火种计划结题报告

Unsupervised Bout Classification of Zebrafish Locomotor Behavior under Head-fixed Condition

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ABSTRACT
Zebrafish presents rich locomotor behavior to orient itself, which could be categorized into different classes of bouts. Here we propose a method pipeline to classify bouts using the tail angle and eye angle data without resorting to the resulting data of bout like the movement distance or orientation angle, which is aiming to benefit the bout classification of head-fixed fish.

KEYWORDS
zebrafish, head-fixed, bout, classification, unsupervised clustering

1. Introduction

Zebrafish uses rich locomotor behavior sets to help it achieve different movement goals, which sheds insights on the neural mechanism underlying behavior. Previous study has revealed 13 kinds of bouts using a very comprehensive datasets including different contexts and parameters setting (Marques et al. 2018). However, it uses raw data including not only the tail angle, eye angle, but also movement result like orientation angle and travel distance. However, the latter raw data is not accessible under head-fixed condition. Therefore, the bout map provided by previous study could not apply to head-fixed fish data. Here we establish a method pipeline for clustering and classifying fish bout without resorting to movement result data, aiming at providing a bout map for head-fixed fish data.

2. Method

2.1. *Experiment*

Here we have two dataset available: 1. free moving fish dataset; 2.head-fixed fish dataset. In the former dataset, the sampling rate is 100 Hz, and fish could freely swim in the pool. While in the latter dataset, the sampling rate is 50 Hz, and fish is mostly constrained by agarose with eye and part of tail freed.

2.2. *Image Processing*

From the raw video, we first obtained the average background from the first 3000 frames and subtracted the average background from each frame along the whole video to get the foreground which is most likely to be fish. Next, as for the foreground, we look for the brightest point which is usually one eye of fish which locates the fish in the whole image. Next, we define a large ROI around the found brightest point to include the whole fish in and threshold this ROI to find the whole fish. Within the ROI, we skeletonize the objects to find out the whole body centerline of the fish. In addition, we also use a larger threshold to find only the anterior part of body and find the third brightest point within the anterior part of body which is usually the swim bladder. By locating the swim bladder along the whole body centerline, we could obtain the tail centerline. We select 10 points with equal distance along the tail centerline and use their angle data as input for the following pipeline.

2.3. *Feature Extraction*

We choose a method pipeline based on consideration of factors: 1.computational efficiency: in this view, the method like performing dynamic time warping (Mearns et al. 2020) and clustering based on between-bout distance is time consuming (10000 bouts requires computation of 10000*9999 dynamic time warping). 2.accuracy: in this view, method like (Johnson et al. 2020) is not considering the whole bout tail dynamics. Therefore, we basically followed the method in (Marques et al. 2018) and made further modifications like discarding features involving trajectory, adding frequency domain features and adopting different clustering methods.

2.3.1. Bout Detection

Bout detection is mainly based on curvature data. First, the curvature data is summed across tail segments, thus resulting in a one dimensional time series which is named as *sum_curv*. Second, we performed Hilbert Transform to *sum_curv* to extract its envelope. Third, considering that fish is either moving or not, a two component Gaussian Mixture Model is fitted to the distribution of envelope and the threshold to identify tail moving is the mean of the component with the larger mean value subtracted by two-fold standard deviation. The code script related to this is getPrepData.m and BoutDetectorCurvatureFunction.m.

2.3.2. Kinematic Parameters Extraction

The tail angle and convergence angle of each bout then is used to extract 179 kinematic parameters, which mainly refers to (Marques et al. 2018). The list of the parameters is in Appendix. A. The main script is boutKine_calc.m.

2.3.3. Frequency Domain Feature Extraction

For each tail segment and each bout, the amplitude in frequency domain is obtained via Fourier Transform. The amplitude is then binned every 5 Hz to yield 10 average amplitude feature in each bin. Including all tail segments, it gives 100 features. The corresponding script is extractFrequencyDomainFeature.m.

2.3.4. Bout Selection

The features above is performed twice: one for original angle data, the other for its opposite. Bouts which are detected to have opposite half beat direction are considered as successful feature extraction. Those not successful are discarded, which occupy about 18% of all detected bouts. Most of the discarded have abnormal raw tail angle. And the remaining bouts all are ensured to have the same upward tail deflection by making the bouts detected as downward in original angle data opposite in sign.

2.4. *Dimension Reduction*

PCA is first applied and the first 50 PCs served as input to tSNE to reduce the dimension to two.

2.5. *Clustering*

2.5.1. *Step one*

Kernel density estimation with the bandwidth of 4.5 is performed to the boutx2 feature given by tSNE. The estimated probability distribution function (pdf) is then binarized by the adapting threshold using MatLab built-in function adaptthresh. The binarized pdf is transformed to distance measure by Matlab built-in function bwdist. Finally, watershed segmentation is applied to the distance-transformed binary pdf to segment the pdf into clusters.

2.5.2. *Step Two*

Watershed segmentation usually detects more clusters than we perceive the pdf. The potential reason may be noise. Or even some detected clusters are really distinct clusters, it's difficult to interpret since their tail dynamics and other aspects we could recognize is qualitatively not very distinct from its neighborhood. Therefore, we perform a secondary clustering of watershed clusters. We used two simple measures: sum_angle (the sum of time-varying angle across the whole bout) $abs(max\_angle)$ (absolute maximum angle across the whole bout) of the 8th tail segment. The mean measure value of each cluster comprised a new feature matrix with size of (number of clusters x 2). Hierarchical clustering is performed on the new feature matrix and recommend the secondary cluster assignment. Combining the tail dynamics plotted, the user should finally decide how to perform the secondary cluster assignment.

2.6. *Classification*

The procedure above would build a bout map with bout kinematic parameters and its cluster label. New data would first be transformed into feature vector and search the nearest 20 training bouts in the bout kinematic parameter space, and is labeled as the cluster most frequently detected.

3. Result

Since free moving fish data has both the tail dynamics and movement result. And movement result is a good measure of the classification/clustering result. Therefore, to validate the pipeline, we first train the clustering pipeline on a subset of free moving fish data, then test it on more free moving fish data. Finally, we applied the method to head-fixed fish data.

3.1. *Free Moving Fish Training Dataset*

We used five free-moving fishes to build the bout map as shown in Figure 1. 50 PCs account for about 90% variance. Kernel density estimation demonstrates several density clusters, indicating that there exists several distinct stereotyped bouts instead of a continumm. And this cannot be explained by fish individual difference (not shown here). The estimated pdf is segmented into 16 clusters, among which cluster 10 has high eye convergence angle and thus is supposed to be J-turn/approach/capture strike. Checking the corresponding video indicates a J-turn class. Next, we aim to group the remaining 15 clusters into larger clusters. The upper panel in Figure 1(e) suggests a group of [14 16], [12 13 15],[6 9 11],[1 2],[3 8 4 5 7]. Although the corresponding tail dynamics is not consistent in some group like [12 13 15], we think that this is an satisfying, though not perfect, grouping. The final clustering also presents a good clustering on PCA plane, and movement result: angle-distance plane which is not used in clustering. This separation on angle-distance plane cannot be achieved by randomness (not shown here).

3.2. *Free Moving Fish Test Dataset*

Next, we apply the bout map obtained in last section to a large dataset of 117 free moving fishes. The information of this dataset is listed in Table 1. After projecting the bout kinematic features onto tSNE plane, we find out significant effect of fish individual difference, e.g. Figure 2(a). To investigate this further, we performed a hierarchcial clustering according to the pdf similarity on tSNE plane between fishes. The highest level of hierarchical tree separates two main clusters of pdf farthest. Among the possible impacting factors including species, age, and body length, species plays a major role in this separation. One cluster is mainly composed of h2bg6f fish (62/66, i.e., 94%) with all ages and body length nearly equally distributed, while the other is mainly composed of nacre fish (33/51, i.e., 65%). Species could not account for all the separation, the nacre fish in cluster 1 and the h2bg6f fish in cluster 2 cannot be accounted for by age or body length difference from their main group, thus attributed to noise. The relatively high proportion of h2bg6f in cluster 2 is likely due to more h2bg6f fish in total. If we randomly select equal number of h2bg6f to nacre fish, we found out that the number of h2bg6f in cluster 2 is largely reduced (from 35% to 22%), although still higher than nacre in cluster 1 (12%). Because our bout map built in last section is mainly of h2bg6f species and h2bg6f and nacre has distinctly different pdf distribution, here we mainly focus on applying the bout map to the h2bg6f fish. Knn search in bout kinematic feature space could also segment the tSNE plane well in Figure 3(a). The classification result could also segment the orientation angle-travel distance plane into six distinct area, thus validating the efficacy of the application of bout map to new dataset. Although in the test dataset, the eye convergence angle of bout classified as J turn don't have a high convergence angle, we found out that those detected bouts are indeed J turn by manual check, suggesting that eye convergence may not be the necessary condition of J turn.

3.3. *Head-fixed Fish Test Dataset*

Finally, we applied the trained bout map to head-fixed fish dataset, whose information is listed in Table 2. However, current head-fixed dataset has the sampling rate at 50 Hz. According to Figure 4 (a), the tail dynamics of normal freely moving fish spans

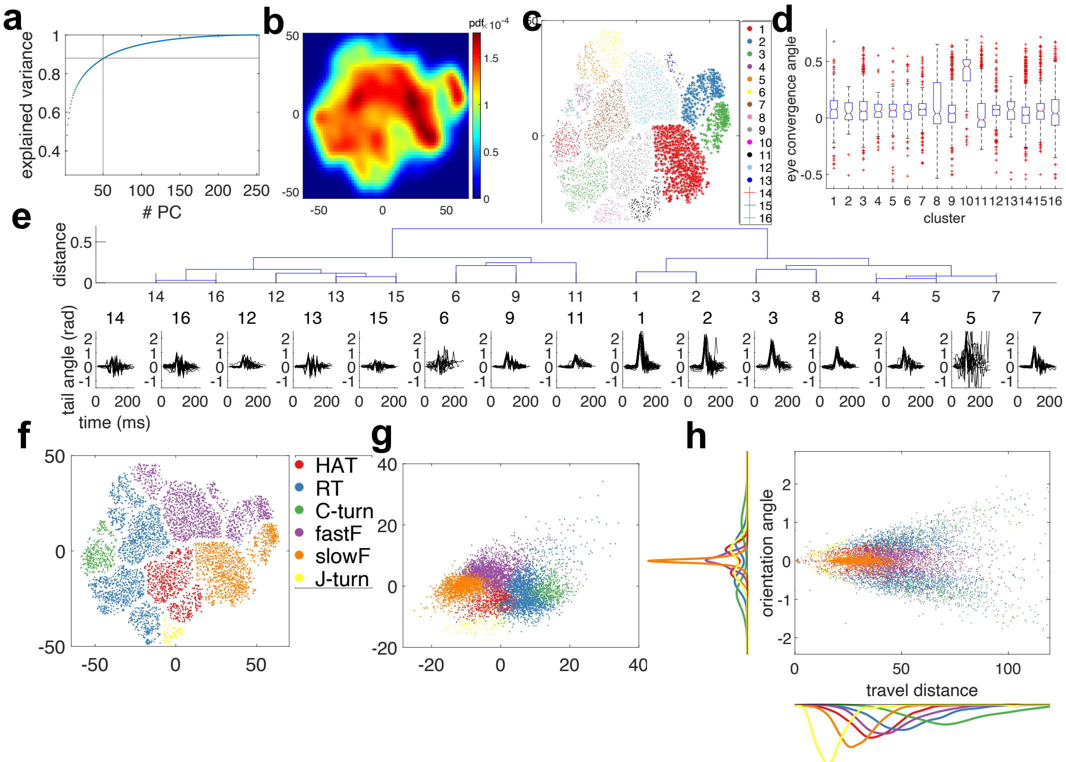

Figure 1.: Build bout map from free moving fish (a) Explained variance obtained by PCA on extracted kinematic features. (b)Estimated probability density function on tSNE plane. (c) First stage clustering by watershed segmentation. (d) Eye convergence angle of bouts classified into each class in (c). (e) Recommended second-stage clustering. The upper panel demonstrates the hierarchical tree across clusters obtained in (c). The bottom panel is the angle of 8th tail segment varying with time for bouts at the center of each class in (c). (f) Final clusters obtained from (e) on tSNE plane. (g) Similar to (f) but on PCA plane. (h) Travel distance and orientation angle coming from bouts. Each dot is one bout, colored by its class in (f). The image at the left and bottom of the scatter plot demonstrates the probability distribution of the orientation angle and travel distance across bouts belonging to each class. (g-h) has the same color scheme as (f).

Table 1.: Free moving fish information

Experiment Date	age(dpf)	species	number of Fish
20201217	12	h2bg6f	8
20201222	9	h2bg6f	14
20201223	10	h2bg6f	12
20210105	9	h2bg6f	5
20210106	10	h2bg6f	8
20210107	11	h2bg6f	8
20210108	12	h2bg6f	4
20210128	11	h2bg6f	11
20210129	12	h2bg6f	11
20210130	9	nacre	10
20210131	10	nacre	10
20210201	11	nacre	10
20210202	12	nacre	7

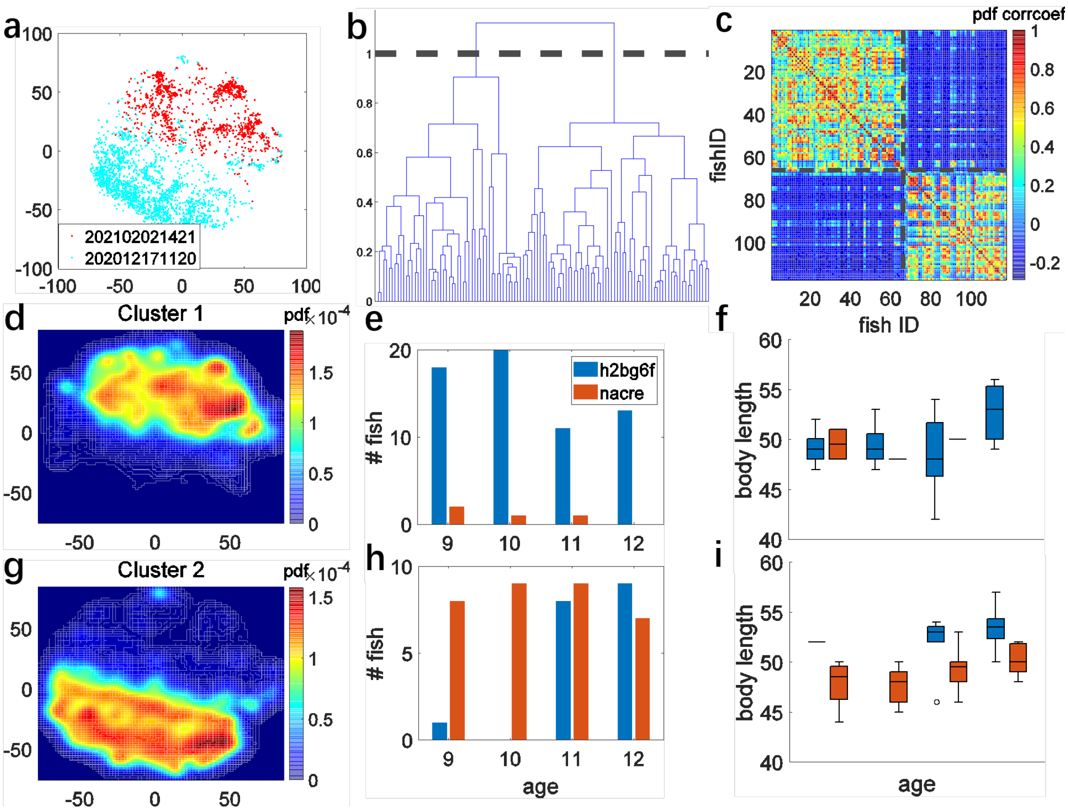

Figure 2.: Different species swim differently. (a) The bouts from two fishes are distributed separately on tSNE plane. (b) Hierarchical tree based on pdf correlation coefficient across all fishes. The horizontal dash line at y=1 denotes the distance where the two main clusters are separated. (c) Pdf correlation coefficient matrix sorted by the two clusters in (b). The dash line separates two clusters. (d) The pdf of cluster 1, which is well separated from the pdf of cluster 2 in (g). (e) The count number of fish of different age and species belonging to cluster 1. (f) The body length distribution of fish of different age and species belonging to cluster 1. (g-i) Similar to (d-f).

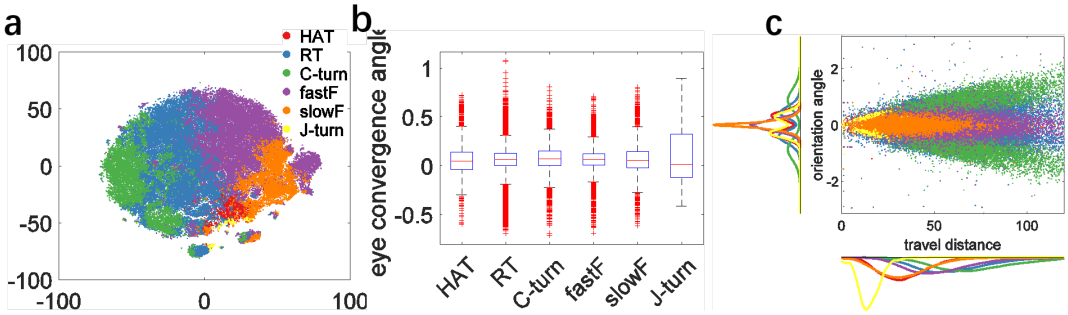

Figure 3.: Application of trained bout map to same species test dataset. (a)Classified test bouts on tSNE plane. (b) Eye convergence angle (rad) of each class. (c) Orientation angle and travel distance of each classified test bouts.

Table 2.: Head-fixed Fish Dataset Information

Experiment Date	age(dpf)
200618	8
200626	9
200705	8
201116	7
201117	8
201125	9
201126	10

spectrogram till 65 Hz, thus requiring at least 130 Hz sampling rate. 50 Hz sampling rate would lose the wave propagation information along the tail and distort the tail dynamics, which is very harmful for the feature extraction of half beat dynamics. Therefore, current head-fixed dataset cannot restore the real information about the tail dynamics and thus is unusable. Although the current dataset cannot account for the real tail dynamics, the same pipeline applied to head-fixed data demonstrates qualitative separation between fishes on tSNE plane. This separation would also make it difficult to build a bout map based on head-fixed dataset itself. This separation still needs more head-fixed data to validate.

4. Discussion

4.1. *Drawbacks of current pipeline*

As for pure techniques, current pipeline has some drawbacks. First, the recognition of J-turn class relies much on eye convergence. However, according to empirical observation, J-turn-like swim does not only occur when eye converged. Second, the estimated pdf in 1(b) is actually complex and does not form distinct clusters as we expected for clustering. Consequently, watershed segmentation requires a further clustering. To have a better estimated pdf, the dataset, the feature could be improved.

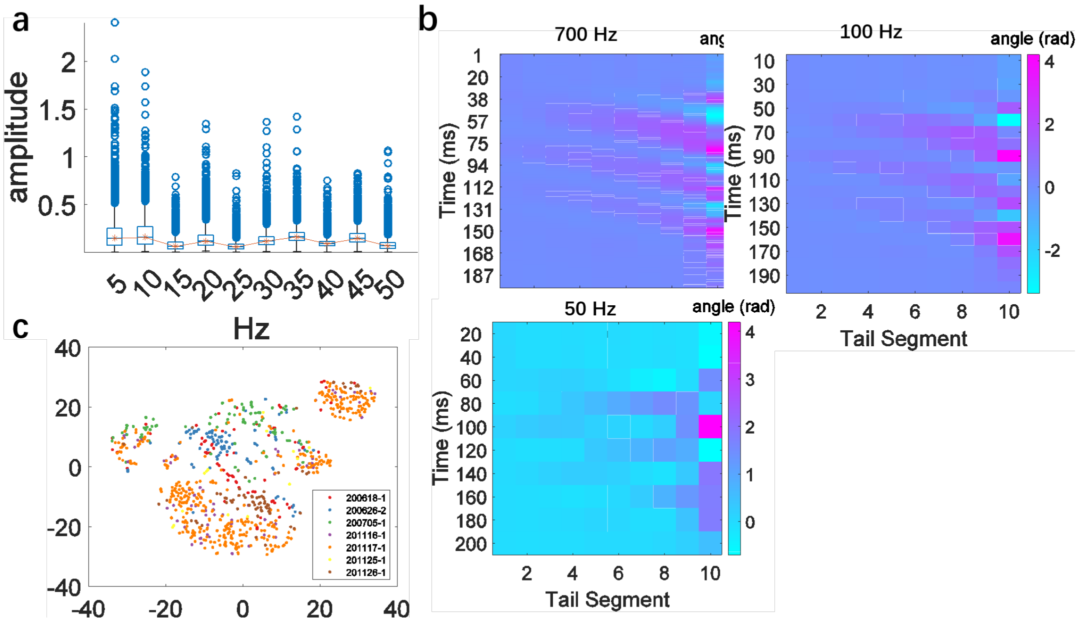

Figure 4.: Head-fixed fish dataset does not have enough sampling rate and have large individual difference. (a) Amplitude distribution at frequency domain across 10000+ free moving bouts sampled at 100 Hz. Each bout has one sample at each frequency. (b) Tail angle of one example bout sampled at 700 Hz, which is then downsampled to 100 Hz and 50 Hz. 50 Hz cannot demonstrate the wave propagation along the tail while 100 Hz generally preserve the dynamics. (c) Bout kinematic features projected on tSNE plane and colored by fish identity. Different fish is well separated on tSNE plane.

4.2. *Head-fixed fish bout classification*

The idea of applying free moving bout classification map to head-fixed fish seems too naive now, especially considering that head-fixed fish does not have a totally free tail thus unlikely to present similar tail movement pattern as free moving fish. Some small tail movements like J-turn or slow approach may not be affected much.

If the goal is to classify the fish tail movement under embedded situation, with regards to current experiment platform, we must make improvement towards two conditions: 1.improve the sampling rate to at least 120Hz as shown in last section of Result; 2.embed each fish as similarly as possible to ensure the same free tail segment. Empirically, different tail length would leave fish with different degree of freedom to use its tail, leading to different pattern of tail movement. With the two conditions improved, next step is to collect data of around 20 head-fixed fishes. Current dataset is still too small, no more than 500 bouts available across all head-fixed fishes, which may cause the failure of density-based clustering techniques. For example, some less frequently appeared bout class may not be able to collect enough samples to form an overall density distribution.

4.3. *Comparison to literature*

In contrast to our unsupervised way, previous study mostly addressed this problem using a supervised way. Semmelhack et al. (2014) trained a Support Vector Machine (SVM) classifier to distinguish prey capture swim from spontaneous swim. A recent study further perform five classes classification using supervised soft-clustering algorithm (Jouary Adrien 2016). They tried unsupervised clustering method, and found out that there is no discrete clustering but a continumm on tsne map. We don't have the similar experiment data to confirm their result. If their result is true, then there is no point to treat tail movements as discrete classes, which will even mislead our investigation of the underlying neural mechanism. Instead, we should think about how to regress the movement to neural activity. If their result is wrong, we should try to use multiple feature extraction methods in addition to the dynamic time warping methods used in that paper to confirm it and see if it could form discrete clusters based on new feature. Therefore, we think that the classification in that paper requires: (1)exclusion of the possible effects of the different embedding tail length (individual difference is not dominantly affecting tsne map); (2)use other feature extraction methods to validate the result. There are some intuitive thoughts: from empirical observation of head-fixed fish tail movement, there is no stereotyped bouts. Instead, tail movements is very flexible, for example, fish could continuously launch several biased tail swing (if we call it a bout) when prey is at its side and fish under head-fixed situation, which never happen under free moving situation. Therefore, there may not exist stereotyped behavior under head-fixed situation and insistence on discrete classification may mislead our investigation. An intuitive way to solve this puzzle is to collect enough data and perform feature extraction as here and do tsne again.

5. Conclusion

In this report, we propose a pipeline for clustering the bouts of head-fixed fish in an unsupervised way, which allows for application of clustered bout map to new datasets. We validated its performance under free moving fish dataset. For the application to head-fixed fish, we still need more high-quality head-fixed fish data using multiple methods to see if head-fixed fish exploits stereotyped tail movements or not.

Supplementary Figure

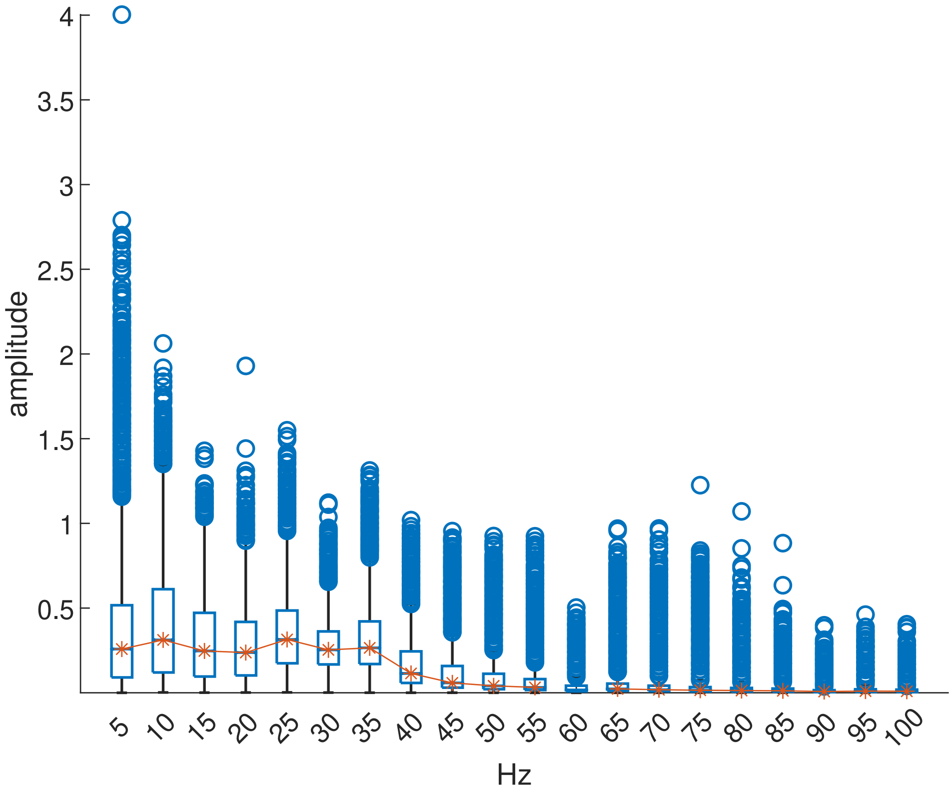

Figure 5.: Amplitude distribution at frequency domain across 30000+ free moving bouts sampled at 700 Hz. The dataset comes from (Marques et al. 2018)

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Appendices

Appendix A. Kinematic Parameters

(Note that ’hb’ is equal to half beat)

numbHalfBeats, boutDuration, maxbeatFreq, minbeatFreq, meanbeatFreq, maxTailChangeAvr, meanTailChangeAvr, maxTailChangeMax, meanTailChangeMax, maxboutTailWaveSpeedAll, minboutTailWaveSpeedAll, meanboutTailWaveSpeedAll, maxboutTailAngularVelocityAll, minboutTailAngularVelocityAll, meanboutTailAngularVelocityAll, maxboutTailAmplitudePositionRateAll, minboutTailAmplitudePositionRateAll, meanboutTailAmplitudePositionRateAll, maxboutHalfBendingPosAll, minboutHalfBendingPosAll, meanboutHalfBendingPosAll, maxboutCruvatureAll, minboutCruvatureAll, meanboutCruvatureAll, maxboutCurvatureRateAll, minboutCurvatureRateAll, meanboutCurvatureRateAll, boutAbsAmplitude1Avr, boutAbsAmplitude2Avr, boutAbsAmplitude3Avr, boutAbsAmplitude4Avr, boutAbsAmplitude5Avr, boutAbsAmplitude6Avr, boutAbsAmplitude7Avr, boutAbsAmplitude8Avr, boutAbsAmplitude9Avr, boutAbsAmplitude10Avr, boutAmplitude1Max, boutAmplitude2Max, boutAmplitude3Max, boutAmplitude4Max, boutAmplitude5Max, boutAmplitude6Max, boutAmplitude7Max, boutAmplitude8Max, boutAmplitude9Max, boutAmplitude10Max, hb1_ud, hb1_numBeat, hb1_dur, hb1_freq, hb1_amp1, hb1_amp2, hb1_amp3, hb1_amp4, hb1_amp5, hb1_amp6, hb1_amp7, hb1_amp8, hb1_amp9, hb1_amp10, hb1_ampAvr, hb1_ampMax, hb1_maxTailAngle, hb1_minTailAngle, hb1_halfBendAngle, hb1_maxTailPos, hb1_minTailPos, hb1_halfBendPos, hb1_waveSpeed, hb1_angularVel, hb1_ampPosRate, hb1_auc1, hb1_auc2, hb1_auc3, hb1_auc4, hb1_auc5, hb1_auc6, hb1_auc7, hb1_auc8, hb1_auc9, hb1_auc10, hb1_aucAvr, hb1_aucMax, hb1_maxTailChange, hb1_meanTailChange, hb1_maxCurv, hb1_maxCurvRate, hb2_ud, hb2_numBeat, hb2_dur, hb2_freq, hb2_amp1, hb2_amp2, hb2_amp3, hb2_amp4, hb2_amp5, hb2_amp6, hb2_amp7, hb2_amp8, hb2_amp9, hb2_amp10, hb2_ampAvr, hb2_ampMax, hb2_maxTailAngle, hb2_minTailAngle, hb2_halfBendAngle, hb2_maxTailPos, hb2_minTailPos, hb2_halfBendPos, hb2_waveSpeed, hb2_angularVel, hb2_ampPosRate, hb2_auc1, hb2_auc2, hb2_auc3, hb2_auc4, hb2_auc5, hb2_auc6, hb2_auc7, hb2_auc8, hb2_auc9, hb2_auc10, hb2_aucAvr, hb2_aucMax, hb2_maxTailChange, hb2_meanTailChange, hb2_maxCurv, hb2_maxCurvRate, hb3_ud, hb3_numBeat, hb3_dur, hb3_freq, hb3_amp1, hb3_amp2, hb3_amp3, hb3_amp4, hb3_amp5, hb3_amp6, hb3_amp7, hb3_amp8, hb3_amp9, hb3_amp10, hb3_ampAvr, hb3_ampMax, hb3_maxTailAngle, hb3_minTailAngle, hb3_halfBendAngle, hb3_maxTailPos, hb3_minTailPos, hb3_halfBendPos, hb3_waveSpeed, hb3_angularVel, hb3_ampPosRate, hb3_auc1, hb3_auc2, hb3_auc3, hb3_auc4, hb3_auc5, hb3_auc6, hb3_auc7, hb3_auc8, hb3_auc9, hb3_auc10, hb3_aucAvr, hb3_aucMax, hb3_maxTailChange, hb3_meanTailChange, hb3_maxCurv, hb3_maxCurvRate, firstBeatAmplitudeDiff, boutAmplitudeDiff, meanBoutFreqCorr, maxBoutFreqCorr, minBoutFreqCorr, eyeConvAvrThisBout, eyeConv20FramesBeforeBout, eyeConv20FramesAfterBout, eyeConvDiff2