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Multivariate classification of animal communication signals: A simulation-based comparison of alternative signal processing procedures using electric fishes

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ABSTRACT

Evolutionary studies of communication can benefit from classification procedures that allow individual animals to be assigned to groups (e.g. species) on the basis of high-dimension data representing their signals. Prior to classification, signals are usually transformed by a *signal processing* procedure into structural features. Applications of these signal processing procedures to animal communication have been largely restricted to the manual or semi-automated identification of landmark features from graphical representations of signals. Nonetheless, theory predicts that automated time-frequency-based digital signal processing (DSP) procedures can represent signals more efficiently (using fewer features) than can landmark procedures or frequency-based DSP – allowing more accurate classification. Moreover, DSP procedures are objective in that they require little previous knowledge of signal diversity, and are relatively free from potentially ungrounded assumptions of cross-taxon homology. Using a model data set of electric organ discharge waveforms from five sympatric species of the electric fish *Gymnotus*, we adopted an exhaustive simulation approach to investigate the classificatory performance of different signal processing procedures. We considered a landmark procedure, a frequency-based DSP procedure (the fast Fourier transform), and two kinds of time-frequency-based DSP procedures (a short-time Fourier transform, and several implementations of the discrete wavelet transform -DWT). The features derived from each of these signal processing procedures were then subjected to *dimension reduction* procedures to separate those features which permit the most effective discrimination among groups of signalers. We considered four alternative dimension reduction methods. Finally, each combination of reduced data was submitted to classification by linear discriminant analysis. Our results support theoretical predictions that time-frequency DSP procedures (especially DWT) permit more efficient discrimination of groups. The performance of signal processing was found to depend largely upon the dimension reduction procedure employed, and upon the number of resulting features. Because the best combinations of procedures are dataset-dependent and difficult to predict, we conclude that simulations of the kind described here, or at least simplified versions of them, should be routinely executed before classification of animal signals - especially unfamiliar ones.

1. Introduction

Animal communication signals exhibit variation among and between individuals, sexes, populations, and species. This variation underlies many evolutionary and ecological processes, including sexual selection, reproductive isolation, and speciation (e.g. Henry et al., 1999; Shaw, 2000; Panhuis et al., 2001; Coyne and Orr, 2004). Biologists therefore need dependable methods for assessing the probability of a given signal belonging to one or more categories of signalers (e.g. population, species etc.), and for quantifying the extent to which categories of signals are divergent.

Multivariate classification procedures, such as linear discriminant analysis (LDA), are designed to meet these needs (Huberty, 1994). For example, an evolutionary biologist might employ LDA to investigate the extent to which geographically isolated populations of a given species exhibit divergent signals. Likewise, an ecologist studying a small group of animals might utilize LDA to ask whether individuals can be reliably recognized, and hence

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Abbreviations: DSP, digital signal processing; DWT, discrete wavelet transform; FFT, fast Fourier transform; EOD, electric organ discharge; LDA, linear discriminant analysis; PPF, peak power frequency; PSD, power spectral density; STFT, short-time Fourier transform.

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monitored, on the basis of subtle but consistent differences among their signals.

Acoustic, seismic, and also electrical signals are especially amenable to multivariate classification. Unlike visual or chemical signals, they comprise oscillations that can be readily converted to electrical voltages in a recording device, and stored digitally as an amplitude-time waveform. Typically, such a waveform comprises a few hundred to thousands of digital samples, recorded at a sampling rate high enough to adequately represent the shortest (highest frequency) events (Smith, 1999). Digitized signals are subjected to classification in three separate procedures. First, in *signal processing*, the raw amplitude-time waveform is converted to representative structural features in the time, frequency, or time-frequency domain. In the second procedure, these features are subjected to *dimension reduction*, in which features not conducive to discrimination are eliminated from consideration. Finally, the remaining features are subjected to a *multivariate classification* procedure, e.g. LDA. At this stage, posterior probabilities of group membership are computed for each case, and misclassified cases are identified. In this paper we use a model dataset of electric fish signals to explore the extent to which different signal processing and dimension reduction procedures influence the performance of subsequent multivariate classification using LDA. We quantify the performance of LDA as the total proportion of correctly classified cases.

The freshwater electric fishes of Africa (Mormyriformes) and the Neotropics (Gymnotiformes) generate a continuous train of weak, stereotyped, electric organ discharges (EODs). In combination with an associated array of electroreceptors, these EODs facilitate both electrolocation and communication, including mate attraction (Bullock et al., 2005). The EOD comprises a pulsed or continuous quasi-sinusoidal three-dimensional electrostatic field, with considerable near-field complexity (e.g. Assad et al., 1999; Rodriguez-Cattaneo et al., 2008). However, for comparative studies of signal evolution, the EOD is usually characterized as a two-dimensional voltage-time waveform recorded from electrodes placed in the far field, anterior to a fish's head, and posterior to its tail. Having served for several decades as a model group for many key areas of neurobiology (Bullock et al., 2005), electric fish are now demonstrating great promise in evolutionary studies, including those of speciation (e.g. Arnegard et al., 2005), and sexual selection (e.g. Curtis and Stoddard, 2003). There is therefore a specific and growing demand for signal processing and dimension reduction procedures that permit accurate multivariate classification of electric fish signals.

We begin with a review of the principles of signal processing, dimension reduction, and classification – discussing procedures suitable for animal signal analysis, with examples where relevant (Section 2). We then conduct a large-scale computer simulation to test the performance of alternative procedures, using a model dataset of EODs (Sections 3–4). The dataset was gathered from an assemblage of five sympatric and syntopic species of the pulse-generating gymnotiform genus *Gymnotus*, from floodplain habitats of the Central Amazon basin. In the discussion (Section 5) we explore reasons why some procedures may perform better than others, and discuss the practical implications for animal signal analysis. We also explore the extent to which different signal processing tools offer advantages – not just in their discriminatory performance but also in the extent to which they are dependent upon assumptions of homology among signal features, and the extent to which they may approximate the neurological mechanisms employed by electric fish (or other animals) to discriminate groups of individuals. The biological significance of signal divergence in the model data set is not discussed here beyond general considerations relevant to the performance and suitability of different analytical approaches; an evolutionary investigation of signal divergence in Central Amazonian *Gymnotus* is forthcoming in a separate paper. Also, the ability of *Gymnotus* to discriminate signals based on other aspects aside from waveform, such as pulse repetition rates and amplitude, will be discussed elsewhere.

2. Background

2.1. Signal processing

Digitized animal signal waveforms can be converted into features amenable to multivariate classification through landmark-based models or digital signal processing (DSP) techniques based on Fourier or wavelet analysis. DSP procedures transform a signal from the time domain (the signal itself) to the frequency domain (by the discrete Fourier transform) or the time-frequency domain (by the short-time Fourier or wavelet transform) (Smith, 1999). These procedures yield a complex coefficient (representing the magnitude and phase) for each frequency, which permits reconstruction of the signal by inverse transform. Often only the magnitudes of these coefficients are retained, effectively ignoring the phase component, which can be understood as relative timing information – indicating when a particular frequency occurs. DSP techniques can provide efficient representations of the signal in that only few of the components may be essential for the recovery of the signal.

Landmark approaches select features using a priori biological information about how signals vary within and among groups of signalers. They may also incorporate hypotheses of evolutionary homology based on an understanding that specific signal features have common inherited developmental and physiological or mechanical substrates in the animal taxa under consideration. In contrast, DSP procedures make no assumptions about signal homology (Section 5.2.2). The only imposed assumption for the Fourier or wavelet procedures is that all signals are aligned to a single time reference point, which itself may be selected on the basis of assumptions of homology (Section 5.3).

2.1.1. Digital signal processing (DSP)

2.1.1.1. Fast Fourier transform (FFT) and power spectral density (PSD). The fast Fourier transform (FFT), by which we mean any efficient implementation of the discrete Fourier transform, expresses a time-varying signal waveform in the frequency domain as a combination of its component frequencies; e.g. the Fourier transform may report that a given frequency has a certain energy somewhere in the signal. Associated with this energy or magnitude is a phase component, giving very rough timing information, which together permit the waveform to be reconstructed by an inverse transform (Bracewell, 2000). The magnitudes alone of the FFT coefficients, commonly called the power spectral density (PSD), constitute the data traditionally discussed in electric fish research. The PSD allows a rapid assessment of the peak power frequency (PPF), or harmonics of this. This is biologically relevant because the frequency responses of some classes of tuberous electroreceptors are closely tuned to the PPF (or higher harmonics in some wave-EOD species) in both gymnotiform and mormyriform fishes (Bastian, 1976; Hopkins, 1976; Watson, 1979).

The PSD is non-invertible, i.e. once the phase information has been removed, the original signal waveform cannot be recovered by inverse transform. Hence the PSD effectively contains no information about the timing of events in the signal. For example, the PSD alone is unable to discriminate the reversed versus original versions of an asymmetrical waveform because the Fourier phase components are absent. Many species of electric fishes with wave type discharges have more or less symmetrical waveforms repeated as stationary waves (including many species of Sternopygidae and Apteronotidae, and also *Gymnarchus niloticus*). Here, the PSD alone should represent signals with great efficiency. However, most EOD waveforms are highly asymmetrical, especially those produced by the pulse-EOD generating electric fishes (Mormyridae, Gymnotidae, Rhamphichthyidae, Hypopomidae), and also by many representatives of the wave-EOD generating fishes (Sternopygidae and Apteronotidae). In these cases, multivariate classification of signals based on DSP is expected to benefit from the inclusion of the phase components in the FFT, or from one of the time-frequency approaches (Sections 2.1.1.2, 2.1.1.3 and 5.3).

2.1.1.2. Short-time Fourier transform (STFT) and spectrogram. If a frequency in a signal is present only over a short period, the FFT will report this transient frequency, but in addition will suggest the existence of many other frequencies, all of which are physically meaningless and serve only to restrict the transient frequency, mathematically, to a specific area of the entire signal. Hence, the FFT will have actually multiplied, rather than reduced, the dimensionality of the data. Consequently, the FFT tends to become less efficient as a signal becomes increasingly spatially heterogeneous (Gabor, 1946; Smith, 1999). The short-time Fourier transform (STFT) corrects this deficiency by analyzing only a small portion of the signal at a time – a procedure called windowing. Utilizing a window function, which slides through the signal, STFT maps separate portions of the signal into the frequency domain, ultimately mapping the entire signal into the time-frequency domain (Smith, 1999). An analog to the PSD, the similarly non-invertible spectrogram is defined as the magnitudes of the STFT. The spectrogram is a plot of frequency components against time, with the magnitude of the frequency components usually expressed as gray-tone intensity. The fixed window length means that the STFT is limited to a specific frequency and time resolution. Short window lengths provide poor resolution of low frequencies while longer windows allow short events to be only approximately localized in time. Hence STFTs are predicted to be inefficient in comparing groups in which the differentiating characteristics are of different lengths (Mallat, 1999; Smith, 1999).

Despite these drawbacks, the spectrogram is the dominant tool for interpreting acoustic or seismic animal signals. Spectrograms provide a convenient graphical representation of longer signals from which features can be identified and quantified. The spectrogram is occasionally used in electric fish research to interpret field recordings (e.g. Schwassmann, 1978; Tan et al., 2005), but has not been employed to generate features for multivariate classification.

2.1.1.3. Discrete wavelet transform (DWT). Wavelet transforms address the limitation of the fixed window length in STFT by utilizing variable-sized windows: i.e. long windows where information about low-frequency events is required, and shorter windows where more precise high-frequency information is required. Unlike the FFT, a wavelet transform reflects variations specific to particular regions of a signal, while, unlike the STFT, it simultaneously represents variations on small and large scales. Instead of breaking up a signal into sine waves, a wavelet transformation breaks up a signal into a shifted and scaled version of a brief and irregular waveform called the wavelet base. There are many such bases, differentiated primarily by their shape and smoothness, and their efficiency will depend on the type of signal to be analysed. The mathematical bases of wavelet analysis are reviewed by Mallat (1999).

The discrete wavelet transform (DWT), any digital implementation of the analytic wavelet transform, is applied to a signal with a length equal to a power of two. As does the STFT, the DWT considers portions of the signal and maps each of these into the frequency domain, so that the DWT maps the entire signal into the time-frequency domain. However, unlike the STFT, the DWT's window size is variable; if the signal contains 2^J sampling points, then there are J such window lengths or "scales", and the DWT will contain 2^J coefficients. An increase in scale corresponds to a halving in window length, and as the scale increases, wavelet coefficients begin to uncover features in the signal that were of too short a length to appear in broader windows. In this manner DWT usually provides very efficient representations of a wide variety of signals, and is often the tool of choice in signal processing.

Because DWTs can successfully reveal otherwise hidden low and high-frequency features of signals, they are popular in a broad range of disciplines – including digital image analysis, speech recognition, and the analysis of geophysical data (Graps, 1995). Surprisingly, applications of wavelet tools to the analysis of animal communication signals have been limited. Some authors have utilized wavelets to de-noise recordings of marine mammal calls (e.g. Weiss and Dixon, 1997). Saldamando et al. (2005) used wavelets to facilitate the automated recognition of boundaries between the short syllables of grasshopper calls. Likewise, Alt et al. (1998) used wavelets to identify the boundaries of pulse components in the courtship songs of *Drosophila melanogaster*. However, wavelets have not hitherto been used as a basis for multivariate classification of non-human animal signals.

2.1.2. Landmark-based signal processing

Landmark-based models involve manual or automatic selection of salient signal features that, based on a priori biological knowledge, are suspected to discriminate groups of signalers. Landmark procedures are routinely used to analyze the spectrograms of acoustic signals (e.g. Beecher, 1988; Hopp et al., 1998; Gerhardt and Huber, 2002). Features identified from spectrograms typically include the beginnings and ends of events, e.g. pulses or syllables, and detailed aspects of them such as their length and frequency limits, or the slope of frequency modulations (Azevedo and Van Sluys, 2004; Runciman et al., 2005; Siemers and Schnitzler, 2004).

Landmark models have also been used in electric fish research. Crawford (1992) demonstrated that individual members of a captive reproductive colony of the mormyrid *Pollimyrus isidori* could be classified correctly by means of discriminant function analysis of landmark features of the EOD waveform and its PSD. From this he inferred that the EOD waveform may serve as a reliable "signature" in the context of an information system. The model comprised six landmark features from the EOD waveform, including the duration and amplitude of phases, and three additional features of the PSD. McGregor and Westby (1992) published a similar landmark model of EOD waveform shape to demonstrate that inter-individual variation greatly exceeds intra-individual variation in a small captive population of *Gymnotus carapo*. Their model included features that were predicted to be discriminatory based on "extensive experience of EODs of many species", including the timing of zero-crossings between phases of alternating polarity, the amplitude and timing of the peaks, and the area of phases.

Arnegard and Hopkins (2003) used a landmark procedure to log the timing and amplitude of prominent features in the EOD waveform of the mormyriform genus *Paramormyrops* from Gabon (previously assigned to *Brienomyrus* – see Hopkins et al., 2007). These features were then subjected to multivariate classification (by LDA) to investigate the extent to which signals were divergent among species. Arnegard and Hopkins' model was based on the assumption that the sequence of EOD phases is homologous among *Paramormyrops* species. The zero-crossing between phases containing the maximum and minimum voltages was chosen as a reference event for comparing temporal EOD parameters. This event is known to be generated in all species of *Paramormyrops* by inward current flowing through the posterior faces of the electrocytes when they are depolarized by anterior face firing (Bass, 1986; Bennett, 1971).

Turner et al. (2007) employed a novel landmark procedure to describe and classify the diversity of EOD waveforms and EOD modulations in Apteronotidae. They documented features from the PSD of the wave-type EOD waveform (including the fundamental frequency, and ratios of the amplitudes of higher harmonics), and from two types of EOD modulations usually associated with social interactions: chirps and gradual frequency rises. These modulations involve brief or protracted frequency and amplitude modulations from which several landmark measurements were taken using a semi-automated procedure. Landmark measurements from all these sources were then subjected to a principal component analysis (PCA) and the resulting PCA factors used in a discriminant function-based classification model. This model investigated the extent to which individual fishes could be accurately assigned to species on the basis of multiple components of their electric signals.

Landmark-based multivariate classification models are expected to be effective in discriminating groups of signals, because a priori assumptions about signal structure and variation can be used to define the features used to discriminate the groups. However, landmark-based models are also extremely dependent upon the validity of the built in assumptions. In cases where these assumptions are incorrect, the comparison of groups of signals may be rendered meaningless (see Section 5.2.2).

2.2. Dimension reduction

DSP procedures typically generate large numbers of components, only a few of which are essential for the discrimination of groups of signals with divergent structural properties. A dimension (data)-reduction procedure is required before any attempt at classification for two reasons. First, most multivariate classification procedures become unstable or non-functional if the number of features greatly exceeds the number of cases; for instance, LDA stipulates that the number of features may not exceed the number of cases minus the number of groups. Second, meaningful information in high-dimensional DSP data is typically well-approximated by only a few features. The removal of the superfluous (non-informative) ones will therefore usually result in greatly increased classificatory power. In other words, data-reduction retains features that are highly discriminatory and eliminates those that are not. In general, dimension reduction must be automatic or "blind" since the computations involved are prohibitively complex, and even educated guesses at what features should remain do little to enhance them. Dimension reduction techniques are numerous, and in this study we focus on two broad categories: measures of variability, such as simple variance; and approximations of discriminatory power, such as ANOVA.

2.3. Classification by linear discriminant analysis

Linear discriminant analysis (LDA), an implementation of the more general discriminant function analysis, is a popular and powerful technique for classification. In LDA, cases are divided a priori into groups (Huberty, 1994), and linear predictors are created to allow for classification of new observations. LDA offers two important tools. First, LDA generates linear discriminant functions that separate each pair of groups; these may be visualized simply as lines drawn between the groups. These functions may be useful outside the training set; i.e. they may be used to classify individuals whose group identity is not yet known. In this study, for instance, we randomly pretend ignorance of a fish's species identity and attempt to ascertain it from the signal alone. The utility of this kind of classification for field-work is clear. Second, given reasonable statistical assumptions, the classification procedure provides Bayesian posterior probabilities of group membership for each case, and allows a quantification of the extent to which two groups 'overlap' in signal space. This notion of overlap is particularly useful when considering, for example, the extent to which species or populations partition signal space.

3. Materials and methods

3.1. Overview

We begin with a description of the model data set of electric fish EOD waveforms, and data conditioning procedures. We then describe the three steps involved in the classification of waveforms: (1) Signal processing, which transforms the raw waveforms into sets of features. (2) Dimension reduction, which removes the features that do not carry information useful for subsequent classification. (3) Classification of waveforms by LDA on the basis of the remaining features. All data manipulation, analysis, and simulations were performed with custom written programs in MATLAB 7.0 (The Mathworks, Natick, MA). Relevant source code is available on request from the first author.

3.2. Model data set

263 specimens of five syntopic species of *Gymnotus* (Gymnotiformes, Gymnotidae) were captured from floating macrophytes in floodplain lakes of the Central Amazon of Brazil, near the confluence of the Rio Solimões (Amazon) and Rio Japurá (within 15 km of 03°07′27″S, 64°47′29″W) during the period 1992–2001 (*Gymnotus arapaima*, $n = 53$; *Gymnotus jonasi*, $n = 80$; *Gymnotus mamiraua*, $n = 87$; *Gymnotus obscurus*, $n = 18$; *Gymnotus varzea*, $n = 25$). All five species were recently described (Albert and Crampton, 2001, Crampton et al., 2005). Juvenile specimens and those with damage to the caudal appendage exceeding an estimated 20% total length were excluded from analysis. Specimens were placed in a nylon-mesh sock suspended in the middle of a 80 × 40 × 30 cm cooler and recorded in water from the capture locality (conductivity 90–110 $\mu S\ cm^{-1}$) at a standardized temperature of 27.0 +/− 0.1 °C. The EODs were detected from silver/silver-chloride electrodes and amplified with a custom built AC-coupled differential amplifier (Wells and Crampton, 2006) or a CWE-Instruments BMA-200 amplifier, in AC-coupled mode. EODs were digitized with a Sony TCD D7 or D8 Digital Audio Tape recorder at a sample rate of 48 kHz (and later transferred to a computer), or directly to a computer with an Edirol UA-5 analog–digital converter at 96 kHz and with 24-bit resolution. EODs were all saved as ASCII files.

3.3. Data conditioning

Any DC offset in the signal was eliminated by subtracting the mean amplitude of the first ten samples of the waveform from each sample in the waveform. All signals were then converted to a common length (2048 samples), sample rate (96 kHz), and root-mean-square normalized amplitude. For the DSP procedures we aligned all signals to the dominant positive peak, P1, (see Fig. 1). This was based on the assumption that P1 is evolutionarily homologous among all species under comparison (see Section 5.2.2). We considered the P1–P2 zero-crossing as an alternative alignment position, but prefer the P1 peak for its wider applicability in Gymnotidae; adults of some species of this family, e.g. *Gymnotus cylindricus* and *Electrophorus electricus*, and post-larval specimens of all species (for which data are available) have monophasic signals, comprising only the P1 phase (Crampton and Albert, 2006).

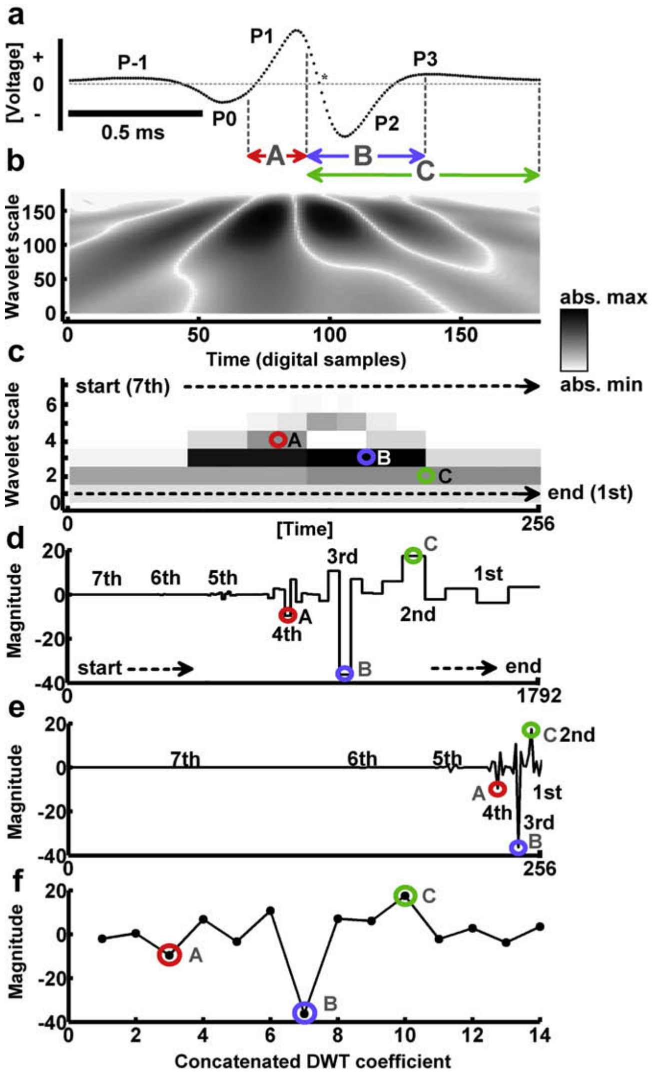

Fig. 1. Procedure for the discrete wavelet transform (DTW): (a) electric organ discharge waveform of *Gymnotus arapaima* with 182 digital samples and 5 phases of alternating polarity P-1 through P3. Asterisk marks the P1–P2 zero-crossing. A–C refer to ranges of samples centered on the middle of a range of repeated DWT coefficients in c (and also d–f), with coefficients from higher scales (e.g. A) referring to a narrower range of samples. In this manner the unique DWT coefficients used as features in classification correspond not to individual samples in the original waveform/s but to ranges of samples (regions of signal), which are narrower (provide better resolution of high-frequency events) at high wavelet scales, (b) continuous wavelet transform (CWT) with 182 samples on abscissa and 182 scales on ordinate. While the CWT is a useful graphical representation of the signal, it is also an over-complete representation of any discrete signal, and therefore not appropriate for statistical analyses in which dimensionality is already problematic, (c) DWT generated with the Symmlet-4 wavelet base, with waveform resampled to 256 digital samples on abscissa, and represented by 7 dyadic (series of powers of two) wavelet scales on ordinate (e.g. scale 1 = 1 unique coefficient repeated 256 times, scale 7 = 128 unique coefficients each repeated twice). The DWT summarizes the CWT with little loss of descriptive accuracy by employing scales and temporal positions based on this dyadic relationship. Absolute (abs.) coefficient magnitudes are represented in grey-tone for CWT and DWT, (d) all 7 × 256 = 1792 DWT coefficients were concatenated in order scale 7 through 1 (dotted arrows in c), with magnitudes represented on ordinate, and (e) 255 unique DWT coefficients. (f): DWT coefficients remaining after dimension reduction (in this case 14 features for subsequent classification).

We defined the beginning (and end) of a waveform as the point at which it first rose (and last fell) to 1% of the P1 amplitude. We truncated all signals at the earliest beginning (*B*) and the latest end (*E*). Each waveform was then forced to a new baseline by subtracting the line segment connecting *B* and *E*, then by converting flanking samplings to zero. All resulting waveforms contained 182 samples.

3.4. Digital signal processing

3.4.1. Fast Fourier transform (FFT) and power spectral density (PSD)

We generated the FFT and PSD of the conditioned waveforms, yielding 182 and 91 features, respectively. For each of the 263 specimens, we also computed the peak power frequency (PPF) of the PSD, to assess the extent to which the five species of *Gymnotus* may be partitioned on the basis of dominant frequency components of the EOD.

3.4.2. Short-time Fourier transform (STFT) and spectrogram

We generated the STFT and spectrograms of the conditioned waveforms, yielding 728 and 364 features, respectively. We utilized a Gaussian window function (a well-understood and common choice), with a width defined as one quarter of the signal length. The window width choice was largely arbitrary, though informed by the average length of a waveform phase. There are arguments to be made for other lengths, but this debate recalls the primary limitation of the STFT, i.e. the necessity of a fixed window length.

3.4.3. Discrete wavelet transform

Because the DWT requires the length of the signal to be a power of two, we first resampled the waveforms from 182 to 256 (the next largest power of two). Using commands from WaveLab, a free library of MATLAB routines for wavelet analysis developed by the Department of Statistics, Stanford University (http://www-stat.stanford.edu/~wavelab/), we executed the DWT, generating 256 DWT coefficients, with one scaling coefficient. The schematic in Fig. 1 visualizes the output of an example DWT. To explore the effect of wavelet-base on classification performance, we chose four wavelet bases with distinct geometric characteristics: the Haar wavelet, the Symmlet wavelet with four vanishing moments (Symmlet-4), and the Daubechies wavelets with four and ten vanishing moments, respectively (Daubechies-4 and Daubechies-10) (Fig. 2).

3.5. Landmark-based signal processing

We designed a program to automate the measurement of landmark features from EODs (Fig. 3). For each signal, we designated the peak of the dominant positive phase (P1) by finding the maximum of the signal's amplitude. We then defined a total of four additional phases of alternating polarity on either side of this

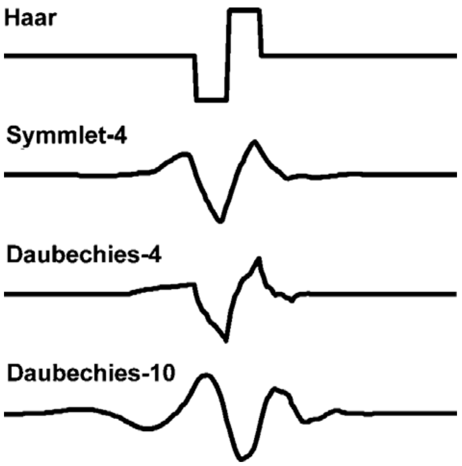

Fig. 2. Four wavelet bases used in this study. 4 and 10 refer to the number of vanishing moments (not applicable to the Haar wavelet). Note the Symmlet-4 and Daubechies-10 wavelets represent smoother functions than Daubechies-4.

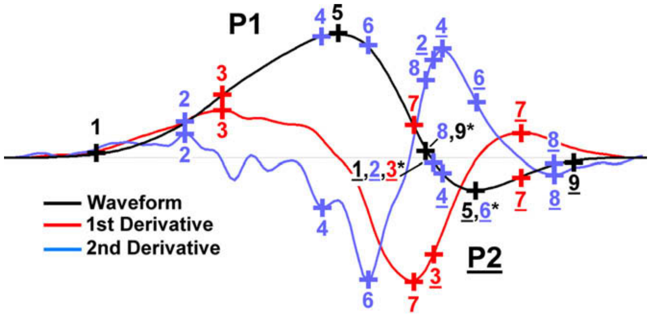

Fig. 3. Procedure for landmark signal processing. Electric organ discharge waveform of *Gymnotus obscurus*, with two phases: P1 and P2. Thin gray line denotes zero volts. Nine landmark positions on the waveform are defined for each phase, each represented by its amplitude and timing: (1) beginning and end (two black crosses) at a 1% threshold of the P1 peak amplitude; (2) peak (one black cross); (3) maximum first derivatives to left and right of peak (two red crosses); (4) maximum second derivatives to left and right of (3) (four blue crosses). Red and blue crosses and are labeled with the landmark number both on the waveform and also at the corresponding timings on the first and derivative plots. Labels in P2 phase are underlined to distinguish them from positions in P1. Note that two or more landmarks are coincidental at positions marked with asterisks. Where two or more landmarks are coincidental for all individual fishes in the analysis, only one is retained for classification – the others being redundant.

dominant phase (Fig. 1). Within each phase, we identified nine landmarks as the local extrema of the signal, and of its first and second derivatives (see Fig. 3 for explanation). The timing (relative to preceding landmark) and amplitude of each landmark were tabulated in a matrix comprising five phases ordered P-1 through P3 (see Fig. 1). Each phase comprised 18 amplitude/timing measures, yielding a total of 90 features. However, some features were statistically redundant (i.e. no variability among all cases, and/or all cases identical to those in another feature, see Fig. 3); these were discarded, reducing the number to 86. Some individual fishes did not present all phases, in which case all measurements for missing phases were set to zero.

3.6. Dimension reduction

Each of the signal processing procedures discussed above generates a certain number of features, which we denote by m. Note that m is equal to the length of the original signal n for FFT, less than n for PSD and landmark analysis, and greater than n for STFT, spectrogram, and DWT.

The objective of dimension-reduction techniques was to choose $M < m$ features for classification. Statistical considerations may motivate the choice of M, though where clear indications are lacking (as is usually the case) it will be necessary to vary M until an optimum is found. Here we let M vary exhaustively, not because we recommend this in practice, but to illustrate the full range of classification performances.

3.6.1. Dimension reduction based on variance or coefficient of variance

To reduce dimension by variance, we simply retained the M features with the highest variances. When the features are all pulled from the same sort of data, this may suffice to select the most meaningful features. We defined the coefficient of variance as the variance divided by the mean of the absolute value of the feature, and reduced dimension by the coefficient of variance by retaining the M features with the highest such values.

3.6.2. Dimension reduction based on ANOVA and pairwise ANOVA

For each feature we computed a standard one-way ANOVA (see e.g. Casella and Berger, 2002), which yielded a p-value estimating the likelihood that the feature contains no group-specific information. We reduced dimension by retaining those M features which have the smallest p-values; i.e. those features which mostly likely contain group-specific information. Note that some features may be clearly useful for distinguishing between, for instance, groups 1 and 2, but useless for separating between any other group. Such a feature would have a very small p-value because it contains group-specific information and it may therefore be retained. If there are M such features, the LDA over these features might clearly discriminate between groups 1 and 2, but not others.

As a possible compensation, we generated pairwise ANOVAs – i.e. we calculated the probability p_{ij} that a feature does not discriminate between groups i and groups j, for all combinations $i \neq j$. We then calculated the sum of these values for each feature and retained the features with the smallest values, which can be conceptualized as the average probability that the feature does not discriminate among two given groups.

3.7. Multivariate classification

3.7.1. Linear discriminant analysis

We performed LDA on the M features derived from signal processing, and retained by dimension reduction. Individual fishes, represented as rows of features, were organized into five groups by species and a priori probabilities of group membership were assumed to be equal for all of the groups. To ensure that the group membership rules were meaningful outside of the training sets, we implemented a cross-validation procedure in which we partitioned individuals into a training set and a testing set. The rules generated by the LDA on the training set were examined for accuracy on the testing set. We designated two-thirds of the cases to the training set for the simulations, but also investigated other proportions. For this data set there was a large range of proportions that gave a clear view of the differences among the procedures themselves, and we chose the smallest representative of these.

3.7.2. Monte Carlo simulations

To assess the performance of each combination of parameters, we conducted 100 Monte Carlo simulations, in which members of the training sets were randomly chosen prior to LDA (i.e. 100 separate classifications by LDA). This approach ensured that the classification rules were not skewed by outlying individuals, but generalized to all members of the group. For each case (fish), LDA assigned a posterior probability of group (species) membership – allowing a simple calculation of the total number of correctly classified individuals among all 263 fishes. For instance, if one fish were misclassified during one simulation, the total proportion of correctly classified cases would be $1 - (1/263) = 0.996198$. We averaged this proportion over 100 simulations. Hence, if 98 simulations exhibited perfect classification (all 263 fish correctly classified) and two simulations each exhibited one misclassified case (0.996198), the overall proportion of correctly classified cases would be $1 - (2/(263 * 100)) = 0.999924$ (i.e. 0.76 errors in 10,000 cases simulations). Using the same model data set, we ran simulations to test LDA's classificatory performance in all permutations of the following procedures and variables:

(i) Signal processing procedure:
   1. landmark model,
   2. fast Fourier transform (FFT),
   3. power spectral density (PSD),
   4. short-time Fourier transform (STFT),
   5. spectrogram,
   6. discrete wavelet transform (DWT) with Haar-4 wavelet base,
   7. DWT with Symmlet-4 wavelet base,
   8. DWT with Daubechies-4 wavelet base,
   9. DWT with Daubechies-10 wavelet base.

(ii) Dimension reduction procedure:
  1. variance,
  2. coefficient of variance,
  3. one-way analysis of variance (ANOVA),
  4. pairwise analysis of variance (pairwise ANOVA).

(iii) Number of features (M) remaining after dimension reduction:

For each dimension reduction procedure, we attempted to generate data for $M = 1–15$ features in increments of 1, then $M = 20–60$ features in increments of 5. We could not exceed $M = 35$ in the variance-based procedures, however, because these procedures often selected sets of coefficients that were linearly dependent within the tolerances of the LDA. In practice it would be reasonable to precondition the coefficients so as to prevent selecting dependent sets. However, this is a dimension reduction procedure in itself. To fully explore these procedures per se, here we allowed them to fail in this manner.

The combinations above yielded 774 permutations of parameters for classification by LDA, each repeated 100 times to yield measures of central tendency and dispersion to allow for comparisons among the permutations. Thus we performed $774 \cdot 100 = 77{,}400$ separate LDA classifications in this study. For the single best-performing combination, we also explored the effect of the relative size of the training versus test sets in a separate simulation, with the proportion of the entire sample assigned to the training set varied from 0.05, to 0.95 at increments of 0.05.

4. Results

4.1. Graphical representations of DSP procedures

EOD waveforms for each of the five species of *Gymnotus* are illustrated in Fig. 4, along with representative plots of the PSD, spectrogram, and DWT.

4.2. Univariate analysis of peak power frequency

The peak power frequencies of the EODs of all five species are plotted in Fig. 5. Two-tailed Student's T-tests reveal that the

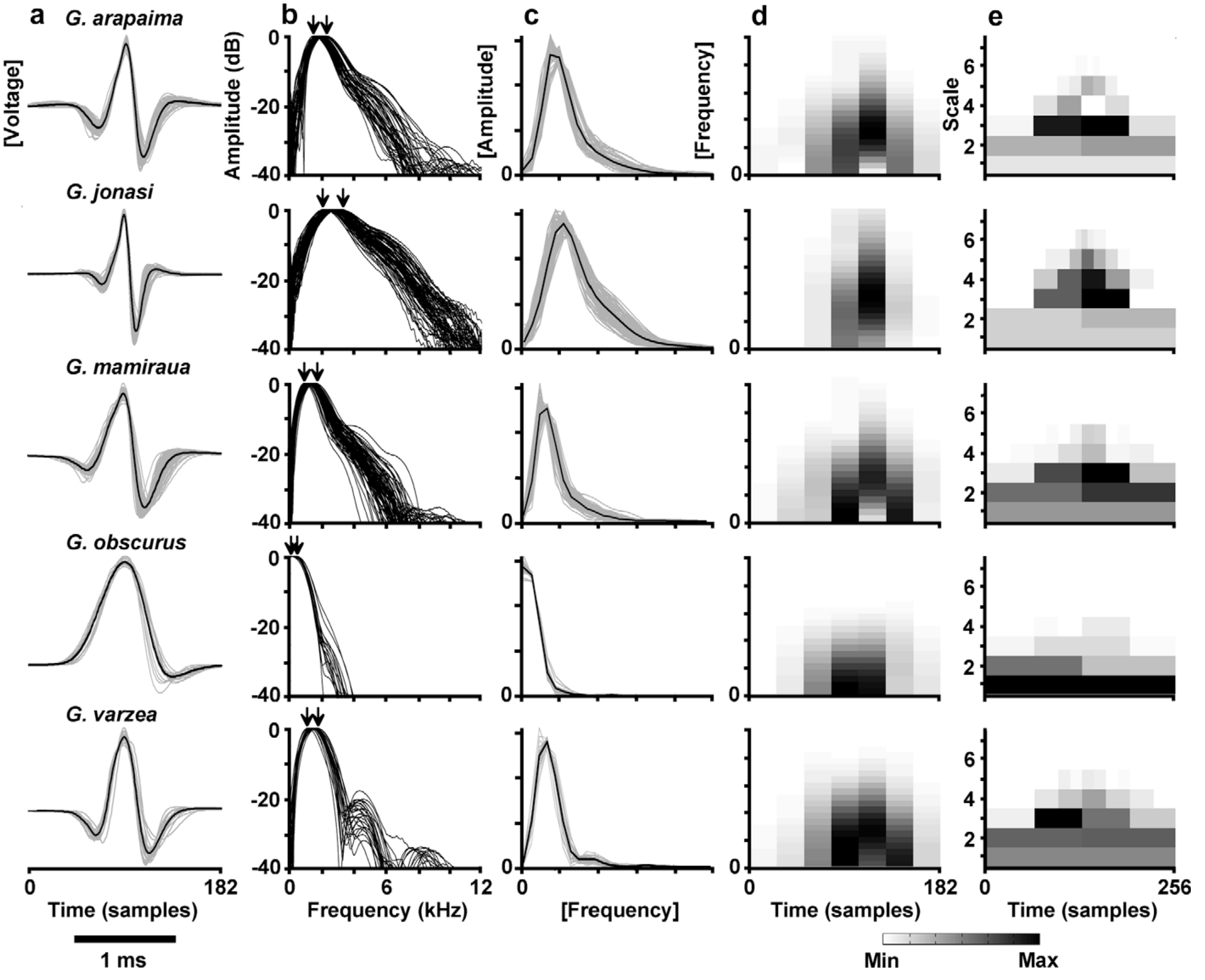

Fig. 4. (a) Electric organ discharge (EOD) waveform for five syntopic species of *Gymnotus* (grey = EODs from individual recorded fishes, black = species average). To generate the species average, EODs were aligned to the P1 peak following conditioning, and a mean amplitude was computed at each sample, (b) power spectral density (PSD) with peak power frequency (PPF) normalized to 0 dB in the form usually used to represent EODs (but not used for analyses in this paper). Arrows indicate species-range of PPFs, (c) PSDs in non-normalized format used for analysis in this paper (black = species average), (d) spectrogram of the species average EOD, and (e) discrete wavelet transform (DTW) of the species average EOD using the Symmlet-4 wavelet base. Axis legends in square brackets refer to those with arbitrary values.

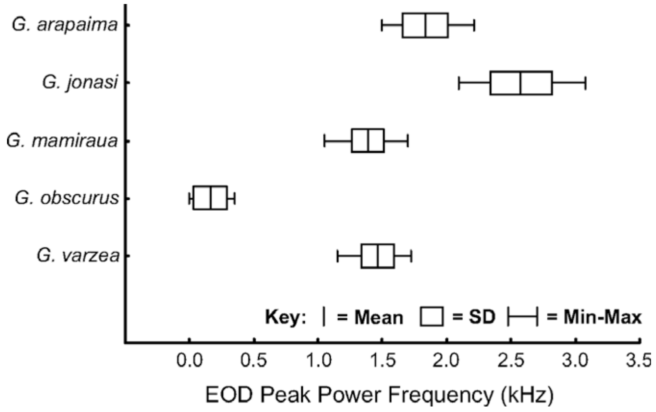

Fig. 5. Peak power frequency (PPF) ranges for the EODs of five syntopic species of *Gymnotus*. The min-max values correspond to the arrows in Fig. 4b. Note overlapping ranges between several species.

mean PPF of all five species are significantly different ($p < 0.05$). However, there is considerable overlap among several species, with *G. mamiraua* and *G. varzea* exhibiting almost complete overlap.

4.3. Multivariate classification simulations

The performances of 774 permutations of procedures are plotted in Figs. 6 and 7. Here performance is represented as the mean percentage of correctly classified cases in 100 simulations. The plots are divided, for the sake of clarity, into the results of landmark and non-wavelet based DSP procedures (Fig. 6) and wavelet DSP procedures (Fig. 7). The top fifty permutations are listed in Table 1. The performances of each of the nine signal processing procedures and four dimension reduction procedures are compared in Table 2 for different ranges of M features. The performance of 36 permutations of signal processing and dimension reduction processes are averaged for features 10 through 15 (inclusive) in Table 3 (see also Table 2.b). In Table 3, combinations that are significantly better-performing than lower-performing combinations are indicated. We emphasize 10–15 features because this is the range over which the single best-performing combination (Symmlet-4 wavelet; pairwise ANOVA) performs optimally (Table 2.b; Fig. 7d (i)). The rankings reported in Tables 1–3 were consistent when the entire simulation was repeated.

The data in Figs. 6 and 7 and in Tables 1–3 are based on using two-thirds of each group to train the LDA. In Fig. 8 we vary this proportion from 0.05 to 0.95 for the best-performing combinations among all 774 permutations (Symmlet-4 wavelet; pairwise ANOVA; $M = 14$). For this best combination, one pair of discriminant functions separates all five species into distinct clusters, in just a two-dimensional representation of signal space (Fig. 9); i.e. $M = 2$. The data presented in Figs. 6–8 and Tables 1–3 indicate the following salient patterns:

(1) The precision of a given combination of signal processing and dimension reduction procedure increases with M features, but usually reaches a peak, beyond which additional components provide no extra discriminatory information and instead serve to reduce classificatory power ('overtraining').

(2) Each signal processing procedure varies considerably in its performance according to the dimension reduction procedure (and vice versa). As such, some permutations of procedures reach considerably better classification precision than others. Below we highlight some of these patterns:
   (i) Landmark-based signal processing yields consistently inferior performance to most DSP procedures under a wide range of conditions, especially under variance-based dimension reduction. For instance, the landmark procedure is represented only twice in the highest ranked 50 of all 774 permutations, in both cases involving relatively high numbers of features (30–35) (Table 1). Also, it is ranked towards the bottom of Table 2 when averaged over both wide and narrow ranges of M. Likewise, in the 10–15 feature range, the landmark procedure only appears in the seven worst-performing combinations (Table 3). For higher number of features, similar patterns are evident (Figs. 6 and 7). Landmark-based SP tools outperform only the worst-performing DSP combinations (see below).
   (ii) Among DSP procedures, the time-frequency-based procedures (STFT and DWT) exhibit superior performance. The Symmlet-4 wavelet and STFT account for 38 of the top 50 procedures in Table 1 (and all of the top 12), and most of the top-performing combinations in Tables 2 and 3. Symmlet-4 with pairwise ANOVA performed significantly better than the 35 other combinations in Table 3 ($p < 0.001$), while STFT with coefficient of variance performed significantly better than the remaining 34 combinations ($p < 0.01$). FFT exhibits an intermediate level of performance, occurring in the middle-ranked positions of Tables 1–3. Performance of FFT is especially low under ANOVA (Fig. 6c (ii)). Fourier procedures with the phase components removed (PSD and spectrogram) generally achieved the worst performances, and over a broad range of conditions.
   (iii) The performance of wavelet transforms depends largely on the choice of base wavelet. The Symmlet-4 wavelet achieves the highest discriminatory performance in the entire simulation, reaching a mean 100% classification (i.e. 100% classification in all 100 Monte Carlo runs) with pairwise ANOVA at 14 components, and exhibiting close to 100% classification (>99.90%) over the range 10–15 features (Fig. 7d, Table 3). The top six of all 774 permutations are all combinations of the Symmlet-4 wavelet and pairwise ANOVA with 9–15 features (Table 1). With just six features, this combination is capable of reaching 99.954% classification, representing the 11th highest rank. For the dataset explored in this study, no other combination came close to matching this performance.

There are no obviously consistent differences among the remaining wavelet bases (Haar, Daubechies-4, and Daubechies-10), with performance varying considerably according to the dimension reduction procedure. In combination with pairwise ANOVA, the Haar and Daubechies-4 wavelets perform significantly worse than all other wavelet combinations (Table 3).

(3) Of the four dimension reduction procedures, none show consistently superior performance, ceteris paribus. ANOVA often performs worse than other procedures under similar conditions (Table 2). This pattern may be largely because Fourier procedures with the phase components removed (PSD and spectrogram) perform exceptionally badly under ANOVA over a wide range of M (Fig. 6c). Variance typically performs better than other procedures when averaged over many signal processing procedures and ranges of M (Table

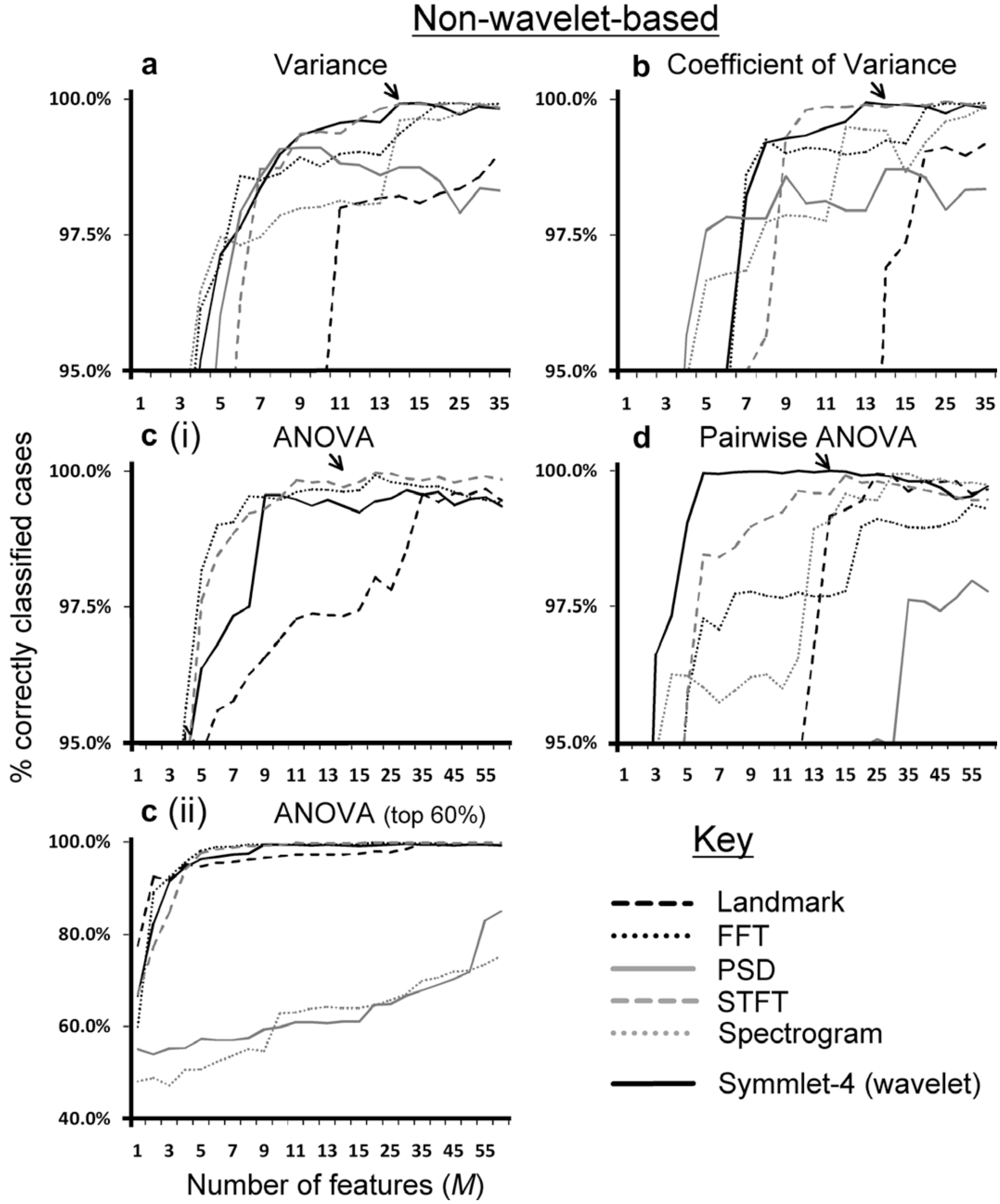

Fig. 6. Classificatory performance (in linear discriminant analysis of the model data set of *Gymnotus*) for: five combinations of non-wavelet signal processing (SP) procedure, four types of dimension reduction (DR) procedure, and up to $M = 60$ features (remaining after DR). Data points refer to the mean classificatory performance of each combination of SP, DR, and M as the percentage of correctly classified cases among all 263 cases (individual fishes) · 100 Monte Carlo simulations (e.g. 99.9% = 13 misclassifications out of 26,300 [263 · 100] permutations). For comparison to Fig. 7, classificatory performance is also reported for the Symmlet-4 wavelet transform (the best-performing signal processing tool under Pairwise ANOVA). For reference, arrows point to 100% classification, which is achieved only with Symmlet-4 wavelet transform and Pairwise ANOVA at 14 features. Classificatory performance refers to the top 5% of correctly classified cases for each combination, except in c (ii), which refers to the top 60%. Lower performing combinations in a, b and d do not show relevant variation (see Fig. 7, dii), and are therefore not represented.

2). However, most of the top ranking combinations in Table 3 involve pairwise ANOVA.

(4) Performance increases as the ratio of the training set to the total population increases (Fig. 8). We first observed consistent 100% classification at the 0.66 training ratio, and 100% for all higher ratios. Training ratios below 0.4 resulted in significantly inferior performance.

In summary, and with many exceptions, the wavelet DSP procedures and STFT generally outperform the landmark procedure, and remaining DSP procedures. FFT exhibits intermediate performance, and the lowest performances of all are with the Fourier procedures in which phase components are removed (PSD and spectrogram).

5. Discussion

5.1. Performance differentials among signal processing procedures

The performances of the DSP procedures we compare in this paper are entirely concordant with theoretical predictions. The DWT and the STFT, both time-frequency procedures, are superior under a wide range of conditions. The lower performance of FFT was anticipated because transient frequencies (signal components

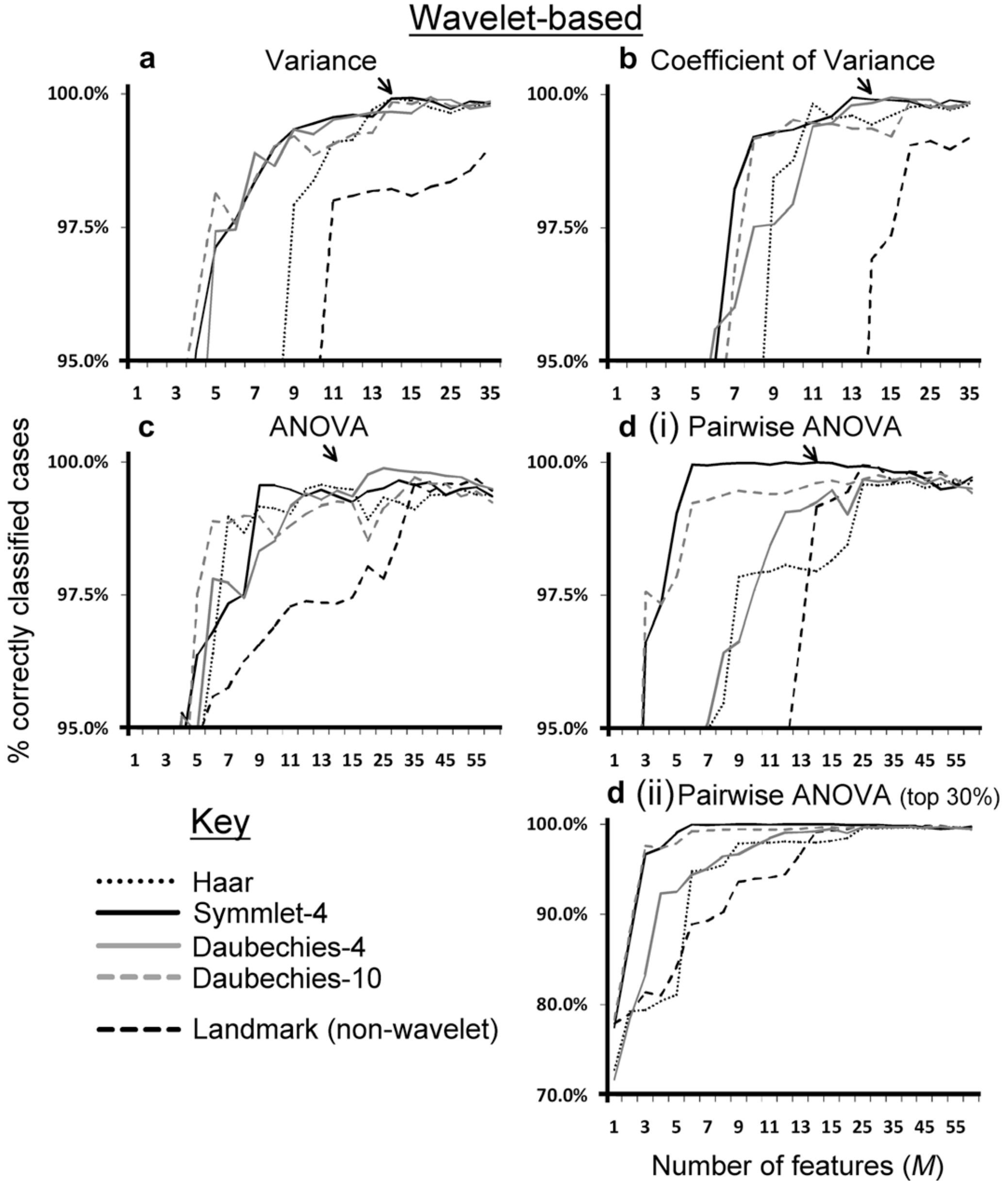

Fig. 7. Classificatory performance (in linear discriminant analysis of the model data set of *Gymnotus*) for: four combinations of wavelet transform signal processing (SP) procedure, four types of dimension reduction (DR) procedure, and up to $M = 60$ features (remaining after DR). Data points refer to the mean classificatory performance of each combination of SP, DR, and M as the percentage of correctly classified cases among all 263 cases (individual fishes) · 100 Monte Carlo simulations (e.g. 99.9% = 13 misclassifications out of 26,300 [263 · 100] permutations). For comparison to Fig. 6, we also report the classificatory performance of landmark analysis. For reference, arrows point to 100% classification, which is achieved only with Symmlet-4 wavelet transform and pairwise ANOVA at 14 features. Classificatory performance refers to the top 5% of correctly classified cases for each combination except in d (ii), which refers to the top 40%. Lower performing combinations in a-c show similar patterns to d (no relevant variation) and are therefore not represented.

present in only short sections of a signal) require that FFT report physically meaningless frequencies, hence failing to reduce the dimensionality of the data, and therefore reducing classificatory performance in subsequent classification.

For the data set of *Gymnotus* considered here, each phase of alternating polarity approximates part of a sine wave. For this reason FFT alone can, with relatively low numbers of features, achieve reasonably high classificatory power. Nonetheless, while FFT can easily discriminate species with different numbers of phases (e.g. *G. obscurus* and *G. mamiraua*, see Fig. 4), subtle differences in the amplitude and duration of species with similar EOD waveforms (e.g. *G. mamiraua* and *G. varzea*) are not adequately represented by FFT. STFT corrects for the deficiencies of the FFT by windowing, and for this data set provides extremely high performance. Nonetheless, as expected, the highest classification is achieved with DWT, which corrects for the disadvantages of a fixed window length in STFT by the use of a windowing technique with variable-sized regions.

The choice of a wavelet-base is critical in the DWT, with wavelet bases chosen to emulate the smoothness and shape of the signals under comparison. Symmlet-4, with smoothed functions and only four vanishing moments, most closely resembles EOD waveforms (Fig. 2), and, as predicted, performs best. The Haar wavelet, which is discontinuous, generally yields poor performance.

The removal of the phase components from STFT, to yield the spectrogram, results in a distinct decline in classificatory power.

Table 1

Fifty highest-performing of 774 combinations of signal processing procedure, dimension reduction procedure, and *M* features in linear discriminant analysis of the model *Gymnotus* data set. The mean and standard deviations (SD) represent the percentage of correctly classified cases among all 263 cases (individual fishes) · 100 Monte Carlo simulations (e.g. two misclassifications in 263,000 = 99.992%). Tied ranks are marked with an equal symbol.

Rank	Signal processing procedure	Dimension reduction procedure	*M* Features	% Classification	
				Mean	SD
1	Wavelet: Symmlet-4	Pairwise ANOVA	14	100.000	0.000
2	Wavelet: Symmlet-4	Pairwise ANOVA	12	99.992	0.054
5=	Wavelet: Symmlet-4	Pairwise ANOVA	9	99.985	0.075
5=	Wavelet: Symmlet-4	Pairwise ANOVA	10	99.985	0.075
5=	Wavelet: Symmlet-4	Pairwise ANOVA	15	99.985	0.075
6	Wavelet: Symmlet-4	Pairwise ANOVA	13	99.970	0.104
8=	STFT	ANOVA	20	99.962	0.120
8=	Wavelet: Symmlet-4	Pairwise ANOVA	8	99.962	0.115
9	STFT	Coefficient of variance	25	99.956	0.127
11=	Wavelet: Symmlet-4	Pairwise ANOVA	6	99.954	0.125
11=	Wavelet: Symmlet-4	Pairwise ANOVA	11	99.954	0.125
12	STFT	ANOVA	25	99.951	0.134
13	Landmark	Pairwise ANOVA	25	99.941	0.158
15=	Wavelet: Daubechies-4	Coefficient of variance	15	99.939	0.141
15=	Spectrogram	Pairwise ANOVA	35	99.939	0.159
17=	FFT	Coefficient of variance	35	99.935	0.153
17=	Spectrogram	Pairwise ANOVA	30	99.935	0.153
20=	Wavelet: Daubechies-10	Variance	20	99.932	0.166
20=	Wavelet: Symmlet-4	Pairwise ANOVA	7	99.932	0.166
20=	Wavelet: Symmlet-4	Coefficient of variance	13	99.932	0.166
21	FFT	Coefficient of variance	25	99.926	0.160
23=	STFT	Variance	25	99.924	0.162
23=	Wavelet: Symmlet-4	Variance	15	99.924	0.154
24	FFT	ANOVA	20	99.920	0.185
26=	FFT	Variance	20	99.918	0.161
26=	Spectrogram	Variance	30	99.918	0.174
28=	FFT	Variance	25	99.916	0.171
28=	Wavelet: Symmlet-4	Pairwise ANOVA	25	99.916	0.159
31=	FFT	Variance	35	99.913	0.169
31=	STFT	Pairwise ANOVA	15	99.913	0.186
31=	STFT	Coefficient of variance	30	99.913	0.165
35=	Wavelet: Haar	Variance	14	99.909	0.181
35=	STFT	Coefficient of variance	15	99.909	0.176
35=	Wavelet: Symmlet-4	Pairwise ANOVA	20	99.909	0.164
35=	Wavelet: Symmlet-4	Variance	14	99.909	0.164
36	STFT	Variance	15	99.903	0.175
41=	Wavelet: Daubechies-4	Coefficient of variance	20	99.901	0.185
41=	Landmark	Pairwise ANOVA	30	99.901	0.202
41=	STFT	ANOVA	55	99.901	0.195
41=	STFT	Variance	14	99.901	0.180
41=	Wavelet: Symmlet-4	Coefficient of variance	14	99.901	0.185
42	STFT	Variance	30	99.899	0.192
43	FFT	Coefficient of variance	30	99.897	0.178
44	Wavelet: Daubechies-4	Coefficient of variance	25	99.894	0.172
45	STFT	Variance	20	99.892	0.192
47=	STFT	ANOVA	40	99.888	0.197
47=	STFT	Coefficient of variance	13	99.888	0.193
53=	Wavelet: Daubechies-10	Coefficient of variance	20	99.886	0.176
53=	Wavelet: Daubechies-4	Variance	20	99.886	0.176
53=	Wavelet: Daubechies-4	Variance	25	99.886	0.207

This suggests that classification is highly sensitive to the timing of events within each window. Likewise, the PSD yields extremely poor classificatory power because information about the timing of events throughout the entire signal is eliminated. These observations indicate that the five species of *Gymnotus* can be completely discriminated on the basis of waveform shape, but not by the distribution of frequencies in the EOD alone. The similarity of the frequency distributions among several of these species is exemplified by a wide overlap in the PPF of the PSD (Fig. 5).

Landmark-based processing of animal signals is predicted to yield highly discriminatory features. This is because the selection of these features is based on a priori information collected by biologists about how signals vary among the taxa under comparison, and about which signal features are likely to discriminate them. The landmark procedure we designed resembles those previously used in multivariate comparisons of electric fish EODs (e.g. Crawford, 1992; McGregor and Westby, 1992; Arnegard and Hopkins, 2003). However, it compared unfavorably to all automated DSP procedures, with the exception of the PSD and spectrogram.

5.2. Advantages of automated DSP versus landmark-based signal processing

5.2.1. Performance

Although landmark models incorporate biological knowledge about which features are suspected to be discriminatory, informative but subtle differences in signals may not be discernable in graphical representations. In contrast, DSP procedures, especially time-frequency procedures, are predicted to detect such hidden features. These considerations are presumably responsible for the poor discriminatory performance of the landmark-based model evaluated here. Even the inclusion of localizable positions in the

Table 2

Comparison of the performance of nine signal processing (SP) procedures, and four dimension reduction (DR) procedures in linear discriminant analysis of the model *Gymnotus* data set. Mean rank = mean of *n* ranks of given SP or DR procedure among all 774 combinations of SP procedures, DR procedures and *M* features. Top rank = highest rank achieved among all 774 combinations. In top 50 = number of times the procedure appears in 50 highest ranked of all 774 combinations (i.e. Table 1). Mean, Max (maximum), Min (minimum) and SD (standard deviation)% refer to percentage classification among *n* combinations per procedure. For example, in Wavelet Symmlet-4 for 6–60 features (a) the mean% of 99.413 refers to 10,189 misclassifications in 1,735,800 permutations (263 cases [individual fishes] · 100 Monte Carlo simulations · *n* = 66).

Procedure	Mean rank	Top rank	In top 50	% Classification				*n*
				Mean	Max	Min	SD	
(a) Features 6-60 inclusive (594 combinations)								
Signal processing:								
Wavelet Symmlet-4	176	1	16	99.413	100.000	94.958	0.86	66
STFT	179	8	14	99.304	99.962	93.416	1.19	66
Wavelet Daubechies-10	249	20	1	99.210	99.932	92.738	0.96	66
Wavelet Daubechies-4	257	15=	3	98.921	99.939	94.418	1.27	66
FFT	277	17	7	98.998	99.935	94.188	0.95	66
Wavelet Haar	304	35	1	98.204	99.909	85.993	2.68	66
Landmark	422	13	2	95.273	99.941	66.707	6.23	66
Spectrogram	423	15=	3	88.751	99.939	52.293	15.90	66
PSD	527	322	0	87.967	99.110	57.015	15.28	66
Dimension reduction								
Variance	280	20	13	98.602	99.932	87.473	2.07	126
Pairwise ANOVA	298	1	17	98.245	100.000	88.941	2.18	171
Coefficient of variance	299	9	12	97.540	99.956	66.707	4.82	126
ANOVA	362	8	5	91.490	99.962	52.293	14.68	171
(b) Features 10–15 inclusive (216 combinations)								
Signal processing								
STFT	130	31	5	99.695	99.913	99.110	0.23	24
Wavelet Symmlet-4	135	1	10	99.685	100.000	99.240	0.26	24
Wavelet Daubechies-4	255	15	1	99.237	99.939	97.597	0.58	24
Wavelet Daubechies-10	263	73	0	99.309	99.840	98.578	0.30	24
Wavelet Haar	277	35	1	99.044	99.909	97.916	0.70	24
FFT	315	148	0	98.888	99.622	97.656	0.74	24
Spectrogram	450	159	0	89.687	99.645	62.901	15.38	24
Landmark	495	288	0	95.439	99.278	87.114	3.86	24
PSD	516	322	0	88.111	99.110	59.819	15.23	24
Dimension reduction								
Variance	266	23	5	99.048	99.924	93.542	0.978	54
Coefficient of variance	278	15	5	98.305	99.939	87.114	3.121	54
Pairwise ANOVA	342	1	7	98.020	100.000	93.949	1.882	54
ANOVA	374	75	0	90.891	99.835	59.819	15.515	54

first and second derivatives of *Gymnotus* waveforms failed to allow adequate representation of the features associated with inter-specific variability in signal structure.

5.2.2. Assumptions of homology

Implicit in the use of a landmark procedure for evolutionary comparisons of animal signals is that some of the compared features in signals are evolutionary homologies, common to the taxa under consideration. This may be advantageous in studies of animal groups where the evolutionary bases of signal patterns are well understood. For instance, the phases of mormyrid EODs correspond to well-documented and homologous anatomical structures and physiological events (Sullivan et al., 2000; Arnegard et al., 2005). Here, cross-taxon comparisons of signal features based on landmark analysis are expected to reveal divergences or similarities of evolutionary significance (e.g. Arnegard et al., 2005).

However, assumptions of landmark homology may be problematic, because if wrong, the resulting analyses may be difficult to interpret, or meaningless. Specifically, if an apparently similar signal feature is actually homoplastic, an erroneous assumption of homology could result in inflated significance for cross-taxon variation in this feature. For example, several pulse-generating hypopomid taxa have accessory electric organs that generate extra EOD phases flanking P1 and P2 (Crampton and Albert, 2006). These accessory organs occur in different parts of the body in different species (e.g. in the mental region, humeral region, or over the operculum) and the EOD phases generated by them are likely non-homologous. Because these EOD phases could be homoplastic across multiple taxa, landmark-based studies of EOD waveform divergence would require cautious interpretation.

Whether all EOD phases in *Gymnotus* represent homologous events in the discharge cycle is uncertain. The zero-crossings between each phase, the phase peaks, and the inflection points in pulse-generating gymnotiforms are physiologically significant events insomuch as they correspond to changes in the rate and direction of electrical current flowing through the electric organ (Caputi et al., 2005). At least some of these events may also be evolutionarily homologous. Phylogenetic reconstructions indicate that the P1 and P2 phases (see Figs. 1 and 3) are homologous among all pulse-type taxa (review in Crampton and Albert, 2006), with the larval EOD comprising only P1, and with P2 appearing early in development. P1 and P2 also show similar underlying patterns of electromotor neuron innervations and membrane receptor activation patterns among pulse-type gymnotiforms (Caputi et al., 2005), and (as in mormyrids) the P1–P2 boundary corresponds to the reversal of current through the posterior faces of the electrocytes when they become depolarized by the firing of the anterior face (Bass, 1986; Bennett, 1971). A more thorough understanding of the comparative anatomy and physiology of the EOD of *Gymnotus* is now emerging. Rodriguez-Cattaneo et al. (2008) demonstrated that similar events in the electric organ underlie the generation of phases P-2 through P3 in two widespread tropical species: *Gymnotus carapo* and *Gymnotus coropinae*. A southern temperate species *G.* n. sp. “omarorum” generates four phases P0, P1 and P2; of which the latter two are generated by similar mechanisms to the equivalent phases in the tropical species. However, the P0 of *G.*

Table 3
Ranked classificatory performance of signal processing and dimension reduction (DR) procedures in linear discriminant analysis of the model *Gymnotus* data set. Results are pooled for 10–15 features M remaining after DR, and presented as mean and standard deviation (SD). Measures represent the percentage of correctly classified cases among all 263 cases (individual fishes) · 100 Monte Carlo [MC] simulations). For example 99.981% = 15 misclassifications in 157,800 permutations (263 cases · 6 features · 100 MC iterations). A comprehensive test in which all combinations were compared pairwise would collapse under the problem of multiplicity; therefore, p-values from one-sided Student's t-tests here reflect the less strict null hypothesis that the given combination outperforms only the combination ranked immediately below it. We then use this as a proxy for the probability of outperforming *all* lower-performing combinations. Because 35 independent t-tests are conducted here, we consider the 1% level to be strongly significant (double underline), the 5% level weakly significant (single underline), and the 10% level (dotted underline) doubtfully significant. Assumptions of normality for t-tests were confirmed using the Kolmogorov-Smirnov test.

Rank	Signal processing procedure	Dimension reduction procedure	% Classification		p-Value
			Mean	SD	
1	Wavelet: Symmlet-4	Pairwise ANOVA	99.981	0.083	0.000
2	STFT	Coefficient of variance	99.858	0.229	0.009
3	STFT	ANOVA	99.744	0.262	0.148
4	Wavelet: Symmlet-4	Coefficient of variance	99.685	0.305	0.394
5	STFT	Variance	99.668	0.319	0.494
6	Wavelet: Symmlet-4	Variance	99.667	0.247	0.141
7	FFT	ANOVA	99.612	0.265	0.097
8	Wavelet: Daubechies-4	Variance	99.544	0.253	0.288
9	STFT	Pairwise ANOVA	99.508	0.380	0.446
10	Wavelet: Daubechies-10	Pairwise ANOVA	99.498	0.304	0.296
11	Wavelet: Haar	Coefficient of variance	99.456	0.462	0.273
12	Wavelet: Symmlet-4	ANOVA	99.408	0.320	0.453
13	Wavelet: Daubechies-4	Coefficient of variance	99.394	0.785	0.471
14	Wavelet: Daubechies-10	Coefficient of variance	99.386	0.318	0.387
15	Wavelet: Haar	ANOVA	99.365	0.390	0.427
16	Wavelet: Haar	Variance	99.346	0.615	0.486
17	Wavelet: Daubechies-10	Variance	99.342	0.479	0.053
18	Wavelet: Daubechies-4	ANOVA	99.195	0.429	0.225
19	FFT	Variance	99.129	0.451	0.379
20	FFT	Coefficient of variance	99.103	0.393	0.142
21	Wavelet: Daubechies-10	ANOVA	99.010	0.468	0.056
22	Wavelet: Daubechies-4	Pairwise ANOVA	98.815	0.735	0.445
23	PSD	Variance	98.798	0.507	0.419
24	Spectrogram	Coefficient of variance	98.770	0.822	0.137
25	Spectrogram	Variance	98.587	0.844	0.014
26	PSD	Coefficient of variance	98.257	0.605	0.007
27	Wavelet: Haar	Pairwise ANOVA	98.006	0.380	0.115
28	Spectrogram	Pairwise ANOVA	97.729	1.591	0.465
29	FFT	Pairwise ANOVA	97.709	0.443	0.086
30	Landmark Analysis	Variance	97.350	1.806	0.411
31	Landmark Analysis	ANOVA	97.290	0.518	0.001
32	Landmark Analysis	Pairwise ANOVA	96.281	2.344	0.000
33	PSD	Pairwise ANOVA	94.655	0.769	0.000
34	Landmark Analysis	Coefficient of variance	90.836	4.798	0.000
35	Spectrogram	ANOVA	63.661	1.536	0.000
36	PSD	ANOVA	60.733	2.022	–

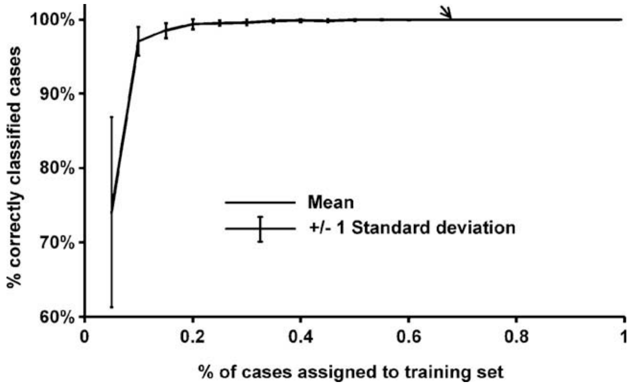

Fig. 8. Effect of the proportion of cases in each group assigned to the testing set on the classificatory performance of linear discriminant analysis (of the model data set of *Gymnotus*). Here data are taken from the single best of all 774 combinations of signal processing procedure, dimension reduction procedure, and M resulting features (Symmlet-4 wavelet, Pairwise ANOVA, $M = 14$). Note the narrowing of dispersion around the mean as it approaches 100%, marked by arrow, at a ratio of 0.66 (at which point standard deviation = zero).

n. sp. "omarorum" comprises two distinct sub-components: V1 (which corresponds to P-2 and P1 in the tropical species), and V2 (which corresponds to P0 in the tropical species). Rodríguez-Cattaneo et al.'s findings suggest wider patterns of homology, but patterns that require a revision of the classic P-2 through P3 model. An additional complication is that phases P-2 and P-1 (and the V1 component of P0 in *G.* n. sp. "omarorum") are weak and difficult to represent consistently in the head-to-tail waveform recording procedures typically used in field studies.

In the landmark procedure adopted here, the construction of the matrix forces a direct comparison of events from each successive phase, P-1 through P3, and assumes homology of each one. However, it is not clear whether the phases flanking P1 and P2 (i.e. P-2, P-1, P0, and P3), where present, correspond to homologous events in the electric organ from one species to another. Without such homology the landmark procedure will force inappropriate comparisons of specific regions of the signals at the expense of a more general comparison of the entire waveform, leading to untrustworthy conclusions.

In addition to the limitations exhibited by landmark-based procedures under conditions of homoplasy, landmarks can also be highly context-dependent. For example, a suite of landmark features that discriminate one set of taxa may not discriminate another, unless additional features are considered. Even the addition of one new taxon with an unusual signal may require a

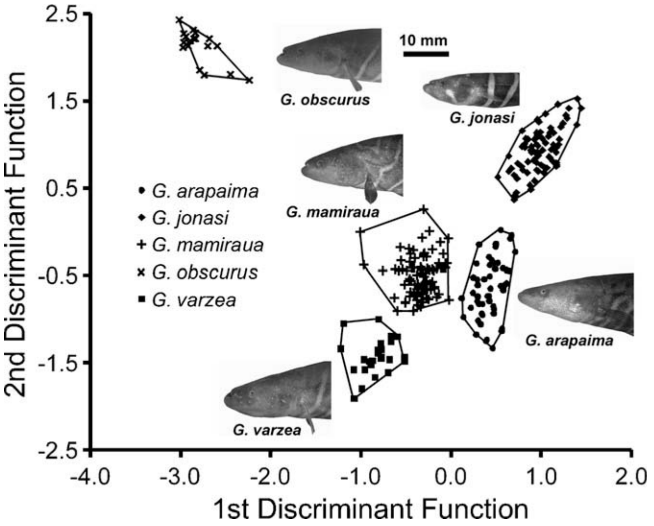

Fig. 9. Ordination of the electric organ discharge (EOD) waveforms of five species of *Gymnotus* in a two-dimensional representation of signal space generated by two of several discriminant functions (from linear discriminant analysis). Each symbol represents the EOD of a single recorded specimen. Here, data are presented for the best of all 774 combinations of procedures (Symmlet-4 wavelet, Pairwise ANOVA, with $M = 14$). Note the complete absence of overlapping EOD parameters among the five species. Inset photographs are of live specimens presented at a common scale.

landmark-based model to be revised. This context-dependence may lead to a lack of standardization in ongoing studies involving multiple taxa and widening horizons of geographical interest. At the very least this context-dependence makes the automation of landmark procedures difficult. For example, the discovery of a species of *Gymnotus* with an additional phase following P3 would require additional programming, because our model assumes that gymnotiforms have no more than six phases (P-2 through P3).

The issues discussed above are largely irrelevant to automated DSP procedures. Since signal features are selected automatically in DSP, subjective choices, or choices that make assumptions of structural homology are limited only to the selection of a single temporal alignment position for all groups of signals. Because all DSP techniques require this reference position, and because this is the only opportunity to allow notions of homology to enter a classification model, its selection should be carefully considered. We chose the P1 peak as a reference position for *Gymnotus* because there are strong grounds for homology of this event across all species in the genus. Having established an alignment point, DSP procedures are thereafter entirely objective. Moreover, they require no special programming. We experimented with using the P1–P2 zero-crossings as an alignment point for the DSP procedures. The overall results appeared very similar, with the Symmlet-4 DWT yielding the highest discriminatory performances, and wavelet procedures or STFT generally outperforming other DSP procedures and landmark analysis. Overall classificatory performance was slightly lower with alignment to the P1–P2 zero-crossing than with alignment to the P1 peak. For comparisons among mormyrids, and among Sternopygidae, the P1–P2 zero-crossing is a robust alignment position because it corresponds to a change in direction of current through the electric organ. Further studies of other gymnotiforms are required to evaluate the extent to which alignment positions such as the P1 peak and P1–P2 crossover are homologous within and between families.

5.2.3. Interpretation of wavelet and landmark analysis

In this section we describe what may be perceived as a drawback of wavelet transforms. Dimension reduction does not always yield intuitively meaningful coefficients, since its goal is finding the maximum amount of information in a given number of coefficients, rather than providing convenient interpretation. As the dimension of the underlying data increases, we may increasingly find that apparently only marginally important coefficients are in fact powerful proxies for multiple numbers of the most obvious discriminating features. Hence, the landmark transformation, in which all coefficients are easily recognizable, very likely yields easily interpreted discrimination rules (e.g. the slope at zero-crossing discriminates between groups 2 and 3), while the DWT may be difficult to interpret (e.g. the 243rd coefficient discriminates between groups 2 and 3). In practice, however, a careful, though separate, analysis can connect these obscure rules with more easily recognized features. Such an analysis would draw from the fact that each wavelet coefficient derived from DWT refers not to a specific position in the waveform (as do landmark features), but instead to a range of positions around a localizable center, which can be graphically represented (see Fig. 1A–C).

5.2.4. Similarity to sensory processing in animal brains

The best system for exploring and understanding cross-taxon differences in animal signal structure should emulate, as far as possible, the sensory and neural mechanisms used by those animals to recognize and discriminate signals. Several early studies reported close matches between the PPF of electric fish EODs and tuberous electroreceptor tuning (Bastian, 1976; Hopkins, 1976; Watson, 1979). This implies that the dominant spectral features of the EOD might encode species identity or allow private interference-free communication channels among ecologically co-occurring species. Consequently, studies of electric signal diversity have historically emphasized univariate divergence of the PPF in ecological assemblages. Several studies of low diversity communities of gymnotiforms noted the complete divergence of PPF and/or EOD repetition rate among co-occurring species (Hopkins and Heiligenberg, 1978; Heiligenberg and Bastian, 1980; Hopkins, 1981; Kramer et al., 1981). However, as first observed by Heiligenberg and Altes (1978), pulse-type gymnotiforms are also sensitive to phase changes in the EOD (where EOD shape is modified while maintaining constancy of the spectral properties). This raised the possibility that electric fish can discriminate species on the basis of temporal (shape) properties of waveforms instead of, or in addition to, spectral (frequency) properties. Hopkins and Bass (1981) demonstrated empirically that temporal characteristics of the EOD mediate conspecific recognition during courtship in a species of the mormyrid genus *Paramormyrops* (previously assigned to *Brienomyrus*). Xu-Friedman and Hopkins (1999) subsequently elucidated a neural pathway for temporal EOD coding in a true species of *Brienomyrus* (*B. brachyistius*).

All 263 individuals of the five ecologically co-occurring species of *Gymnotus* studied in this paper can be classified with no error, on the basis of features derived from DWT, which we recall gives a mixture of time and frequency information simultaneously. However, Fourier transforms with the phase components removed, i.e. stripped of much of their timing information, result in very poor discrimination of the species. Moreover, there is considerable overlap of the PPFs among several species (Fig. 5). These observations imply that the EOD waveforms of these five species exhibit non-overlapping *time*-frequency components but overlapping frequency components. To recognize and discriminate among conspecifics on the basis of the EOD waveform, these five species must therefore use some combination of temporal and frequency cues.

Hopkins and Westby (1986) hypothesized two alternative mechanisms for the detection of temporal properties in gymnotiform EODs. The first, "temporal coding", hypothesized that short EODs might be encoded as a burst of nerve spikes with a recognizable pattern (as in mormyrid temporal coding). In the second, "scan sampling", the receiver detects a signaler's EOD as an amplitude modulation or 'beat' set up by the combination of its own discharge with that of the signaler. The receiver uses the modulation envelope to assess the signaler's waveform. However, the neural mechanisms for EOD analysis are still relatively poorly known for gymnotiform fishes.

Regardless of the precise mechanism of signal decoding involved in the discrimination of conspecifics from non-conspecifics, gymnotiforms acquire information from neighboring fishes via tuberous electroreceptors, which are tuned to specific frequencies, or ranges of frequencies, and then process the information in the brain, presumably in real time, as they are acquired (Caputi et al., 2002). In this sense, signal processing by electric fish brains and afferent nervous systems may resemble, in broad terms, time-frequency-based DSP procedures such as the STFT and wavelet transforms. The multi-scale approach of wavelet analysis exhibits an additional analogy to the mode of electroreception of pulse-type gymnotiforms (and also mormyriforms). These fishes have several types of phase-coding tuberous electroreceptors with different tuning curves; some representing low frequencies, and others high frequencies (Bastian, 1976, 1977; Heiligenberg and Altes, 1978).

Electric fish may be sensitive to specific events in the waveform of a neighboring fish, such as peaks, zero-crossings, and inflection points, but it is unlikely that sensory processing of electric fish (or other animals) resembles the landmark-analysis employed here to detect these events. This would require post-acquisition processing of the entire signal, held in memory. Detection of relatively low frequencies, for example, requires that proportionally long signal samples be retained. Moreover, all of these specific events, such as peaks and inflection points are also equally represented in time-frequency processing.

In sum, landmark-based analyses do not resemble the general sensory processes used by animals to acquire and discriminate signals, whereas time-frequency-based DSP procedures, and in particular wavelet transformations do.

5.3. Other animal signals

Generally speaking, animal communication signals contain a mixture of low and high-frequency signals, with different groups (e.g. species) exhibiting subtle or profound variation in the presence or timing of a mixture of frequency components. Under these conditions, time-frequency analyses, especially the DWT, are predicted to most efficiently represent a signal with a small number of features. Electric fish signals are somewhat unusual in that the repeated elements (pulses or wave cycles) are short (0.4–6 ms), highly structured, and exhibit almost negligible variation between repetitions. Pulse-type electric fish signals comprise discrete pulses, made up of one to several phases of alternating polarity. These phases constitute transient frequencies (events occupying only part of a signal), which are predicted to be represented more efficiently with the time-frequency-based procedures STFT or DWT. Despite these theoretical predictions, this paper is the first to explore non-landmark-based procedures for the multivariate classification of electric signals.

The head-to-tail EOD waveform of wave-type electric fish resembles a sinusoid, although the deviation from a true sine wave introduces harmonics to the PSD. The FFT is predicted to represent wave-type signals with extreme efficiency. However, because they are rarely symmetrical, the Fourier phase components should be included. The EOD waveforms of some wave-type species exhibit extreme deviations from a sinusoid-like wave in the form of bumps or additional 'phases' which may or may not involve additional zero-crossings in a single EOD cycle. More "complex" EOD waveforms typically have peak power frequencies at higher harmonics of the fundamental frequency (Crampton and Albert, 2006; Crampton, 2007; Turner et al., 2007). For the reasons outlined in Section 2.1.1., time-frequency analyses, and especially wavelet analysis are predicted to more accurately represent signals containing these kinds of transients than can the FFT, regardless of the cyclical nature of the waveform. The implementation of the DWT (in the form described here) for wave-type signals requires first that an alignment position is chosen (e.g. the zero-crossing between successive EOD cycles). A cross-species comparison would involve beginning all waveforms at the alignment point, and ending all waveforms at the end of one complete EOD cycle for the individual fish with the longest waveform. For instance, in a comparison of several species in which the highest fundamental frequency was 1000 Hz, and the lowest 10 Hz, the individual with the lowest frequency would be represented by one EOD cycle, and the individual with the highest frequency by 10 cycles. A theoretical prediction is that time-frequency-based analyses would increasingly outperform FFT as the signals become increasingly complex. Nonetheless, for practical purposes, multivariate classification based on FFT might still resolve all but the most subtle variation.

Several authors have noted that the baseline EODs of many species within sympatric + syntopic communities of wave-type gymnotiforms exhibit overlapping properties (Kramer et al., 1981; Crampton, 1998, 2006, 2007; Crampton and Albert, 2006; Turner et al., 2007). As predicted by Crampton (1998), Turner et al. (2007) found substantial species-specific structuring of EOD modulations in apteronotid fishes. In this family, EOD modulations comprise short (10 ms–1 s) 'chirps' or longer (10 ms–60 s) 'gradual rises' that involve frequency and amplitude modulations of the baseline EOD. Incorporation of landmark-features from the time-voltage waveform of these modulations substantially improved discriminant function-based classifications of apteronotid species based otherwise on the EOD alone (Turner et al., 2007).

From the signal analysis perspective, the EOD modulations of apteronotids resemble short acoustic signals, such as insect chirps or anuran vocalization. Like EOD modulations, acoustic or seismic signals are generally longer, more complex, and more variable between repetitions than are single electric fish EODs. These kinds of signals should submit well to multivariate classification based on features derived from automated time-frequency DSP procedures. DWT is predicted to be the most efficient procedure, especially where some groups can be discriminated primarily on the basis of low-frequency components, and others primarily on the basis of high-frequency components. However, the classification of extremely long signals may not perform well with features derived from automated DSP procedures because the number of features that are derived may exceed the number of cases, thus violating statistical assumptions (see Section 2.2). Under these conditions, landmark features identified from the raw signal or spectrogram may be more efficient.

Our expectation is that automated time-based frequency analysis would be optimal for the extraction of a small number of informative features from electric fish chirps or short acoustic signals. In contrast, longer-term patterns such as the repetition rate of a volley of electric fish chirps, the gradual rises of wave-type electric fishes, or the longer acoustic signals of birds may be characterized more profitably from landmark features of the time-voltage waveform or spectrogram. An additional challenge with chirp-like signals is the selection of an alignment position, which as stressed earlier (Section 5.2.2), is a requirement of all DSP procedures. In the case of EOD modulations, valid alignment positions might include the beginning of a modulation (defined at a threshold deviation from baseline EOD frequency or amplitude), or the position of the maximum absolute deviation of frequency or amplitude. Where possible, the choice of the alignment position should be influenced by concepts of the underlying physiological and anatomical mechanisms of the modulations, although these are still relatively poorly known in apteronotids. Automated DSP-based classification of EOD modulations in *pulse*-type electric fish species and also short acoustic signals would require similar alignment considerations. In the case of acoustic signals generated by simple mechanical processes, such as stridulation (e.g. in grasshoppers), alignment positions may be linkable to discrete and potentially homologous mechanical events. We anticipate greater difficulties in aligning the vocalized signals used by many vertebrates, or the seismic signals used by some insects and spiders; although alignment to the beginning of each vocalization may prove to be robust.

Long acoustic signals such as bird calls are usually compared with landmark procedures, and to great success; but it would appear that nobody has attempted to apply automated DSP procedures to acoustic or seismic (non-human) animal signals for the purpose of classification. We predict that the objective nature of automated time-frequency DSP procedures will provide alternative or complementary approaches to traditional landmark procedures for the analysis of non-electric signals. DSP procedures may also permit automated assumption-free 'first passes' for the classification of unfamiliar animal signals.

5.4. The need for accurate classification

Where there is variation in classificatory performance due to the choice of procedures, it is important to choose the best-performing combination. Failure to do so will introduce classificatory error as an artifact of the statistical procedure, and hence inflate the true extent to which groups approximate each other in multidimensional 'signal space'. This is especially important in studies where the extent of overlap in signal space may be representative of an evolutionary process, for instance reproductive character displacement. Theory predicts that closely related, ecologically co-occurring, species will exhibit non-overlapping mate attraction signals (e.g. Shaw, 2000). For the five species of *Gymnotus* discussed here it is possible to use the proportion of misclassified signals between species as a proxy for the degree of signal overlap.

For the single best-performing combination of procedures we observed no misclassified EOD waveforms between any of the five species, conforming to the theoretical prediction of complete signal partitioning. However, with only a small increase in the overall percentage of misclassifications we begin to see overlap between species. For instance, with a 0.01% error we observe misclassifications between one specimen of *G. varzea* and *G. mamiraua*. With a 0.03% error we observe additional reciprocal misclassifications between these two species. At 0.1% error *G. jonasi* and *G. arapaima* begin to overlap. Therefore, for this data set, our simulations clearly indicate that the performances of the classification models vary sufficiently to influence an evolutionary interpretation of the data. Other data sets may be more or less sensitive to the choice of signal processing and dimension reduction procedures, and to the number of features retained. Of course, not all overlap is an artifact of analysis; comparisons of closely related individuals should reflect that commonality. In sum, the choice of the best-performing signal processing and data-reduction tools should minimize any 'false' instances of signal overlap (due to statistical error alone) which could distort evolutionary interpretations. These considerations also apply to classification for the purpose of identification alone; e.g. in ecological field studies where newly encountered signals are assigned to species on the basis of previously defined training sets, or in behavioral studies where signals can be classified to individual animals. Multivariate measures of distance between groups in signal space are also sensitive to 'false' instances of misclassification. For example, the Mahalanobis distances between the centroids of species clusters may be useful in phylogenetic studies of signal divergence, but depend on the accuracy of the inferred distances. Poorly performing models may underestimate the distance between subtly different signals relative to the distance between very distinct signals.

5.5. The need for simulations

The differential performance of alternative signal processing procedures largely fits theoretical predictions for the data set under consideration in this paper. However, as stressed in the preceding Section 5.4, the classificatory power of even the best-performing procedures depends largely, and in a generally unpredictable manner, on both the dimension reduction procedure, and on the number of resulting features. Moreover, different data sets may be expected to exhibit optimal classifications under different models, and again in an unpredictable manner. For these reasons, a 'brute force' simulation approach, similar to the one we employ in this paper, might be profitably applied to any dataset of animal signals as a precursor to multivariate classification. Unless statistical considerations are clear, simulations are likely to provide meaningful approximations to whatever the theoretical best-performing classification scheme is; in this data set and similar sets, there will be no such overwhelming indications. The approach we utilized here was easily programmed and relatively unrestricted by available computing power. For instance, all 774 combinations took approximately 60 h to run. However, many combinations were of course included here only for heuristic purposes and for practical purposes it would be reasonable to drastically simplify the number of combinations explored. For instance, high and low ranges of M features (e.g. below five and above 25) might be eliminated. Likewise it is not necessary to explore every increment of M within the desired range (e.g. increments of five could be considered). Informed decisions or trial and error can also rapidly exclude some signal processing procedures from consideration. For instance, based on our experience of pulse-type EODs we would recommend excluding the non-time-frequency-based DSP procedures, and limit wavelet analyses to bases that resemble the time-voltage waveform of the signal under analysis (e.g. Symmlet 4). From the optimal solution, performance could then be fine tuned by varying M in increments of one (for instance from -4 to $+ 4$ if increments of five had already been explored). By simplifying the simulation in this manner, a 'best solution' could be approximated within an hour or two of computer time (substantially less with streamlined programming and increased computer power). Classificatory studies of other kinds of animal signals, including wave-type EODs or acoustic signals, would probably benefit from more exhaustive initial simulations (resembling the ones we present here) to explore performance trends. Likewise, additional wavelet bases might be considered.

5.6. Mixed-source classification models of signals

Communication 'signatures' such as species identity may be encoded by multiple components of a signaling system. For instance, the stereotyped EOD of *Gymnotus* may be complemented by EOD pulse-rate modulations; although the data we present here suggest that EOD waveform may be in itself sufficient to encode species identity. Multivariate classifications of animal signals can combine features from such mixed sources. For example, Turner et al. (2007) combined landmarks from the EOD waveform and from EOD modulations in order to classify apteronotid signals. By selectively adding or removing different signal elements they then explored the extent to which EOD waveform versus EOD modulations contributed to inter-specific variation. For apteronotids, an alternative and probably better-performing approach would be to combine features derived from time-frequency DSP (e.g. wavelet analysis) of the EOD waveform with DSP *or* landmark-based analysis of the EOD modulations. Likewise, univariate measurements such as the rate of chirps in volleys of modulations could be added to the classification model as additional columns. In this manner, the choice of signal processing tool and dimension reduction procedure can be tailor-made to the structure and duration of each signal component. The resultant features are then combined into a single matrix for multivariate classification.

5.7. Conclusions

We used a large-scale simulation test to explore the extent to which landmark analysis and alternative DSP procedures can discriminate among groups of signalers in a model dataset comprising five species of pulse-type gymnotiform electric fish. We demonstrated that, as predicted by theory, time-frequency DSP procedures outperform landmark analyses, and also outperform frequency-based DSP procedures (i.e. the FFT). We also demonstrated that the classificatory performance of a given signal processing procedure is highly dependent on the dimension reduction procedure involved, and the number of features used in the LDA. Because it is difficult to predict the precise conditions under which the very highest accuracy will be reached, and because it is important to eliminate errors that are due to statistical artifact, we argue that a combination of signal processing tools, under a range of reduced dimension, should be compared in preliminary simulations. We anticipate that the multivariate classification of animal communication signals on the basis of features derived from automated DSP, in particular wavelet tools, will complement and strengthen traditional procedures such as landmark analysis, not only for electric fish signals, but also for short acoustic or seismic signals. We do not advocate the replacement of these traditional procedures but instead maintain that wavelet tools and other automated DSP procedures should have a place in the tool box of those seeking to classify animal signals, quantify signal divergence, or explore evolutionary concepts of signal space.

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References

Albert, J.S., Crampton, W.G.R., 2001. Five new species of *Gymnotus* (Teleostei: Gymnotiformes) from an Upper Amazonian floodplain, with descriptions of electric organ discharges and ecology. Ichthyol. Explor. Freshwaters 12 (3), 241–266.

Alt, S., Ringo, J., Talyn, B., Bray, W., Dowse, H., 1998. The period gene controls courtship song cycles in *Drosophila melanogaster*. Anim. Behav. 56, 87–97.

Arnegard, M.E., Hopkins, C.D., 2003. Electric signal variation among seven blunt-snouted *Brienomyrus* species (Teleostei: Mormyridae) from a riverine species flock in Gabon, Central Africa. Environ. Biol. Fishes 67 (4), 321–339.

Arnegard, M.E., Bogdanowicz, S.M., Hopkins, C.D., 2005. Multiple cases of striking genetic similarity between alternate electric fish signal morphs in sympatry. Evolution 59 (2), 324–343.

Assad, C., Rasnow, B., Stoddard, P.K., 1999. Electric organ discharges and electric images during electrolocation. J. Exp. Biol. 202, 1185–1193.

Azevedo, A.F., Van Sluys, M., 2004. Whistles of tucuxi dolphins (*Sotalia fluviatilis*) in Brazil: comparisons among populations. J. Acoust. Soc. Am. 3, 1456–1464.

Bass, A.H., 1986. Electric organs revisited: evolution of a vertebrate communication and orientation organ. In: Bullock, T.H., Heiligenberg, W. (Eds.), Electroreception. Wiley, New York, pp. 13–70.

Bastian, J., 1976. Frequency response characteristics of electroreceptors in weakly electric fish (Gymnotoidei) with a pulse discharge. J. Comp. Physiol. A 112, 165–180.

Bastian, J., 1977. Variations in the frequency response of electroreceptors dependent on receptor location in weakly electric fish (Gymnotoidei) with a pulse discharge. J. Comp. Physiol. A 121, 53–64.

Beecher, M.D., 1988. Spectrographic analysis of animal vocalizations: implications of the “uncertainty principle. Bioacoustics 1, 187–208.

Bennett, M.V.L., 1971. Electric organs. In: Hoar, W.S., Randall, D. (Eds.), Fish Physiology. Academic Press, New York, pp. 347–484.

Bracewell, R.N., 2000. The Fourier Transform and its Applications, fourth ed. McGraw Hill, Boston.

Bullock, T.H., Hopkins, C.D., Popper, A.N., Fay, R.R., 2005. Electroreception. Springer, New York.

Caputi, A.A., Castello, M.E., Aguileira, P., Trujillo-Cenóz, O., 2002. Electrolocation and electrocommunication in pulse gymnotids: signal carriers, pre-receptor mechanisms and the electrosensory mosaic. J. Physiol. Paris 96, 493–505.

Caputi, A.A., Carlson, B.A., Macadar, O., 2005. Electric organs and their control. In: Bullock, T.H., Hopkins, C.D., Popper, A.N., Fay, R.R. (Eds.), Electroreception. Springer, New York, pp. 410–452.

Casella, G.B., Berger, R.L., 2002. Statistical Inference, second ed. Duxbury Press, Pacific Grove, CA.

Coyne, J.A., Orr, H.A., 2004. Speciation. Sinauer Associates, Sunderland, MA.

Crampton, W.G.R., 1998. Electric signal design and habitat preferences in a species rich assemblage of gymnotiform fishes from the Upper Amazon basin. An. Acad. Bras. Ciências 70 (4), 805–847.

Crampton, W.G.R., 2006. Evolution of electric signal diversity in gymnotiform fishes. II. Signal design. In: Ladich, F., Collin, S.P., Moller, P., Kapoor, B.G. (Eds.), Communication in Fishes. Science Publishers, Enfield, NH, pp. 697–731.

Crampton, W.G.R., 2007. Diversity and adaptation in deep channel Neotropical electric fishes. In: Sebert, P., Onyango, D.W., Kapoor, B.G. (Eds.), Fish Life in Special Environments. Science Publishers, Enfield, NH, pp. 283–339.

Crampton, W.G.R., Thorsen, D.H., Albert, J.S., 2004. Three new species from a diverse, sympatric assemblage of the electric fish *Gymnotus* (Gymnotiformes, Gymnotidae) in the lowland Amazon basin, with notes on ecology. Copeia 2005 (1), 82–99.

Crampton, W.G.R., Albert, J.S., 2006. Evolution of electric signal diversity in gymnotiform fishes. I. Phylogenetic systematics, ecology and biogeography. In: Ladich, F., Collin, S.P., Moller, P., G Kapoor, B. (Eds.), Communication in Fishes. Science Publisher, Enfield, NH, pp. 647–696. 718–731.

Crawford, J.D., 1992. Individual and sex specificity in the electric organ discharges of breeding mormyrid fish (*Pollimyrus isidori*). J. Exp. Biol. 164, 79–102.

Curtis, C.C., Stoddard, P.K., 2003. Mate preference in female electric fish, *Brachyhypopomus pinnicaudatus*. Anim. Behav. 66, 329–336.

Gabor, D., 1946. Theory of communication. J. Inst. Elec. Eng., London 93 (3), 429–457.

Gerhardt, H.C., Huber, F., 2002. Acoustic Communication in Insects and Anurans: Common Problems and Diverse Solutions. University of Chicago Press, Chicago.

Graps, A., 1995. An introduction to wavelets. IEEE Comp. Sci. Eng. 2, 50–61.

Heiligenberg, W., Altes, R.A., 1978. Phase sensitivity in electroreception. Science 199, 1001–1003.

Heiligenberg, W., Bastian, J., 1980. Species specificity of electric organ discharges in sympatric gymnotoid fish of the Rio Negro. Acta Biol. Venez. 10 (2), 187–203.

Henry, C.S., Wells, M.L.M., Simon, C.M., 1999. Convergent evolution of courtship songs among cryptic species of the *Carnea* group of green lacewings (Neuroptera: Chrysopidae: *Chrysoperla*). Evolution 53, 1165–1179.

Hopkins, C.D., 1976. Stimulus filtering and electroreception: tuberous electroreceptors in three species of gymnotoid fish. J. Comp. Physiol 111, 171–208.

Hopkins, C.D., 1981. On the diversity of electric signals in a community of mormyrid electric fish in West Africa. Am. Zool. 21, 211–222.

Hopkins, C.D., Heiligenberg, W., 1978. Evolutionary designs for electric signals and electroreceptors in gymnotoid fishes of Surinam. Behav. Ecol. Sociobiol. 3, 113–134.

Hopkins, C.D., Bass, A.H., 1981. Temporal coding of species recognition in an electric fish. Science 212, 85–87.

Hopkins, C.D., Westby, G.W.M., 1986. Time domain processing of electric organ discharge waveforms by pulse type electric fish. Brain Behav. Ecol. 29, 77–104.

Hopkins, C.D., Lavoué, S., Sullivan, J.P., 2007. Mormyridae. In: Stiassny, M.L.J., Teugels, G.G., Hopkins, C.D. (Eds.), Poissons d'eaux douces et saumâtres de basse Guinée, ouest de l'Afrique centrale (The Fresh and Brackish Water Fishes of Lower Guinea, West-Central Africa), vol. 1. IRD Éditions, Paris, pp. 219–334.

Hopp, S.L., Owren, M.J., Evans, C.S., 1998. Animal Acoustic Communication. Springer-Verlag, Berlin.

Huberty, C.J., 1994. Applied Discriminant Analysis. Wiley Interscience, New York.

Kramer, B., Kirschbaum, F., Markl, H., 1981. Species specificity of electric organ discharges in a sympatric group of gymnotoid fishes from Manaus (Amazonas). In: Szabo, T., Czeh, G. (Eds.), Sensory physiology of aquatic lower vertebrates. Akademiai Kiado, Pergamon, Budapest, pp. 195–219.

Mallat, S., 1999. A Wavelet Tour of Signal Processing. Academic Press, San Diego.

McGregor, P.K., Westby, G.W.M., 1992. Discrimination of individually characteristic electric organ discharges by a weakly electric fish. Anim. Behav. 43 (6), 977–986.

Panhuis, T.M., Butlin, R.K., Zuk, M., Tregenza, T., 2001. Sexual selection and speciation. Trends Ecol. Evol. 16, 364–371.

Rodriguez-Cattaneo, A., Pereira, A.C., Aguilera, P.A., Crampton, W.G.R., Caputi, A.A., 2008. Species-specific diversity of a fixed motor pattern: the electric organ discharge of *Gymnotus*. PLoS One 3 (5), 1–13.

Runciman, D., Zann, R.A., Murray, N.D., 2005. Geographic and temporal variation of the male zebra finch distance call. Ethology 111, 367–379.

Saldamando, C.I., Miyaguchi, S., Tatsuta, H., Kishono, H., Bridles, J.R., Butlin, R.K., 2005. Inheritance of song and stridulatory peg number divergence between *Chorthippus brunneus* and *C. jacobsi*, two naturally hybridizing grasshopper species (Orthoptera: Acrididae). J. Evol. Biol. 18, 703–712.

Schwassmann, H.O., 1978. Ecological aspects of electroreception. In: Ali, M.A. (Ed.), Sensory Ecology. Plenum, New York, pp. 521–533.

Shaw, K.L., 2000. Further acoustic diversity in Hawaiian forests: two new species of Hawaiian cricket (Orthoptera: Gryllidae: Trigonidiinae: *Laupala*). Zool. J. Linn. Soc. 129, 73–91.

Siemers, B.J., Schnitzler, H., 2004. Echolocation signals reflect niche differentiation in five sympatric congeneric bat species. Nature 429, 657–661.

Smith, S.W., 1999. The Scientist and Engineer's Guide to Digital Signal Processing, Second Edition. California Technical Publishing, San Diego.

Sullivan, J.P., Lavoue, S., Hopkins, C.D., 2000. Molecular systematics of the African electric fishes (Mormyroidea: Teleostei) and a model for the evolution of their electric organs. J. Exp. Biol. 203, 665–683.

Tan, E.W., Nizar, J.M., Carrera-G, E., Fortune, E.S., 2005. Electrosensory interference in naturally occurring aggregates of a species of weakly electric fish, *Eigenmannia virescens*. Behav. Brain Res. 164, 83–92.

Turner, C.R., Derylo, M., de Santana, C.D., Alves-Gomes, J.A., Smith, G.T., 2007. Phylogenetic comparative analysis of electric communication signals in ghost knifefishes (Gymnotiformes: Apteronotidae). J. Exp. Biol. 210, 4104–4122.

Watson, D., Bastian, J., 1979. Frequency response characteristics of electroreceptors in the weakly electric fish, *Gymnotus carapo*. J. Comp. Physiol. 134, 191–202.

Weiss, L.G., Dixon, T.L., 1997. Wavelet-based denoising of underwater acoustic signals. J. Acoust. Soc. Am. 101, 377–383.

Wells, K., Crampton, W.G.R., 2006. A portable bio-amplifier for electric fish research: design and construction. Neotrop. Ichthyol. 4 (2), 295–299.

Xu-Friedman, M.A., Hopkins, C.D., 1999. Central mechanisms of temporal analysis in the knollenorgan pathway of mormyrid electric fish. J. Exp. Biol. 202, 1311–1318.