Title: Novel methods to distinguish among stocks of spotted grunter: an overexploited estuarine small-scale fisheries target.

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Background:
Estuaries are productive ecosystems which provide food to many organisms as well as local communities. In South Africa, estuaries are important to small-scale fishers whose livelihoods depend on these productive ecosystems. However, many of the estuarine-dependent fish species on which they depend are overexploited, such as Pomadasys commersonii, the spotted grunter. Despite the importance of estuarine-dependent fish species, not much is known about their stock status, this negatively affects effective management and utilization of these species. It is hypothesised that there are different stocks of spotted grunter among South African estuaries, and thus it is crucial to determine connectivity among estuaries and to identify sub-populations with significantly different traits. Effective management requires stock delineation with possibly spatially explicit management strategies. To determine if spotted grunter have multiple stocks, a holistic approach was used to identify morphological and ecological differences between individuals from different estuaries. The aim of this study was to determine whether the Breede river population differs from the Kei river population by comparing otolith shape morphology and vocal ‘grunt’ dialects between spotted grunter from each estuarine system.

Methods:
Otoliths were removed from individuals captured in the Breede river estuary and the Kei mouth estuary. To capture images of the otoliths, each otolith was raised, with the use of a small pipe, on a black background and captured using a Leica camera. The otoliths (left hand side) were positioned with the rostrum to the left and the sulcus facing upward. The images were captured in full colour and saved as a jpeg file. An R-package software, known as ShapeR, was used to detect the outlines and shape coefficients of the otoliths. ShapeR uses an outline analysis approach which can extract and generate shape data. By quantifying the boundary shapes of the otoliths, we are able to determine the patterns associated within or among groups in terms of stocks. ShapeR has built in functions which are able to transform the outlines using either normalized elliptic Fourier analysis or a discrete wavelet analysis. These analyses will generate coefficients for the otolith outlines. A canonical analysis of principal coordinates (CAP) analysis was conducted to analyse the variation in shape among the populations, this result was displayed on a cluster plot. An ANOVA test was also performed to determine the partition of variation among groups. ShapeR is able to plot the mean shape of otoliths for each area to compare the outlines visually.
For the acoustic method of stock discrimination, a proof of concept study was conducted using recordings downloaded from Fishbase. These short recordings (<10 seconds) were used to determine if soundings from different fish families, genera and species could be differentiated. Acoustic parameters (amplitude, frequency and time) were measured using Raven Pro and then run through a principal component analysis (PCA) to determine whether
individual species could be identified via call types. To determine if different families could be distinguished, the vocal sounds of a dusky kob were compared to those of multiple Haemulidae species. Next, multiple species within the Haemulidae family were compared and lastly species from within the *Haemulon* genus were compared.

**Results:**
The ANOVA test suggested that there are significant differences ($P<0.05$) in otolith shape morphology among spotted grunter populations, this was further substantiated by the CAP analysis which also suggested a significant difference in otolith shape parameters among spotted grunter populations.

There were significant differences ($P < 0.05$) in the acoustic parameters of the dusky kob compared to the Haemulidae species, between different Haemulidae genera and lastly between *Haemulon* species. Frequency parameters (peak frequency, delta frequency and centre frequency) had the highest variable importance contributing to the spread of each species.

**Conclusion:**
The significant differences in otolith shape morphology are most likely influenced by the variability in environmental conditions among the spotted grunter populations. This result suggests that there are multiple stocks of spotted grunter within South Africa. Given the significant differences in the morphology of their hearing apparatus (otoliths) we have further reason to believe that there may be differences in their acoustic repertoires.

The differentiation of vocalisations among *Haemulon* species was successful despite limited data and it is hypothesized that extended recordings should provide enough information to differentiate subpopulations within a species. These results suggest that acoustic parameters may be a useful method of stock discrimination for vocal species such as the spotted grunter. This project adopts acoustics in a novel way, providing a proof of concept for the use of fish vocalisation and acoustics for stock discrimination. This novel technique will hopefully expand the implementation of fish acoustics in South African marine research with the aim to eventually develop a measure of fish abundance using acoustics to provide alternative density estimates to catch-per-unit-effort (CPUE).