	Sample collection and processing	Choice of target gene	DNA sequencing	Bioinformatics
Constraints	➢ Differences in cell and DNA quantity from each specimen/species. ➢ Number of mitochondrial copies from tissue type and species.	➢ Different PCR amplification rates. ➢ Number of target genes/ regions. ➢ Development of universal primers/probes.	➢ Short reads leading to low taxonomic resolution. ➢ Detection sensitivity for rare species. ➢ Tag-jump and contamination.	➢ % of read's cutoff threshold. ➢ Low taxonomic resolution of targeted gene. ➢ Incorrect taxonomic assignment. ➢ Distinct PCR amplification rates.
Solutions	✓ Tissue size normalization. ✓ Biomass estimation. ✓ Total larval flow (TLF).	✓ Probe capture. ✓ Metagenomics (whole genome sequencing). ✓ Highly conserved primer sites. ✓ Two or more target DNA regions.	✓ Longer or paired ends reads. ✓ Increase sequencing depth. ✓ Negative and positive controls .	✓ Minimum % of reads to be included/excluded. ✓ Development of curated databases and use of MOTUS/ASVs. ✓ Correction factors for abundance estimation.