBOAE spectra arose from points clear of the noise floor. A ‘difference spectrum’ was then calculated by subtracting the complex spectrum from the corresponding

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<sup>2</sup> The complex noise spectrum was used to calculate the estimate of the noise floor for both the composite and complex spectra because results of pilot testing had shown that at all three tone burst levels, the greatest noise levels were contained within the complex response.

composite spectrum. Within these difference spectra, suppression is represented by regions of positive values.

Suppression was estimated along the high frequency slope of the response to TB<sub>1</sub> only. To do this a dominant peak within the region of 1 kHz was identified within the composite spectra. Suppression (in dB) was then estimated as the mean difference in spectral level (composite – complex) within an arbitrary 0.5 kHz-wide frequency band above the frequency of the dominant peak. This approach allowed for the predicted between-subject variation in the frequencies at which suppression occurred (e.g. Probst et al., 1986; Xu et al., 1994; Yoshikawa et al., 2000; Killan and Kapadia, 2006). Paired *t*-tests were used to test any differences in suppression obtained for TB<sub>1</sub> and TB<sub>2</sub> ( $S_{TB2}$ ) and suppression obtained for TB<sub>1</sub>, TB<sub>2</sub> and TB<sub>3</sub> ( $S_{TB2+3}$ ) for statistical significance using a Bonferroni-corrected significance level of  $p < 0.01$ .

### 3. Results

The left hand panels of Fig. 1 show the composite (bold) and complex (fine line) response spectra for the combination of  $TB_1$  and  $TB_2$  at 50, 60 and 70 dB p.e. SPL measured from an individual ear. Simultaneous suppression is evident at all three levels as a reduction in amplitude of the complex response relative to the composite spectra, notably along the high frequency side of the response peak at 1.3 kHz. The right hand panel of Fig. 1 shows the resultant difference spectrum (composite – complex). The main feature of these difference spectra is the region of suppression around 1.5 kHz, most notable at 60 and 70 dB p.e. SPL. The left hand panels of Fig. 2 show the spectra obtained for  $TB_1$ ,  $TB_2$  and  $TB_3$  for the same ear as shown in Fig. 1. Again, suppression is evident along the high frequency side of the dominant peak at 1.3 kHz. This is confirmed by the corresponding difference spectra shown in the right hand panels. Visual inspection of these reveals a tendency for peak suppression to increase as a function of increasing tone burst level.

Figs 3 and 4 show the mean results ( $n = 9$ ) for  $TB_1$  and  $TB_2$  and  $TB_1$ ,  $TB_2$  and  $TB_3$  respectively. Similar patterns of suppression to those seen for the individual ear are apparent. In Fig. 3 suppression is present in the region of 1.5 kHz. Mean suppression increases from 1.5 to 2.6 dB as tone burst level increases from 50 to 60 dB p.e. SPL, with a further increase to 70 dB p.e. SPL resulting in a small reduction in suppression to 2.5 dB. Again, mean suppression of the 1 kHz response peak increased as tone burst level increased from 50 to 60 dB p.e. SPL (1.9 to 3.3 dB), with a reduction to 2.2 dB seen for a further increase to 70 dB p.e. SPL. A region of suppression, corresponding to the 2 kHz response peak, is also evident in Fig. 4.

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207 Fig. 5 allows comparison of suppression obtained for TB<sub>1</sub> with TB<sub>2</sub> ( $S_{TB2}$ ) versus suppression  
 208 obtained for TB<sub>1</sub>, TB<sub>2</sub> and TB<sub>3</sub> ( $S_{TB2+3}$ ) at 50, 60 and 70 dB p.e. SPL for all nine subjects  
 209 (open circles). The diagonal dashed line is the line of equality, i.e. the line along which a  
 210 data-point would lie if  $S_{TB2}$  and  $S_{TB2+3}$  were equal. A data-point to the left of this line  
 211 indicates  $S_{TB2+3}$  was greater than  $S_{TB2}$  whilst a data-point to the right shows  $S_{TB2}$  was greater.  
 212 At each of the three tone burst levels, ears that exhibited larger  $S_{TB2}$  tended to also exhibit  
 213 larger values of  $S_{TB2+3}$ . At 50 dB p.e. SPL,  $S_{TB2+3}$  was greater than  $S_{TB2}$  in seven out of nine  
 214 subjects. The data-point representing mean suppression (filled circle) was also located to the  
 215 left of the line of equality. However, the mean paired difference between  $S_{TB2}$  and  $S_{TB2+3}$   
 216 (0.40 dB) was shown not to be significant ( $t = 1.07$ ,  $p = 0.32$ ). Similar results were seen at 60  
 217 dB p.e. SPL, with six ears yielding larger values of  $S_{TB2+3}$ . Mean suppression again indicated  
 218 greater  $S_{TB2+3}$ , though the mean difference (0.67 dB) did not reach significance ( $t = 1.7$ ,  $p =$   
 219 0.16). At 70 dB p.e. SPL four out of nine ears exhibited greater  $S_{TB2+3}$ , with mean  
 220 suppression located to the right of the line of equality, indicating  $S_{TB2}$  tended to be greater  
 221 than  $S_{TB2+3}$ . This small difference (0.24 dB) was not significant ( $t = -0.66$ ,  $p = 0.53$ ). Finally,  
 222 visual inspection of mean results at 50 and 60 dB p.e. SPL confirms the increase of mean  
 223 suppression with increasing tone burst level. However, a further increase to 70 dB p.e. SPL  
 224 resulted in a reduction in mean suppression. This likely reflects a contamination of the  
 225 TBOAE responses by extended stimulus ringing. Because stimulus ringing is essentially  
 226 linearly scaling it would not exhibit suppression.

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#### 4. Discussion

Simultaneous suppression of TBOAEs has been the subject of a number of studies, with suppression of the response to a 1 kHz tone burst (TB<sub>1</sub>) described separately for a single additional higher frequency tone burst (TB<sub>2</sub>) (Yoshikawa et al., 2000; Killan et al., 2012; Killan et al., 2015) and a pair of additional higher frequency tone bursts (TB<sub>2</sub> and TB<sub>3</sub>) (Xu et al., 1994; Killan and Kapadia, 2006). No previous study has measured suppression for both these conditions from the same ears, so that a question that remains unanswered is what effect does the additional presence of TB<sub>3</sub> have on suppression caused by TB<sub>2</sub> alone? A comparison of data from two separate studies of simultaneous suppression of TBOAEs (Killan and Kapadia, 2006; Killan et al., 2015) lends support to suppression being reduced; however, it is not clear whether this simply represents differences between the methodologies and ears used by the two studies. In simple terms, two alternative possibilities exist: TB<sub>3</sub> causes an increase in suppression or TB<sub>3</sub> has no effect on suppression. The results of the present study demonstrate that whilst the additional presence of TB<sub>3</sub> caused both an increase and reduction in suppression in individual ears, it had no significant effect on mean suppression caused by TB<sub>2</sub> at all three tone burst levels. It is therefore considered likely that the apparent reduction in suppression reported for two and three-tone burst combinations by Killan et al. (2015) and Killan and Kapadia (2006) simply reflects methodological and ear differences.

The present study used the same tone burst combinations as previous investigators (i.e. 1 and 2 kHz and 1, 2 and 3 kHz). This allowed the specific question relating to the comparison of data reported by Killan and Kapadia (2006) and Killan et al. (2015) to be addressed.

However, this choice of frequencies was likely to limit the outcomes possible within the present study. For example, for an increase in suppression to occur it can be argued that  $TB_3$  alone has to be capable of producing suppression of either the 1 kHz response component that originates from its tonotopic place (e.g. Kemp and Chum, 1980; Tavartkiladze et al., 1994; Killan et al., 2012; Moleti et al., 2013) or the short-latency basal-source component (e.g. Yates and Withnell, 1999; Withnell et al., 2008; Moleti et al., 2013; Lewis and Goodman, 2015). Contrary to this, previous simultaneous suppression of TBOAEs data show a 3 kHz tone burst caused little or no suppression of response components at 1 kHz (Yoshikawa et al., 2000; Killan et al., 2012; Killan et al., 2015). The current data are also consistent with recent research that has shown the basal-source response component originates from a BM region located approximately 3/5-octave basal to its tonotopic place (Lewis and Goodman, 2015). A 3 kHz tone burst is too remote to cause suppression of those basal-source 1 kHz components that were preserved by the time-window used in this and previous studies. In this regard, it can be argued that the present data are compatible with previously proposed mechanisms for simultaneous suppression of TBOAEs (e.g. Yates and Withnell, 1999; Killan et al., 2012, 2015).

To better understand this suppression behaviour, further investigation is warranted using tone bursts with different frequencies that are more likely to cause interactions necessary for significant suppression to occur. Further investigation could also address whether the results from this small-scale study hold for large numbers of subjects, or whether there are sub-groups that exhibit one of the different suppression behaviours outlined above. Recording techniques that preserve the short-latency basal-source component (e.g. Keefe, 1998; Withnell et al., 2008) and analysis techniques that decompose the TBOAE in the time and frequency domain (e.g. Jedrzejczak et al., 2004; Moleti et al., 2012) should be also be utilised.

277 However, the present results provide data against which the predictions of cochlear models  
278 can be compared.

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**Fig. 1. Composite (bold line) and complex (fine line) spectra and the corresponding difference spectra for TB<sub>1</sub> and TB<sub>2</sub> at 50, 60 and 70 dB p.e. SPL obtained from an individual ear.**

**Fig. 2. Composite (bold line) and complex (fine line) spectra and the corresponding difference spectra for TB<sub>1</sub>, TB<sub>2</sub> and TB<sub>3</sub> at 50, 60 and 70 dB p.e. SPL from the same individual ear shown in Fig. 1.**

**Fig. 3. Mean composite (bold line) and complex (fine line) spectra and the corresponding difference spectra for TB<sub>1</sub> and TB<sub>2</sub> at 50, 60 and 70 dB p.e. SPL.**

**Fig. 4. Mean composite (bold line) and complex (fine line) spectra and the corresponding difference spectra for TB<sub>1</sub>, TB<sub>2</sub> and TB<sub>3</sub> at 50, 60 and 70 dB p.e. SPL.**

**Fig. 5. Scatter plots of  $S_{TB2}$  and  $S_{TB2+3}$  for individual ear (open circles) at 50, 60 and 70 dB p.e. SPL. Mean values ( $\pm 1$  standard error) is also shown (filled circles). The dashed diagonal line is the line of equality.**

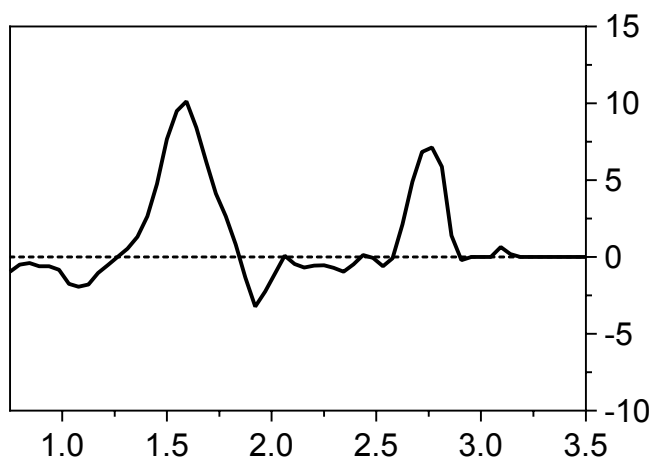
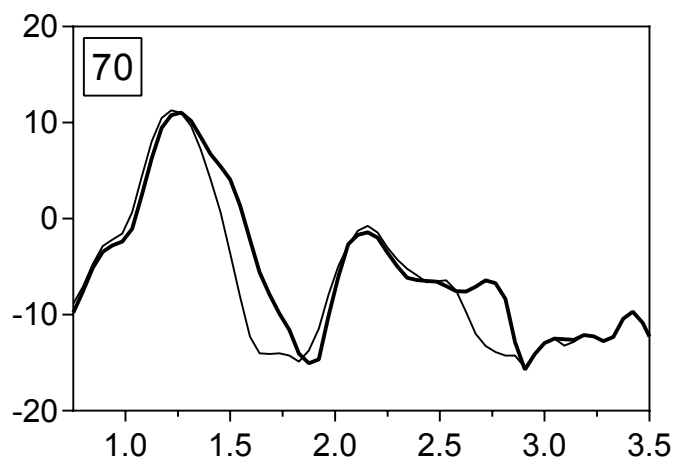
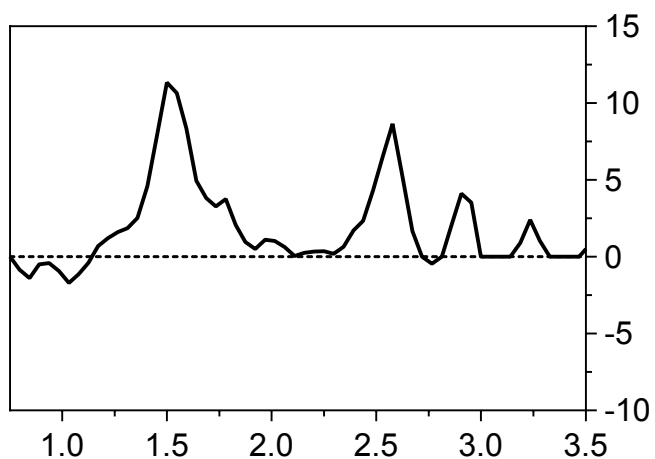
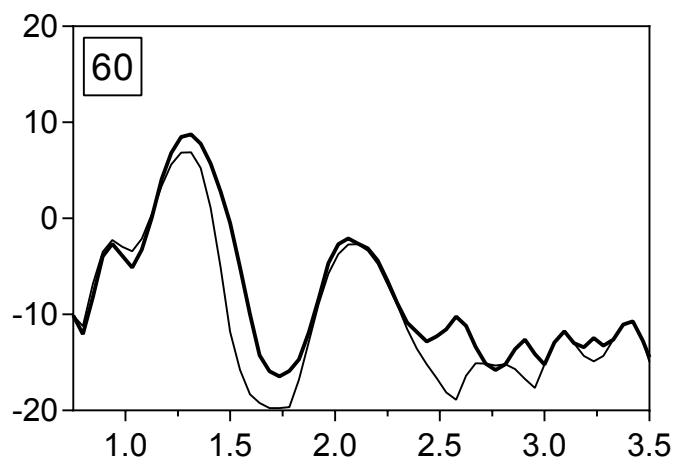
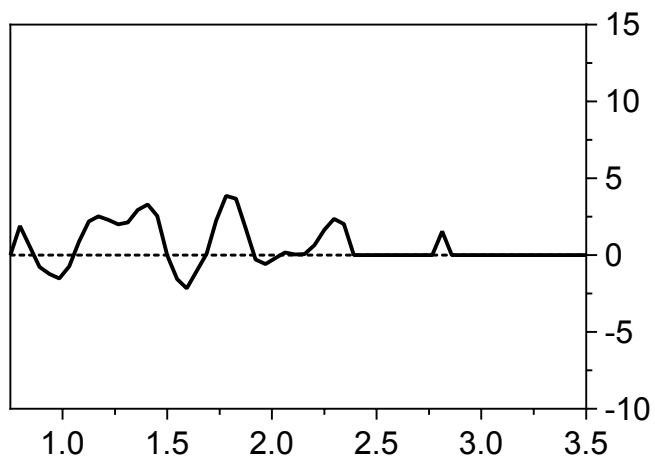
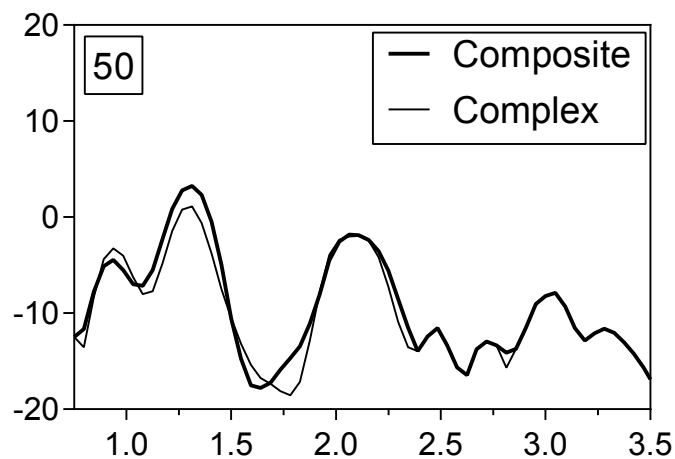
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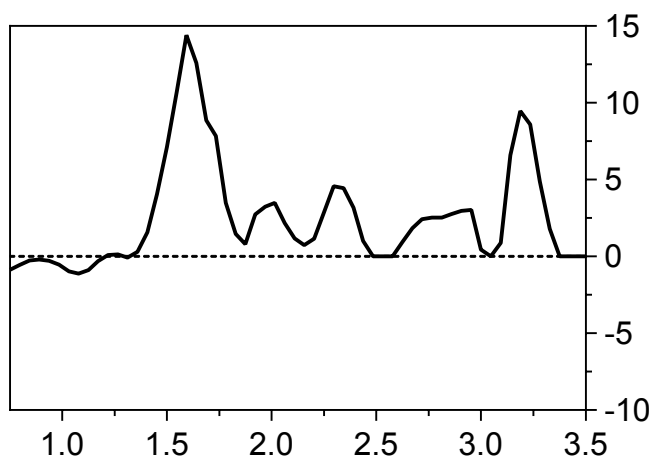
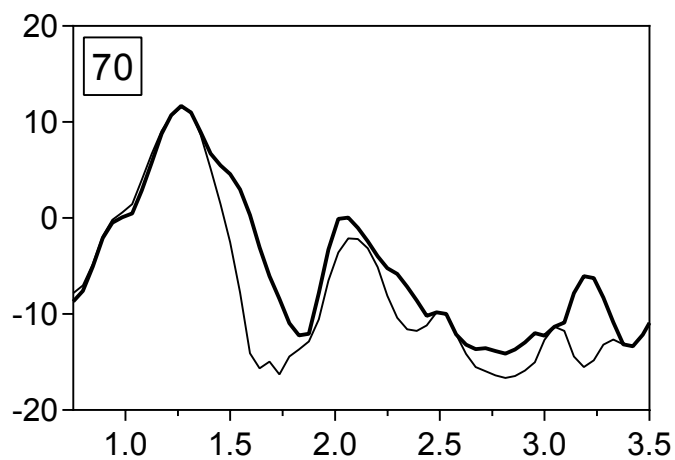
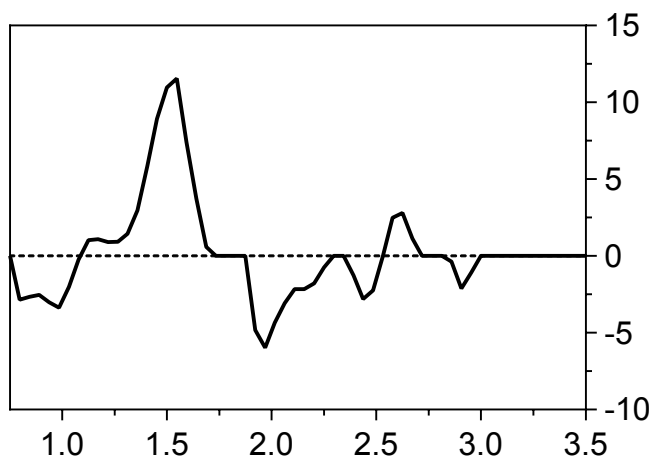
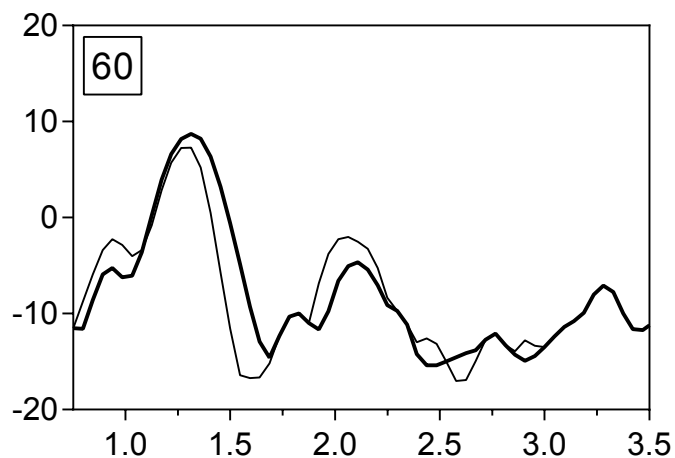
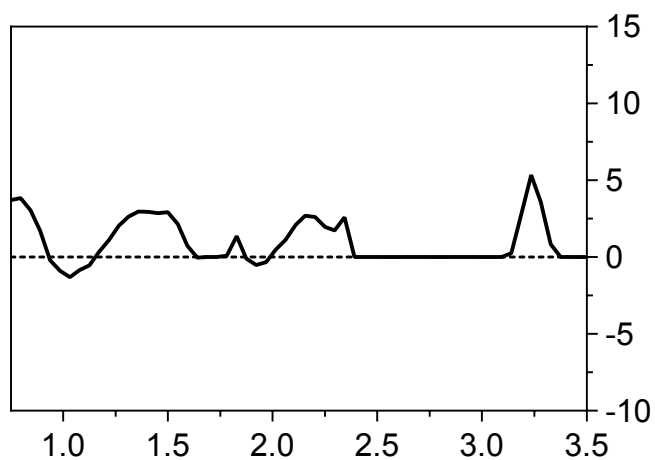
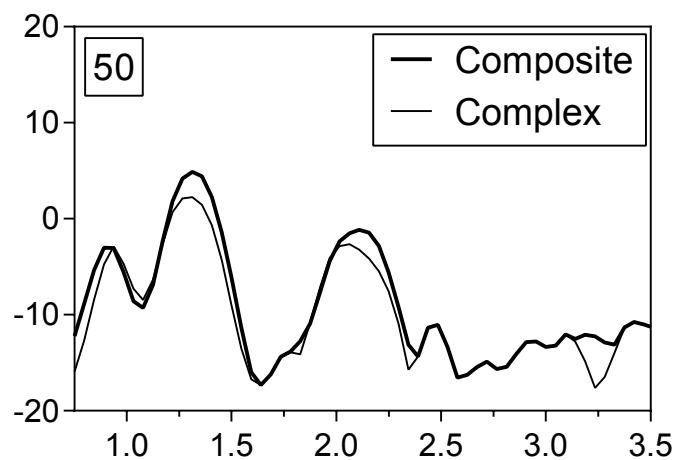
TBOAE level (dB SPL/Hz)



Difference (dB)

Frequency (kHz)

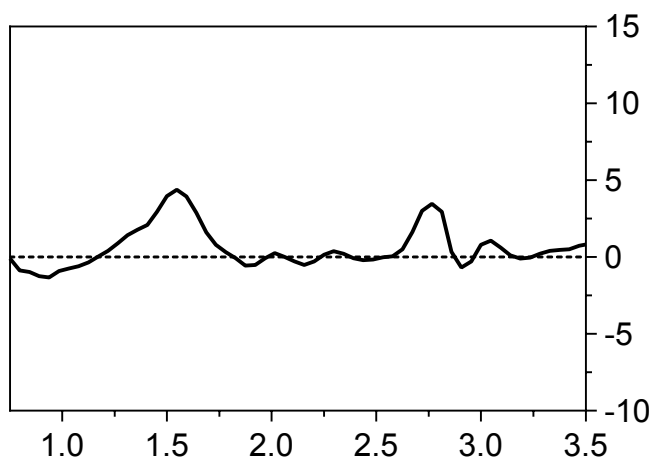
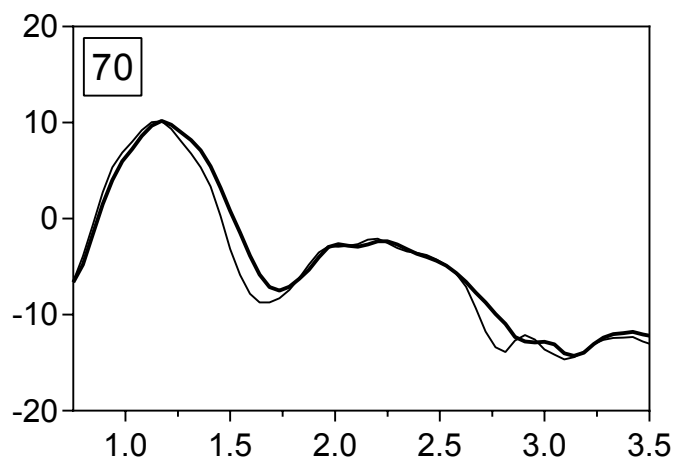
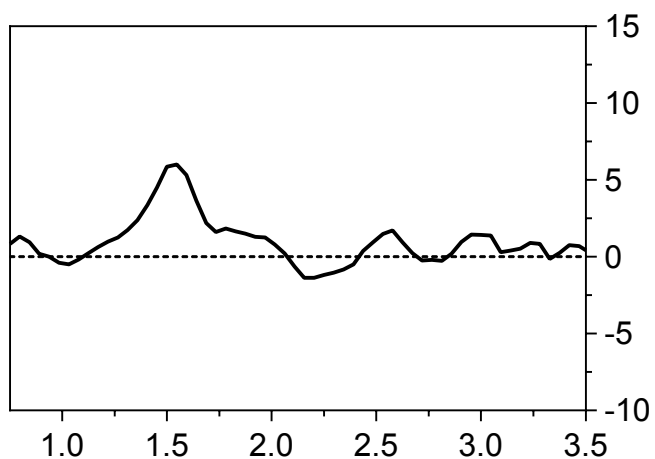
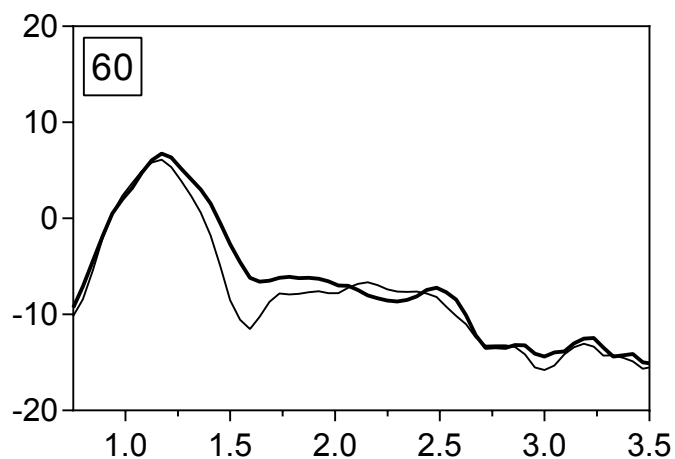
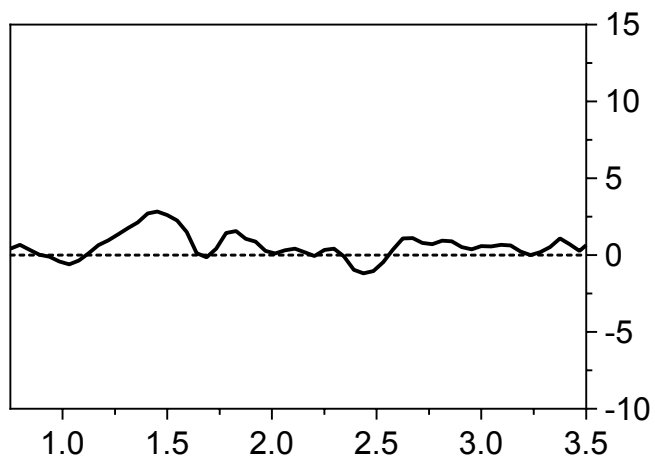
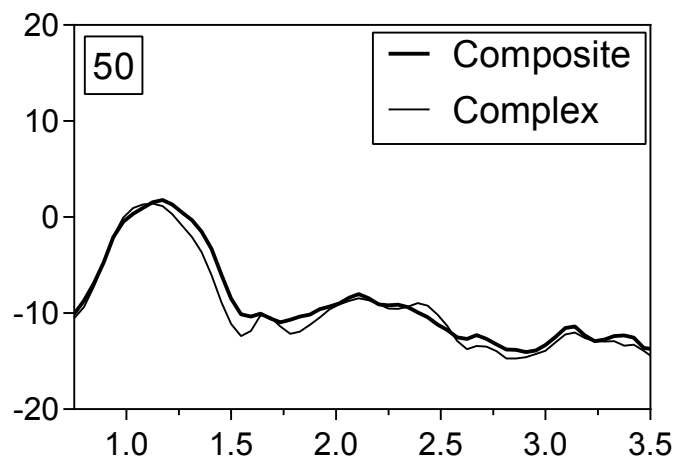
TBOAE level (dB SPL/Hz)



Difference (dB)

Frequency (kHz)

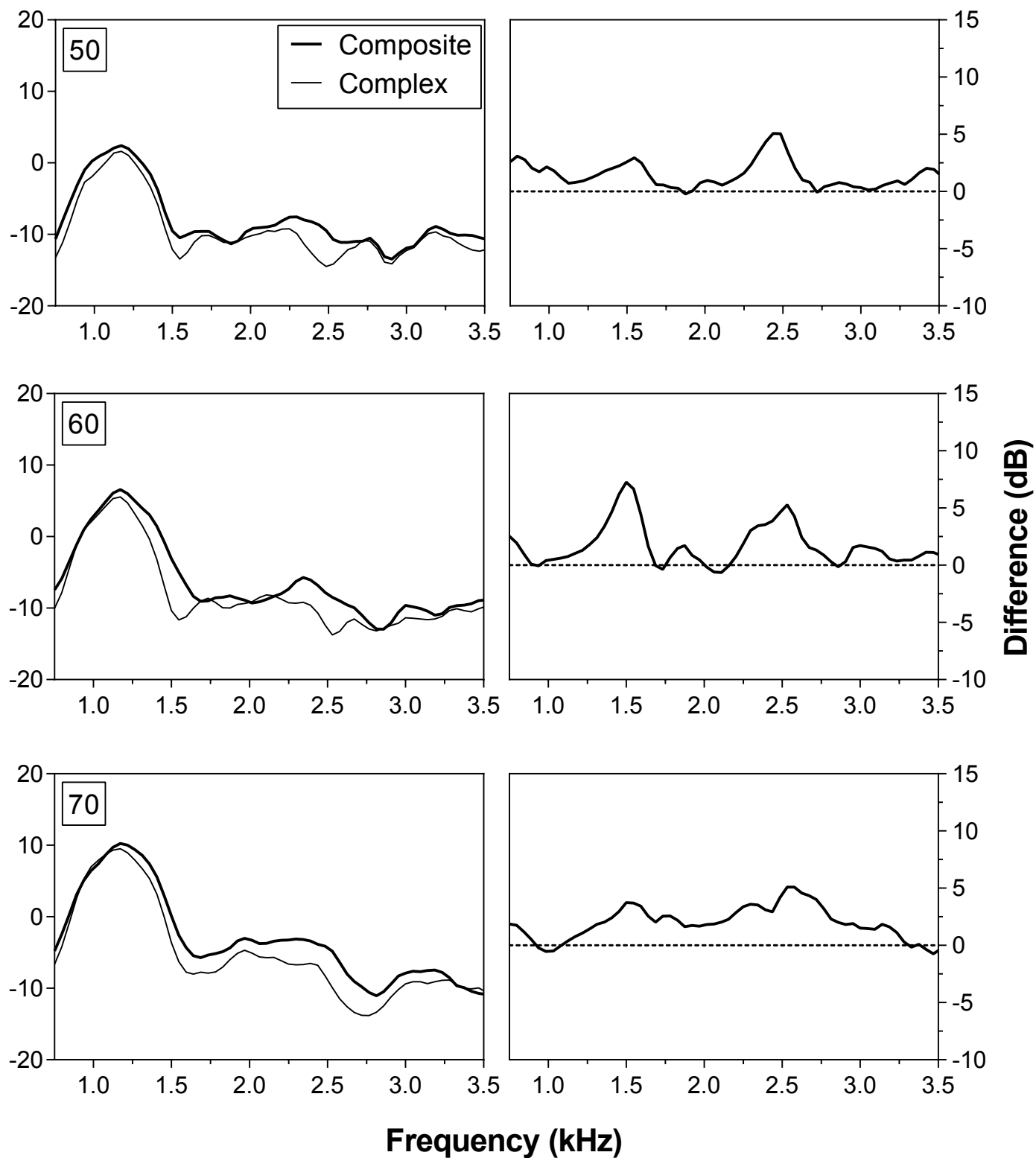
TBOAE level (dB SPL/Hz)



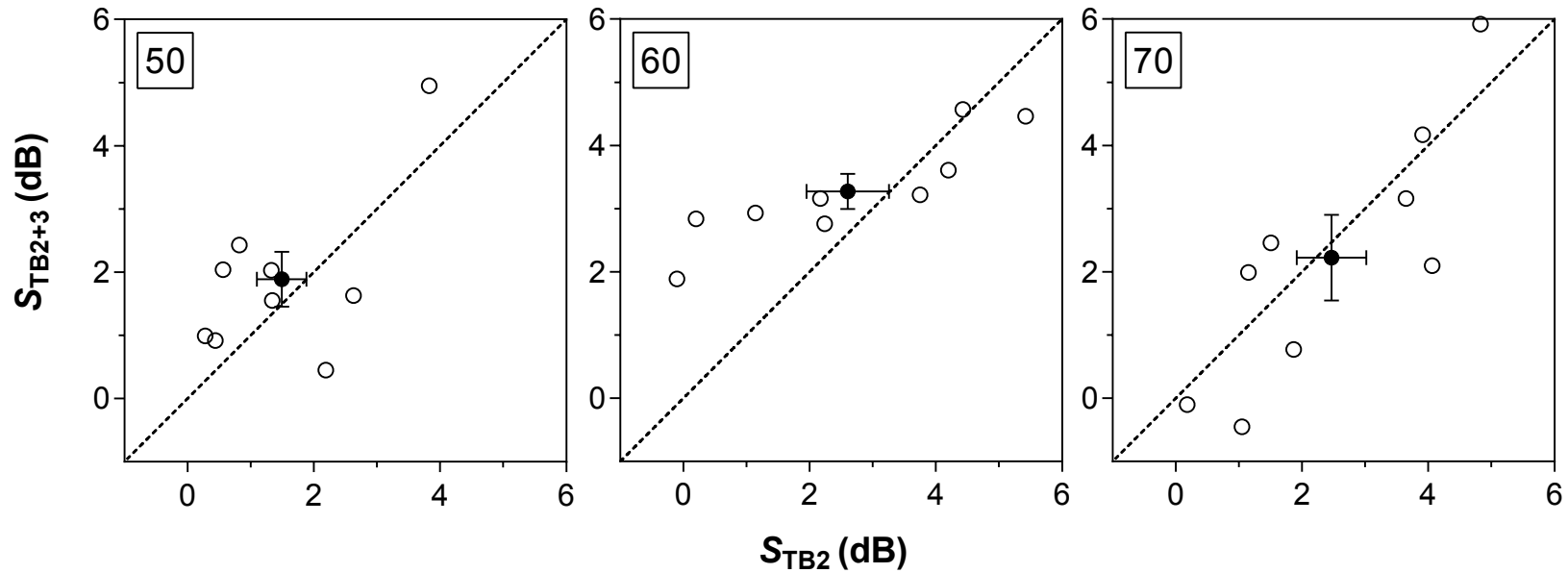
Difference (dB)

Frequency (kHz)

TBOAE level (dB SPL/Hz)







Previous data are unclear regarding tone burst number and TBOAE suppression.

Suppression of TBOAEs is measured for two and three tone burst combinations.

No material difference in mean suppression was observed between combinations.

Previously reported differences reflect ear and methodological differences.