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Research article

RNA sampling time on postmortem avian carcasses in the wild

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Genetic sampling, especially high-quality RNA from wild avian populations, is challenging in wildlife biology due to rapid RNA degradation. Although carcasses could be a potential RNA source, the optimal postmortem sampling time on the avian carcasses under field conditions remains unclear. Here, we carried out a field experiment on the Qinghai-Tibet Plateau (QTP) and evaluated the relationship between PMI and RNA degradation in three tissue types (muscle, brain, and liver) of the domestic chicken *Gallus gallus domesticus* carcasses. In the muscle and brain tissues, we found that the RNA Integrity Number (RIN) of samples collected within 60 h postmortem was more than 7.0, suggesting a high RNA extract quality. The following RNA-seq experiment demonstrated that gene expression profiles of the samples collected within 36 h postmortem were comparable to those of fresh samples (i.e. 0 h), with a low percentage of differentially expressed genes (< 3.0%) observed between samples at 0 and 36 h postmortem. However, in the liver tissue, RNA samples already degraded at 12 h postmortem, showing low RIN values (< 7.0), different gene expression profiles from fresh samples, and a high percentage of differentially expressed genes (15.6%). Therefore, our study suggests that samples from muscle and brain tissues collected within 36 h postmortem are qualified for RNA-seq analyses. In contrast, only the fresh RNA samples from liver tissue are qualified. Our study provides a practicable and efficient sampling strategy for the transcriptome study on avian populations under extreme environment such as the QTP.

Keywords: postmortem sampling time, RNA degradation, RNA-seq, wild avian populations

Introduction

Genetic sampling (e.g. RNA) from wild animals in the field is challenging, especially for the birds with strong flight capability and wide-ranging activity (Pan et al. 2017, Gu et al. 2021, Hu et al. 2022). Opportunistic encounters with wild avian carcasses,



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thus, provide a complementary source for genetic sampling (Camacho-Sanchez et al. 2013, Romero et al. 2014). With the aid of next-generation sequencing technologies, these samples enable us to address the research questions on ecology, evolution, and conservation of wild avian populations (Ouborg et al. 2010, Alvarez et al. 2015, Todd et al. 2016). However, due to the rapid degradation of RNA in samples from carcasses (Wong et al. 2012), obtaining qualified RNA extracts from them had been proven to be challenging in the wild, which hindered downstream RNA sequencing (RNA-seq) analyses (Romero et al. 2014). Therefore, studies on the sampling strategy (i.e. determination of appropriate postmortem sampling times) from carcasses in the wild are necessary for better use of this source in avian transcriptome studies.

Investigating RNA degradation under field conditions could provide clues for this kind of RNA sample collection since various environmental factors such as sunlight, humidity, and temperature could influence the degradation rates of RNA (Bonadio et al. 2021, Heneghan et al. 2021). Although many previous studies had checked the effect of different preservation methods on RNA degradation in the wild (Perez-Portela and Riesgo 2013, Nakatsuji et al. 2019, Passow et al. 2019), very few considered the relationship between postmortem interval (PMI) and RNA degradation. A recent study suggested that samples within six hours could yield relatively high-quality RNA based on the evaluation of RNA Integrity Number (RIN) in muscle and liver tissue in the house sparrow *Passer domesticus* (Chevireon et al. 2011). However, such a time interval (i.e., six hours postmortem) is not enough for most field surveys, especially those conducted in complex environments such as Qinghai-Tibet Plateau (QTP). Furthermore, up to date, there is no study using RNA sequencing data to investigate the effect of PMI on RNA degradation under field conditions.

To address these issues, we designed a field experiment of RNA sampling from domestic chicken *Gallus gallus domesticus* carcasses on the QTP. As a classical extreme environment, QTP provides us valuable opportunities to study species' gene expression changes in response to extreme conditions characterized by low oxygen levels and temperatures (Pan et al. 2017, Hu et al. 2022). On the plateau, wild avian species have been found to die of natural (e.g. predation and starvation), climatic (e.g. snowstorm, Li et al. 2018), and anthropogenic reasons (e.g. electrocution and road kill; Dixon et al. 2013, Kong et al. 2013, Wang et al. 2022a). More importantly, because the QTP is mainly covered by grassland (54–70%, Wang et al. 2022b) with flat topography, the carcasses are more visible to be detected. Therefore, QTP is a valuable place for collecting RNA samples from wild avian carcasses. Here, to provide an estimate of postmortem sampling time on carcasses for transcriptome studies of wild avian populations under the extreme environment such as the QTP, we used the chicken carcass exposed to the natural QTP environment to mimic the scenarios of the carcasses found in the fieldwork. We further evaluated the RNA degradation in three tissues (muscle, brain, and liver) within 120 h postmortem in the wild by examining the RNA quality (RNA yield

and integrity) and analyzing RNA-seq data (read alignment and gene expression).

Material and methods

Study design

Figure 1 shows our study design. Specifically, we purchased three male domestic chicken individuals aged five months old from Xining (elevation: 2200 m), Qinghai Province, China. The chickens were next transported to Madoi County (elevation: 4300 m) and euthanized using a manual cervical dislocation method. The carcasses placed in an outdoor cage were exposed to the field environment. The relative ambient humidity and ambient air temperature were continuously measured using temperature/humidity probes 'Cos-03-*' (Renke Control Technology Co., Ltd., China) (Fig. 1b). This study defines the interval between death and postmortem sampling time points as the postmortem interval (PMI) (Romero et al. 2014, Nakatsuji et al. 2019, Bonadio et al. 2021). Since fieldworkers usually start the survey in the target species' habitats in the morning and return before nightfall (Järvinen et al. 1976, Bårdsen and Fox, 2006), we set 12 h as an interval of postmortem sampling time to simulate typical field survey scenarios. Postmortem sampling began at 0 h with an interval of 12 h until 120 h (Fig. 1b). For each sampling, following Valverde's method (2020), photographs were taken to help examine the decomposition degree of external (i.e. eyeball and tongue) and internal organs (i.e. brain, liver and pectoral muscle) (Supporting information). By adopting the following steps, we tried to minimize the impact of the experimental operation on RNA degradation. Firstly, after the initial sampling, we sew up the cut to minimize prolonged exposure of the organs to external environment. We randomly sampled the tissues from different locations of the organ. The muscle, brain, and liver samples were collected respectively from each individual (n=3) and were immediately stored in liquid nitrogen. All collected samples were transported to the lab on dry ice and stored in a -80°C freezer before use. To verify our application of postmortem RNA strategy to the wild carcasses on the QTP, we have successfully collected the muscle samples from carcasses of electrocuted upland buzzard *Buteo hemilasius* at 0 h (n=2), 12 h (n=2), 24 h (n=2) and 48 h (n=6) postmortem during our field survey in June 2023. The storage conditions are as the same as mentioned above.

RNA extraction

RNA extraction was conducted by the same researcher and used the phenol-chloroform-based method (Rio et al. 2010). The main procedures followed our previous studies (Pan et al. 2017, Hu et al. 2022). Briefly, we added 1 ml of TRIzol reagent (Invitrogen, USA) per frozen tissue sample (30 mg) and homogenized each sample using a homogenizer (Thermo Fisher Scientific, USA) on ice. With 200 µl chloroform, we

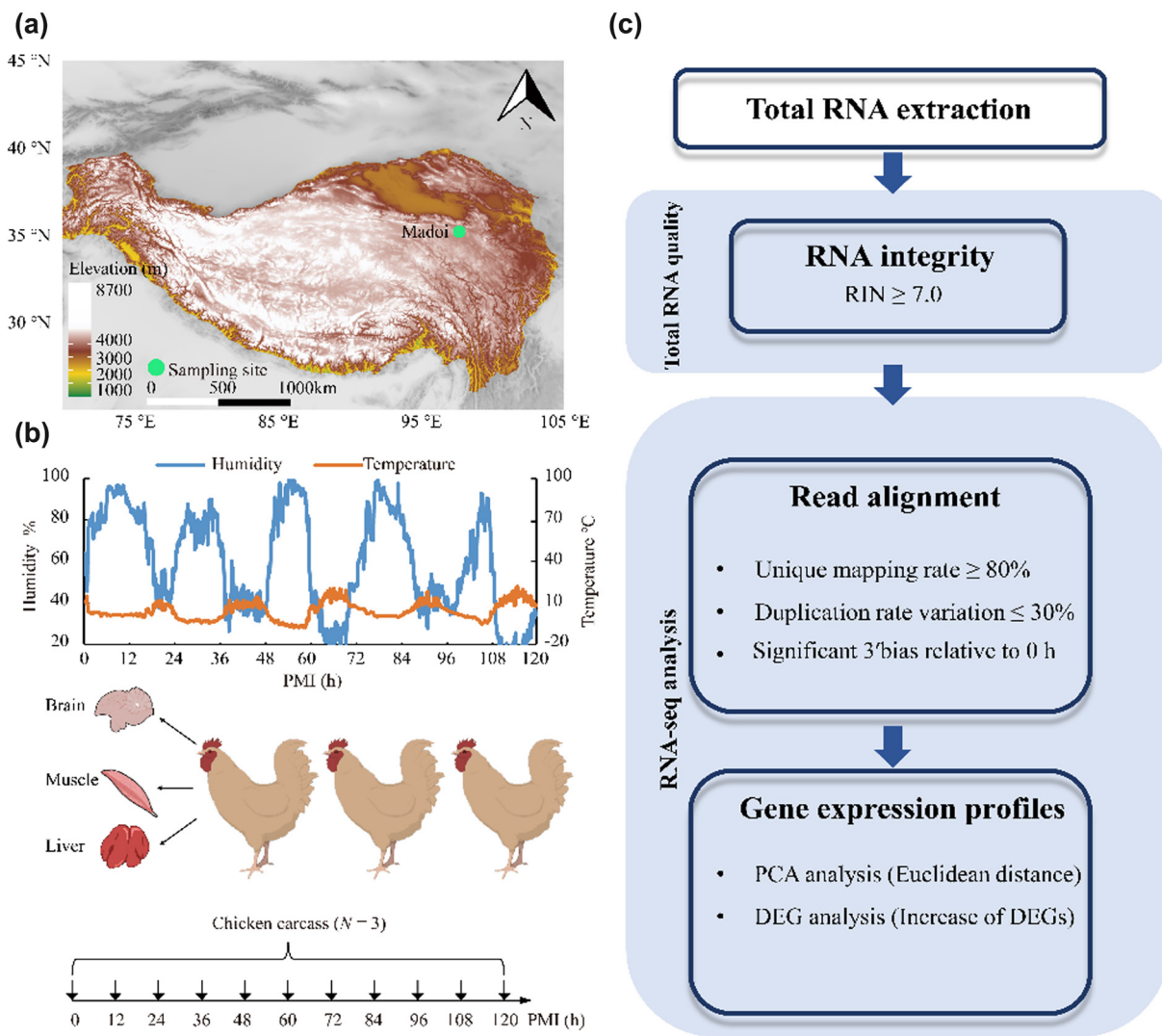


Figure 1. Experimental design for evaluating the relationship between PMIs with RNA degradation for each tissue sample with postmortem interval (PMI) ranging from 0 to 120 h. (a) The study site on the Qinghai-Tibet Plateau. (b) Sample collection for three tissue types at different postmortem sampling times under field conditions. (c) Screening pipeline of RNA quality evaluation.

separated the aqueous phase containing the RNA. Next, we precipitated the RNA from the aqueous phase by mixing it with 500 μ l isopropyl alcohol and washed the RNA pellet with 1 ml 75% ethanol. Finally, we dissolved RNA using 20 μ l RNase-free water and stored them in a -80°C freezer.

RNA yield and integrity evaluation

RNA concentration (ng/ μ l) was measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). RNA yield from each sample was calculated as ng/mg of tissue. RIN as the indicator of RNA integrity was determined using an Agilent Bioanalyzer 2100 with an Agilent RNA 6000 kit. This instrument produces a RIN for each RNA extract using a proprietary algorithm (Schroeder et al. 2006) with values ranging from 10 (completely intact) to 1 (extensively degraded). The relationships between RNA yields and PMI, and between RIN values and PMI were analyzed using

a linear regression model, respectively. Referring to previous studies (Pan et al. 2017, Hu et al. 2022), RNA extracts with RIN values ≥ 7.0 were considered high-quality samples for RNA-seq analyses. At each postmortem sampling time, all samples were sequenced, of which at least two samples' RIN values were ≥ 7.0 . While for the relatively poorer quality samples (RIN < 7), we sequenced samples at 2–3 postmortem time points for each tissue to represent the whole experimental period (Supporting information).

RNA-seq library construction and sequencing

We followed our previous protocol to construct the RNA-seq library (Pan et al. 2017, Hu et al. 2022). Briefly, 1 μ g of total RNA per sample was used for library construction and sequencing. The libraries were generated using NEBNext[®] UltraTM RNA Library Prep Kit for Illumina[®] following the manufacturer's recommendations. The library quality was

assessed on the Agilent Bioanalyzer 2100 system. The constructed RNA-seq libraries were subjected to sequencing on the Illumina NovaSeq 6000 sequencing platform and 150 bp paired-end reads were generated. Library construction and sequencing were performed at Novogene Co., Ltd. (China).

RNA-seq data analysis

After sequencing, we trimmed adapters and removed low-quality reads using 'Fastp' (ver. 0.21.0) with default parameters (Chen et al. 2018). We then mapped the reads against the chicken reference genome (ver. GRCg7b; GenBank accession number: GCA_016699485.1) using 'HISAT2' (ver. 2.2.1) with default parameters (Kim et al. 2019).

The mapping rate and unique mapping rate were estimated by 'HISAT2' (ver. 2.2.1). The samples with $\leq 80\%$ unique mapping rate would be considered low quality (Passow et al. 2019). The duplication rate was estimated by 'Picard' (ver. 1.86) (McKenna et al. 2010) (Supporting information). If the variation of duplication rate between different samples has over 30% disagreement relative to 0 h at the same post-mortem sampling time points, the sample is regarded as an outlier (Conesa et al. 2016). The relationship between read alignment parameters (i.e. mapping rate, unique mapping rate, and duplication rate) and PMI were evaluated by a linear regression model, respectively. We next measured the read coverage uniformity (Conesa et al. 2016), the coverage variation along each transcript from 5' to 3' ends. Because mRNA decays from 5' to 3' ends in eukaryotes (Houseley and Tollervey, 2009), an unbalanced reads accumulation at the 3' ends indicate RNA sample degradation (Conesa et al. 2016). To evaluate the coverage uniformity, we firstly mapped the reads to the transcript database obtained from NCBI (www.ncbi.nlm.nih.gov/genome/?term=chicken) using 'BWA' (ver. 0.7.17) with parameters '-M -P -R' (Li and Durbin, 2009). We then divided each transcript into 100 equally sized bins from 5' to 3' ends. For each bin, the read depth was summarized using the median value across all transcripts. We defined the 3' bias as the area under the curve at the relative position (50–100%) of the normalized transcript sequence as the proxy of the degradation level of the transcript (Supporting information). The 3' bias of each postmortem time point was compared with that of 0 h. If it is not significant, it represents that the RNA quality could be similar to 0 h and vice versa.

We estimated gene expression, transcripts per million (TPM), using the raw counts generated from 'featureCounts' (ver. 2.0.1) (Liao et al. 2014), with parameters '-p -t -exon'. Then, we plotted the distribution of median TPM values for the three types of samples (muscle, liver, brain) at different postmortem sampling time points to assess the overall expression difference with PMI. A linear regression model was used to measure the relationship between the median TPM value and PMI. Furthermore, to identify whether the samples had similar gene expression relative to 0 h, we used a principal component analysis (PCA) to cluster gene expression profiles. We selected the top 50% of genes with the expression greatest variability (i.e. coefficient of variation) at different

postmortem sampling time points for each tissue in the analysis since the genes with less expression variability would introduce the background noise to affect the separation of the high- or poor-quality RNA samples. Additionally, we checked the gene expression similarities of samples between 0 h and any postmortem sampling time point by calculating their Euclidean distances.

To identify differentially expressed genes (DEGs) between 0 h and any other postmortem sampling time points for three different tissues, we adopted three R packages, 'edgeR' (www.r-project.org, ver. 3.40.0) (Robinson et al. 2010), 'Deseq 2' (ver. 1.38.0) (Love et al. 2014), and 'limma' (ver. 3.54.0) (Ritchie et al. 2015). A gene was considered as a DEG only if at least two methods supported it.

Statistical analysis

All statistical analyses were performed using R (www.r-project.org, ver. 4.0.1). The data normality was checked using the Shapiro-Wilk normality test. The significant differences between multiple groups were tested pairwise using a parametric Student's t-test followed by a Benjamini-Hochberg correction with a false discovery rate (FDR) ≤ 0.05 . Genes with $|\text{fold change}| \geq 2$ and $\text{FDR} \leq 0.05$ were considered as differentially expressed with Benjamini-Hochberg correction. The coefficient (R^2) was used to evaluate the linear relationship, and the significance level was calculated using an F-test with the p -value ≤ 0.05 .

Results

Total RNA yield and integrity

The relationship between RNA yield (Supporting information) and integrity (Fig. 2) and PMI for each sample and each tissue is shown. Mean RNA yields of all samples were greater than 1 μg , and there were no significant differences in RNA yields with the increase of PMI for all the tissue types (Supporting information). However, all the tissue types had time-dependent RIN decay based on the RNA integrity evaluation ($R^2 = 0.54$, $p = 1.235 \times 10^{-6}$ for the muscle, $R^2 = 0.75$, $p = 9.9 \times 10^{-11}$ for the brain; $R^2 = 0.55$, $p = 0.037$ for the liver, Fig. 2). Of the three types of tissues, muscle had the slowest degradation in the RIN values, followed by the brain and liver (Fig. 2). The RIN values of muscle and brain samples remained above 7.0 within 60 h postmortem, while the liver samples rapidly declined below 7.0 within 12 h postmortem (Fig. 2).

Read alignment statistics

On average, 5.01, 4.89 and 5.04 Gb clean data were respectively generated for the muscle ($n = 27$), brain ($n = 27$), and liver samples ($n = 15$). From which, 89.78% (86.38–99.36%), 92.59% (90.39–98.16%) and 93.52% (91.70–96.14%) of the total reads could be mapped to the chicken reference genome, respectively. For the unique mapping rate, 89.40%

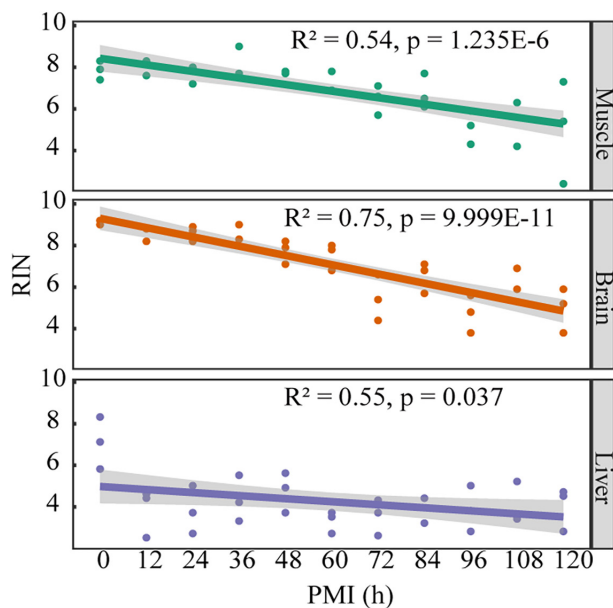


Figure 2. Correlations between RNA integrity (RIN) and postmortem interval (PMI) for muscle, brain, and liver tissues, respectively. The RNA integrity of each sample (dot) is quantified by Agilent Bioanalyzer 2100 is plotted against the PMI (0–120 h), as shown in a linear regression model. The shaded areas represent the 95% confidence intervals.

(86.07–99.06%), 95.45% (61.55–98.62%) and 95.93% (87.91–97.89%) of uniquely mapped reads could be mapped to the chicken reference genome, respectively. The mapping rate and unique mapping rate did not increase significantly with the prolonged PMI for all the tissue types (Supporting information). Despite the increasing duplication rates with the prolonged PMI for all the tissue types (Supporting information), the samples within 120 h postmortem can still meet the standard of following RNA-seq analysis since the variation was less than 30%. For the read coverage uniformity, there was an increased 3' bias with the increase of PMI ($R^2=0.70$, $p=5.403 \times 10^{-8}$) for the muscle within 120 h

postmortem (Supporting information). The significant difference of 3' bias relative to 0 h in muscle samples occurred after 36 h postmortem ($p=0.039$, Supporting information). In contrast, there was no significant 3' bias relative to 0 h observed for the brain ($p=0.116$, Supporting information) and liver ($p=0.35$, Supporting information).

Influence of RNA degradation on gene expression landscape

The gene expression landscape for each sample was demonstrated by its median TPM and PCA clusters. Relative to 0 h, the median TPM of muscle and brain samples decreased, significantly correlated with PMI ($p < 5.166 \times 10^{-4}$, Supporting information). In contrast, we did not observe a PMI-dependent decrease in the liver ($p=0.808$, Supporting information).

PCA of our data also demonstrated that much of the variation for the brain (32.3%) and for muscle (59.6%) in gene expression levels were highly associated with PMI (Fig. 3a; principal component 1 (PC1) associated with PMI ($p=1.022 \times 10^{-8}$ for the muscle; $p=1.992 \times 10^{-8}$ for the brain)). Based on the PCA result, samples of muscle and brain tissues were separated into two groups, respectively: 0–36 and 48–120 h for muscle tissues; 0–60 and 72–120 h for brain tissues. Consistent with the PCA result, the comparison of Euclidean distances showed significance after 36 h postmortem for the muscle samples and after 60 h postmortem for the brain samples (Supporting information, $p < 0.05$). However, for the liver samples, the samples at different postmortem sampling time points were randomly distributed in the PCA plot (Fig. 3a), confirmed by the result of Euclidean distance comparison (Supporting information, $p=0.35$).

Influence of degradation on differentially expressed genes

Our DEG analysis showed that the percentages of DEGs at 12, 24 and 36 h postmortem for muscle and brain samples

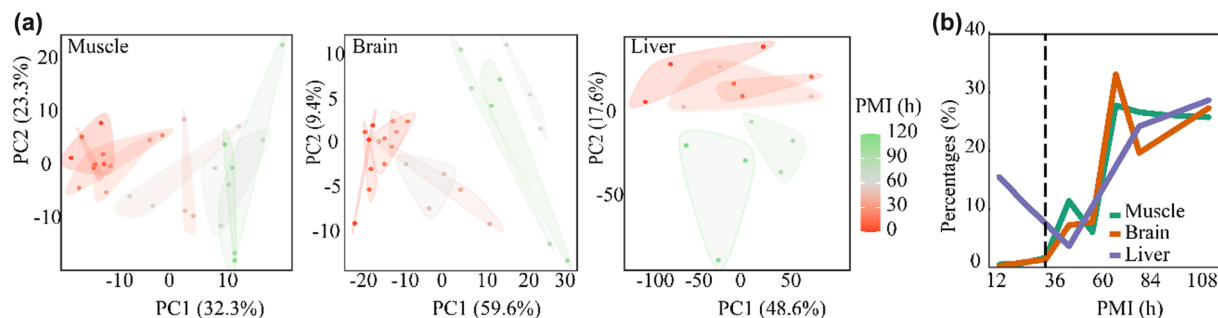


Figure 3. Change of gene expression profiles and differentially expressed genes (DEGs) within 120 h postmortem for muscle, brain, and liver tissues, respectively. (a) PCA plots showing sample clustering for muscle, brain, and liver tissues. Different colors identify different postmortem sampling time points. The shaded areas represent 95% confidence intervals. (b) Percentage changes of DEGs relative to 0 h for the three tissues. The dashed line indicated that muscle and brain samples within 36 h postmortem are optimal for the RNA-seq analysis. DEGs were identified using the three analyses ('edgeR', 'DeSeq2' and 'limma'; the section of RNA-seq data analysis in Material and methods).

were lower than 3% of the total genes (Fig. 3b). In contrast, 15.6% of genes have already differentially expressed in the liver just at 12 h postmortem, although the percentage of DEGs decreased to 3.6% at 48 h postmortem (Fig. 3b). After 48 h postmortem, the percentage of DEGs showed a growing trend for all the tissue types (6.1–33.0%, Fig. 3b).

RNA sample degradation in wild upland buzzard samples

As shown in Supporting information, the RIN values of four upland buzzard muscle samples at 0, 12 and 24 h postmortem remained above 7.0. In contrast, all the RIN values decreased below 7.0 at 48 h postmortem. We also found that the DEG at 12 and 24 h postmortem accounted for a low proportion of the total transcribed genes (3.1%, $n=547$ and 2.3%, $n=416$), while the proportion of DEGs at 48 h postmortem ascended to 7.5% ($n=1332$, Supporting information).

Discussion

Providing an appropriate postmortem RNA sampling time of field carcasses is valuable for transcriptome studies of wild avian populations. Our study is the first to provide the practical postmortem RNA sampling time on the QTP for different tissues by examining the relationship between a long PMI (i.e. 120 h) and RNA degradation using the RNA quality measurement and RNA-seq data in the three tissue types of chicken carcasses (brain, muscle and liver).

Evaluation of RNA quality based on the RNA integrity number

Using the RIN value, our study suggested that the samples in the muscle and brain collected within 60 h showed high RNA integrity (RIN value ≥ 7.0). Under similar temperatures (4°C on average), the postmortem sampling time window in our study is shorter than those (seven days) reported under laboratory environments (Bahar et al. 2007, Shen et al. 2018, Nakatsuji et al. 2019). This is likely because 1) in contrast with less temperature fluctuation under the laboratory condition, the temperature in the wild changes a lot (Fig. 1, range from: $-7.8 \sim 21.9^\circ\text{C}$), which could accelerate the RNA degradation due to not only the cycling thawing of carcasses (Botling et al. 2009) but also the transient high temperature in the environment; 2) Other fluctuated environmental factors such as sunlight and humidity in the field could also accelerate RNA degradation (Bonadio et al. 2021). Thus, unlike past studies conducted in the lab, our study provides a more practical time window for RNA sample collection on the muscle and brain tissues of carcasses under extreme environment such as the QTP. However, for liver tissue, only RNA extracts from fresh samples (i.e. 0 h) showed a high RNA integrity, which is consistent with previous studies under laboratory conditions (Thompson et al. 2007, Sobue et al. 2016), and a fast RNA degradation probably results from the highest

RNase concentration in the liver compared with other tissue types (Bahar et al. 2007, Sampaio-Silva et al. 2013).

Evaluation of RNA quality based on RNA-seq data

Different from the RIN value, the RNA-seq data can provide more information for the assessment of RNA quality because 1) RIN value is less suitable for evaluating the integrity of mRNA than rRNA, with the former being the primary input for RNA sequencing (Wang et al. 2016); 2) RIN value is an overall assessment of RNA quality based on the assumption that the RNA degradation occurs at the relatively constant rate across all the transcripts. However, the mRNA decay rate varies between transcripts (Opitz et al. 2010, Romero et al. 2014). Thus, the RIN value is insufficient for assessing differential RNA degradation between transcripts in downstream gene expression analyses (Wang et al. 2016). In this study, PCA results showed similar gene expression levels of samples within 36 h for muscle tissues and 60 h postmortem for brain tissues (Fig. 3a), suggesting a limited RNA degradation during these periods for these two types of tissues. Different from the RIN estimate, the assessment of RNA quality through gene expression profiles reduced the postmortem sampling time window from 60 to 36 h for the muscle tissues. In contrast, for the brain tissues, the postmortem sampling time window based on the gene expression comparison was the same as that estimated by the RIN value. It is possibly because muscle tissues have a richer ribosomal RNA content than brain tissues (Bortoluzzi et al. 2001), which could enhance the stability of rRNA in the muscle tissues. Given that the RIN focused more on evaluating rRNA quality, we may observe a longer sampling window in muscle according to the RIN measurement.

Consistent with the PCA result, we observed the same sampling window (36 h) for the muscle, as evidenced by the low proportion of DEGs ($< 3\%$) during this period (Fig. 3b). But for the brain tissue, less than 3% of DEGs found within 36 h postmortem suggested a shorter postmortem sampling time window than this estimated by the PCA analysis (60 h). The inconsistency between the PCA and DEG analyses may be because the brain has a high density of cells with tightly interconnected networks that allow for complex signaling and regulation (Pozhitkov et al. 2017). Therefore, the tight regulation by several genetic factors, such as epigenetic modifications, post-transcriptional regulation, and feedback loops, could result in a stable overall gene expression landscape even for a long time since death (Gavin and Akbarian 2012) but great expression change of individual genes sensitive to death for a short time (Catts et al. 2005). Together, our results suggested that for the gene expression research, brain samples within 60 h could be selected; if they want to minimize the false positive of DEGs (Dachet et al. 2021), samples within 36 h could be optimal. Our postmortem sampling window for muscle and brain tissues based on the gene expression level was shorter than the previous study, which was conducted in blood cells under stable laboratory conditions (4°C, Shen et al. 2018). Except for fluctuating

environmental factors that could change gene expression levels by accelerating RNA degradation (Opitz et al. 2010, Romero et al. 2014), different tissue types probably could lead to different time windows due to the unique cellular composition and physiological functions in the different tissues (Zhu et al. 2017, Ferreira et al. 2018).

In contrast, gene expression in liver did not significantly decline with the increase of PMI as in the other two tissues (Supporting information). There are two possible reasons. First, it is possible that different cell types in the organ have different RNA decay rate (Pozhitkov et al. 2017). To control the effect, in this study we have tried to randomly sample different locations in the organ (the section of Study design in Material and methods). Second, this result maybe also because the liver is an immune and metabolic organ whose gene expression can be altered in response to organismal death. Some previous studies have demonstrated that many genes related to apoptosis and signaling were significantly up- and/or down-regulated in the liver within 24 h post-mortem (Javan et al. 2015, Sobue et al. 2016). In addition, a recent transcriptomic study has also shown that human liver tissues exhibit sustained gene upregulation related to metabolism and chemokine activity pathways over 15 h post-mortem (Ferreira et al. 2018). Similar responses may explain a sustained postmortem gene expression and a rise of DEG number that we found in chicken liver tissues. Nonetheless as time progresses, the function of these genes should gradually decrease due to the degradation, and we have observed a decrease of the total DEG number at 24, 36 and 48 h post-mortem, when compared with its preceding PMI. Similarly, Pozhitkov et al. (2017) has also found that the mouse liver transcript profile had a significant increase at 12 h post-mortem, followed by a decline at 24 and 48 h postmortem. However, it is noted that we have only three samples per replicate in the RNA-seq analysis, this standard has been widely used in previous studies (Opitz et al. 2010, Romero et al. 2014, Sigurgeirsson et al. 2014) but may underestimate the number of differentially expressed genes.

To verify the efficiency of recommended sampling time on the postmortem RNA collection on the QTP, we have compared RNA quality of wild upland buzzard samples within and after 36 h postmortem from the RNA integrity and DEG analysis aspects. As expected, both results were similar to our findings in domestic chickens, suggesting our sampling strategy is more applicable for the fieldwork.

In summary, our study presents a practicable and efficient sampling strategy for high-quality RNA collection in the fieldwork on the QTP. Upon detecting avian carcasses in the wild (e.g. QTP), researchers could roughly estimate the time of death by examining their eyeballs and tongues (Supporting information). The muscle and brain samples within 36 h postmortem are recommended, while only fresh liver samples could be harvested from the avian carcasses.

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Permits – All procedures performed were under the guidance of the Ethics Committee of the Institute of Zoology, Chinese Academy of Sciences (IoZ, CAS) and were in full compliance with the Institutional Animal Care and Use Committee at the IoZ, CAS (IOZ-IACUC-2021-121).

Author contributions

Ziying Shao: Conceptualization (equal); Data curation (equal); Formal analysis (equal); Investigation (lead); Methodology (equal); Visualization (equal); Writing – original draft (lead); Writing – review and editing (lead). **Xian Hou:** Conceptualization (equal); Project administration (equal); Supervision (equal); Writing – original draft (supporting); Writing – review and editing (equal). **Yangkang Chen:** Data curation (equal); Formal analysis (equal); Methodology (equal); Software (lead); Visualization (equal); Writing – review and editing (supporting). **Zhenzhen Lin:** Conceptualization (equal); Project administration (equal); Supervision (equal); Writing – review and editing (supporting). **Xiangjiang Zhan:** Conceptualization (equal); Funding acquisition (lead); Project administration (equal); Supervision (lead); Writing – review and editing (equal).

Transparent peer review

The peer review history for this article is available at <https://publons.com/publon/10.1002/wlb3.01157>.

Data availability statement

Data are available from the GenBank Sequence Read Archive (Bioproject PRJNA1005767, SRP455282, https://www.ncbi.nlm.nih.gov/Traces/study/?acc=SRP455282&o=acc_%3Aa) (Shao et al. 2023).

Supporting information

The Supporting information associated with this article is available with the online version.

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