

农业害虫环境 DNA 监测技术的 标准化研究现状与展望

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摘要: 环境 DNA (environmental DNA, eDNA) 技术以其高灵敏性、非侵入性等优势在农业害虫监测领域具有重要应用价值与巨大潜力。目前, eDNA 技术在自然生态系统 (涵盖水生及陆生生态系统) 中的标准化研究已取得较大进展, 但在农业生态系统 (涵盖作物病虫害、畜禽病虫害、土壤健康及传粉生物监测等) 中的标准化研究仍相对缓慢。该文聚焦于农业虫害监测, 系统对比了水生生物与农业害虫 eDNA 研究在野外采样、样品处理及分子试验等阶段各个环节的异同。农业害虫 eDNA 技术虽展现出良好的应用前景, 但其标准化发展仍面临多重挑战, 尤其在样本采集规范制订、实验室检测流程优化及质量控制体系完善等方面亟需突破。该文基于农业害虫监测实际需求, 从构建全流程标准化操作规程、探索 eDNA 技术与传统监测手段的协同应用模式, 以及强化监测结果向防控决策的有效转化等方面提出展望, 旨在为推动 eDNA 技术在农业害虫监测领域的研究与应用提供参考。

关键词: 农业害虫; 环境 DNA; 标准化; 监测

Current status and prospects of standardizing eDNA-based monitoring for agricultural pests

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Abstract: Environmental DNA (eDNA) technology, known for its high sensitivity and non-invasive sampling, shows great promise for monitoring agricultural pests. While notable progress has been made in standardizing eDNA applications within natural ecosystems (including aquatic and terrestrial ecosystems), standardization within agricultural ecosystems remains comparatively limited. This review focuses on pest monitoring in agricultural settings, comparing practices across key stages such as field sampling, sample processing, and molecular analysis between aquatic organisms and agricultural pests. Despite its potential, eDNA-based pest monitoring faces challenges in areas such as protocol development, laboratory workflow optimization, and quality control. In response, this article outlines future directions including: (1) establishing comprehensive, standardized operating procedures; (2) promoting synergistic use of eDNA with conventional pest surveillance methods, and (3) enhancing the integration

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of monitoring results into pest control decision-making. These recommendations aim to promote the standardized adoption of eDNA technology and support sustainable pest control strategies in agricultural systems.

Key words: agricultural pest; environmental DNA (eDNA); standardization; monitoring

近年来,受耕作制度变革、气候变化、国际贸易频繁以及经济全球化等因素影响,农业害虫的扩散性和破坏性日益增强,对粮食安全和生态环境的威胁与日俱增(Skendžić et al., 2021)。及时有效的监测预警是防范农业害虫暴发成灾的关键前提(Zhang et al., 2018; Yang et al., 2024),对守护国家粮食安全、生态安全和经济安全,促进农民增收与农业可持续发展具有重要意义(封洪强等, 2023)。然而,传统农业害虫监测方法主要依赖人工观察和经验判断,存在效率低、准确性差等缺点(Kudoh et al., 2020)。随着生物技术的发展,快速分子检测方法逐渐应用于物种早期检测、诊断与监测(Li et al., 2017; Blackman et al., 2020),但现有分子检测技术仍需捕获害虫个体,操作过程耗时耗力(杨力风等, 2023)。近年来,环境DNA(environmental DNA, eDNA)技术由于具有高灵敏性、非侵入性等特点,逐步被用于农业害虫监测,为解决上述难题提供了新的技术路径(Allen et al., 2021; McPherson et al., 2022)。

eDNA是指从土壤、粪便、植物材料、水或空气等生物基质中分离鉴定出的DNA(Barnes & Turner, 2016)。该技术起源于20世纪80年代,最初用于研究微生物多样性(Rondon et al., 2000)。随着PCR等分子生物学技术的发展,eDNA技术的应用范围不断拓展,逐渐成为生物监测的重要手段(Yan et al., 2024)。由于水体样品采集便捷,且生物释放的DNA能在水中均匀分布,eDNA技术在水生生态系统监测中得到了广泛应用(Bell et al., 2024; Espinosa et al., 2024)。如用于调查水生生态系统的生物多样性(陈晓等, 2021)、评估水体的生态健康状况(Senapati et al., 2019; Xiong et al., 2024)以及监测水生入侵物种等(Mahon et al., 2014)。随着eDNA技术的深入研究和完善,其应用领域不断拓展,逐渐从水生生态系统延伸到陆地生态系统,并在农业领域得到应用。目前,eDNA技术已用于作物病虫害(Tordoni et al., 2021; Young et al., 2021)、畜禽病虫害(Gamage et al., 2020)、土壤微生物(Sternhagen et al., 2020)和传粉生物监测(Evans & Kitson, 2020)等多个方面。其中,在农业害虫监测方面,传统人工巡查方法难以及时发现斑衣蜡蝉 *Lycorma delicatula*

和美洲斑潜蝇 *Liriomyza sativae* 等隐蔽性害虫,而eDNA技术只需采集土壤、植物表面等环境样品即可检测到害虫DNA片段,可及时发现虫情并采取相应防控措施,有效降低害虫暴发带来的经济损失(Valentin et al., 2018; Pirtle et al., 2021)。这种监测方式显著提高了农业害虫监测的准确性和效率(Kestel et al., 2022),同时大幅降低了人力、物力和时间成本,提升了害虫管理的经济效益(Mauvisseau et al., 2022)。

当前,eDNA技术研究正处于标准化发展阶段。完整的eDNA研究流程通常包括野外采样、样品处理和分子试验等阶段,涵盖采样设计、样品前处理、样品保存、引物扩增以及测序分析等多个环节。实现这些环节的标准化有助于提高监测结果的准确性和可靠性,促进不同研究间的数据共享与对比分析(Jackman et al., 2021)。目前,eDNA技术标准化研究主要集中于自然生态系统,尤其是水生生物领域,而在农业系统中的相关研究相对较少。因此,本文以水生生物eDNA技术标准化为参照,系统比较了农业害虫eDNA技术在野外采样、样品处理和分子试验各环节的异同,探讨农业害虫eDNA技术标准化面临的挑战,以期为推动eDNA技术在农业害虫监测领域的研究与应用提供参考。

1 野外采样阶段

样品是影响eDNA技术监测结果的重要因素,这是由于野外环境因素差异较大,再加上采样条件不易控制,使得eDNA技术监测结果的可重复性、准确性以及精确性易受到影响。因此,规范的采样标准在eDNA监测过程中扮演着不可或缺的角色(Poyntz-Wright et al., 2024)。例如,统一规定采样的时间、地点、深度、频率等参数,可以避免因采样条件的差异导致某些物种的eDNA被过度采集或采集不足,进而影响对物种分布和丰度的判断(杨海乐等, 2023)。

1.1 采样时间

在水生生物eDNA的监测中,采样时间的选择关键考量研究对象的特定洄游周期、繁殖期等季节性变化规律(Biggs et al., 2015; Carvalho et al.,

2024)。而在农业昆虫的eDNA监测中,试验需求及目的直接决定采样时间。例如,Rasmussen et al. (2021)选择在开花期与收获期进行采样,原因在于这2个阶段生态系统中的生物互动更频繁,有利于观测现有管理制度对果园生态系统多样性的影响;Zenker et al. (2020)为全面评估巴西所有地区昆虫和主要生物群系的多样性,采用自动光诱捕法将采样时间安排在湿季和旱季;Crisol-Martínez et al. (2016)在评估澳大利亚坚果园同域鸟类取食及减害服务时,将采样时间与澳大利亚主要坚果害虫发生数量最多的时期对应,通过分析鸟类粪便中昆虫种类,精准评估鸟类的减害作用。

1.2 采样点

在水生生物eDNA的监测中,采样点的选择需要综合考虑研究目的、水体类型、水生生物分布特点等多种因素(Xu et al., 2018; Rehill et al., 2024)。农业害虫eDNA采样点的设计同样需要综合考量研究目的、研究对象及环境条件等多种因素。例如,Todd et al. (2020)在监测土壤无脊椎生态系统时,选取8个猕猴桃果园与8个苹果园采集样本,在每个果园500 m²的范围内随机采集约1 L土壤,这种多点随机采样方式既规避了单一采样点的局限性,又能有效反映果园土壤生态系统的整体特征。而Crisol-Martínez et al. (2016)在分析澳大利亚坚果果园鸟类捕食的节肢动物构成时,根据果园空间布局和害虫活动规律,每日在全园均匀布设6个雾网,通过优化采样点分布显著提高了鸟类粪便及捕食目标的采集效率,确保了eDNA数据的完整性与代表性。

1.3 样品类型选择

在水生生物eDNA的监测中,通常将其生存环境里的水体、池塘沉积物等当作监测样品(Mahon et al., 2013; Lim et al., 2016; Kusanke et al., 2020)。在农业害虫eDNA的监测中,则需根据目标物种的生活史特征选择多样化的非生物基质,如天敌粪便、土壤、诱捕器残留等,突破了水体采样的局限性(van der Heyde et al., 2020)。例如,利用天敌粪便eDNA检测到了茶翅蛾 *Halyomorpha halys* (Maslo et al., 2017);从土壤eDNA中检测到了阿根廷蚁 *Linepithema humile* (Yasashimoto et al., 2021);从运输容器eDNA中检测到了谷斑皮蠹 *Trogoderma granarium* (Trujillo-González et al., 2022);在诱捕器上残留的eDNA中成功检测到入侵昆虫番茄潜叶蛾 *Phthorimaea absoluta* (Butterworth et al., 2022)。

1.4 样品采集量

在水生生物eDNA的监测中,水样采样量差异大但研究较为全面。运用eDNA技术研究鱼类物种多样性时,在淡水生态系统等场景下,采样量从15 mL到10 L不等,以1~2 L的采样量最为常见(Rees et al., 2014),国内研究的采样量多为2 L(徐念和常剑波, 2016)。在野外采样中,采样量越大,eDNA技术的有效性越高(How et al., 2024)。然而,较大的采样量会增加工作量,因此需选择合适的采样量。采样量主要受目标物种密度(Díaz-Ferguson et al., 2014)、物种eDNA释放与分布规律(Biggs et al., 2015)、水体流速(Mauvisseau et al., 2022)、温度(Lamb et al., 2022)以及酸碱度(Poyntz-Wright et al., 2024)等因素的影响。

农业害虫eDNA样本的采集量还没有统一标准且相关研究较少,同一类型样品的采样量差异较大。在自然系统中研究昆虫eDNA样本的采集量也没有统一标准。例如,同样都是在土壤样品中检测eDNA,检测阿根廷蚁的活动痕迹时采集了大约25 g的土壤样品(Yasashimoto et al., 2021),而检测果园中的无脊椎生物时则采集了约1 L土壤(Todd et al., 2020)。产生这种差异主要归因于3个方面,一是目标物种的生境分布特性,如阿根廷蚁在土壤中的巢穴密度决定了25 g样本的有效性;二是基质类型的影响,如果园生态系统中1 L土壤即可覆盖500 m²范围内的无脊椎生物多样性;三是样本处理策略的差异,如粪便样本需通过去杂称重(Crisol-Martínez et al., 2016)或风干保存(Montauban et al., 2021),为确保eDNA的提取效率,初始的采集量就会较大。

1.5 样品采集方式

在水生生物eDNA的监测中,常使用简单的工具在海岸或船上收集水样(Everett & Park, 2018)。随着eDNA监测技术的应用发展,已经开发了各种水样收集装置(Lu et al., 2024)。近年来,无人机领域诸多关键技术瓶颈已被突破,这为未来水样采集设备的研发提供了极具潜力的发展方向(Shelare et al., 2021)。农业害虫eDNA样品的采集方式主要有以下3种类型:一是直接将植物材料、表层土壤等收集在容器内(Milla et al., 2022);二是利用喷雾收集法、树干滚筒法(Valentin et al., 2020)、拭取叶片法(Lynggaard et al., 2023)等有针对性地采集样品,间接收集植物材料上残留的eDNA;三是使用无人机、空气采集器等机器辅助人工采样,例如利用无人机采集树冠上的eDNA样品(Aucone et al., 2023),使

用自动光诱捕器在同一个地区的农业生境和自然生境中从黄昏到黎明连续5个晚上进行害虫诱捕(Zenker et al., 2020),使用空气流量为1.1 m³/h和3.5 m³/h的2台机器采集空气样本,以监测动物园和森林中的昆虫多样性(Lynggaard et al., 2024)等。

1.6 样品重复数

在水生生物eDNA的监测中,样品重复数多由环境类型决定。实验室样方通常设3个重复,河口采样点同样建议设3个重复,而海洋采样点则增加至4个重复(Goldberg et al., 2013; How et al., 2024)。关于农业害虫eDNA监测中样品重复数的设计往往由于检测目的、面积等不同而有所不同。当检测目的仅为确认特定害虫是否存在时,调查范围小、需求简单,1个重复即可满足要求。如通过检测同一棵树果实的淋洗液,以单次重复判断茶翅蝽的存在与否即可(Valentin et al., 2018)。而当检测目的变为调查一定区域内某种害虫的地理分布范围及寄主范围时,因涉及较大的调查面积和多种寄主类型,样品重复数则需大幅增加。例如,Pirtle et al. (2021)在研究美洲斑潜蝇在紫花大翼豆 *Macroptilium atropurpureum* 上的分布时,每个地点采集了15个重复样本;在后续研究其寄主范围时,又针对多种植物分别采集5个重复样本。

2 样品处理阶段

样品处理的目的一般是富集样品里eDNA含量,同时延缓其降解速度,进而提升eDNA监测效率(Coble et al., 2019)。在样品处理阶段,农业害虫eDNA样品往往也面临着技术衔接障碍、成本投入限制、标准规范差异等与水生生物eDNA样品处理中一样的问题(Shu et al., 2020; 杨海乐等, 2023; Thamke et al., 2024)。

样品处理阶段通常涉及样品前处理与样品保存2个方面。水生生物eDNA样品前处理最常用的方法是水体过滤,但滤膜材质和孔径会影响处理结果(Capo et al., 2020; Mauvisseau et al., 2022),其中0.45 μm滤膜较为常用(李红婷等, 2022)。当水体中目标种群数量较低时不宜过滤,否则易产生假阴性结果(Kawato et al., 2021)。农业害虫监测则因样本类型多样性高且eDNA稳定性较强,多数情况下无需进行样品前处理,一般情况下土壤、沉积物、固体混合物、痕迹样品采集后直接密封冷冻即可(Deiner et al., 2017)。对于样品的保存,无论水生生物eDNA监测还是农业害虫eDNA监测,eDNA信号的

降解均受时间、生物类群、初始浓度、赋存介质、紫外线、温度和pH等诸多因素的影响(Nagler et al., 2022),两者的保存条件也较为相似。水生生物eDNA样品保存方式主要有低温保存(Hinlo et al., 2017; Weldon et al., 2020)和化学试剂保存(Hinlo et al., 2017);另外,苯扎氯铵、氯化十六烷基吡啶和溴化十六烷基三甲基铵等阳离子表面活性剂也被用于延缓eDNA的降解(Thamke et al., 2024);DNeasy Power Water Kit等商业化裂解缓冲液也适用于eDNA保存。对于农业害虫eDNA样品的保存,除了上述低温、化学保护剂等保存方法外,还有Whatman® FTA卡片、干燥植物材料等保存方式。在监测潜叶类害虫时,收集具有取食痕迹的叶片后拍照并保存在100%乙醇或Whatman® FTA卡片上,这2种方法都被证实为适宜的保存技术(Pirtle et al., 2021)。近年来,研究发现茶叶中可以检测到昆虫eDNA,这表明干燥植物材料也可作为一种昆虫eDNA的保存方式(Krehenwinkel et al., 2022)。

3 分子试验阶段

任何样品都需要进行标准化的eDNA提取-引物扩增-结果分析流程。由于eDNA的提取方法与具体操作程序、PCR扩增的退火温度和循环次数、测序平台以及测序技术、分析流程和具体参数等,都会对最终解析出的结果产生特定影响(Shi et al., 2024)。因此,唯有统一标准化的eDNA提取-引物扩增-结果分析程序,才能使所得结果具有完全的可比性(Zhang et al., 2020)。

3.1 eDNA提取

在水生生物eDNA提取过程中,eDNA样品中的抑制性物质、不同的提取方法及纯化方式等多重因素均可能对eDNA的提取效率与品质产生影响(Pawlowski et al., 2022)。农业害虫eDNA的提取需要注意以下3个方面:一是要根据样品类型选择适宜的提取方法,例如在提取土壤样品中eDNA时借鉴了从水生沉积物中提取eDNA的方法(Yasashimoto et al., 2021);在提取植物材料样品中eDNA时使用了改良的Chelex提取方法,确保充分研磨样品,让潜道中的DNA暴露出来(Pirtle et al., 2021);二是根据样品量加入裂解缓冲液和蛋白酶K进行裂解,并尽量排除样品中干扰物质的影响(Thomsen & Sigsgaard, 2019);三是有必要在专门处理低DNA浓度样本、痕量样品的实验室进行eDNA提取,并建立定期的去污程序,从提取环节防止样品污染。

3.2 扩增引物选择

应根据DNA监测对象和目的选择引物,若需检测特定分类群及其种群数量时,应选用物种特异性引物,避免使用对部分分类群亲和力低、易扩增非目标DNA的宏条形码通用引物(Saccò et al., 2022);同时,鉴于环境中DNA持续降解,基于eDNA的引物设计多针对小片段,以适应PCR扩增的要求(Jo & Minamoto, 2021)。

在检测单一的、特定的农业害虫目标时,eDNA引物可以使用选择物种特异性引物,如利用特异性引物监测土壤中的阿根廷蚁活动范围(Yasashimoto et al., 2021)、蝙蝠*Pipistrellus pygmaeus*粪便中的稻水象甲*Lissorhoptrus oryzophilus*(Montauban et al., 2021);也可以利用节肢动物特异性引物扩大检测范围,通过高通量测序进行数据对比,确定多种节肢动物的存在(Madden et al., 2016)。

3.3 扩增-测序-数据分析

在扩增环节,由于试验目的和引物特性的不同,试验条件和参数需依据研究的实际需求设定。例如,若研究聚焦特定物种的有无时可选用特异性引物(Valentin et al., 2020; Rourke et al., 2023);若旨在分析群落多样性则需采用通用引物(Madden et al., 2016; Sato et al., 2021)。鉴于引物特异性和稳定性的差异,也需要研究者根据具体试验情况,对退火温度、循环次数等参数进行综合考量与优化。同时,诸如沉积物、悬浮颗粒、无机物或高密度的靶DNA这些环境化合物也可能抑制PCR,干扰引物扩增(Lance & Guan, 2020),可通过稀释或净化样品,在降低抑制效果的同时保证检测灵敏度(Goldberg et al., 2015)。

在测序环节,一般情况下PCR产物常委托商业生物技术公司完成测序。对于特定物种的监测,只需通过物种特异性引物进行PCR定性检测,再配合实时荧光定量PCR(real-time quantitative PCR, qPCR)或微滴式数字定量PCR(droplet digital PCR, ddPCR)技术进行分析,即可获取目标信息,无需后续复杂的测序、序列分析及注释流程(Larson et al., 2020),该策略有效简化了操作流程,提升了检测效率。

在数据分析环节,原始数据首先要经过严格的质量把控。通过评估碱基平均质量进行过滤,去除低质量序列;对过滤后的序列进行拼接,并剔除含有错误碱基和错配的部分,同时去除嵌合体,从而得到高质量的可用序列(宋飏和黄原,2016)。近年来,扩

增子序列变体(amplicon sequence variant, ASV)降噪分析因其在提高序列分辨率和准确性方面的优势,逐渐成为新的研究方向(杨海乐等,2023)。

3.4 序列比对注释

在多物种监测时,引物扩增之后需要进行比对注释,从而确定所监测物种的种类与数量,在此情形下数据库显得尤为重要。若要对大类群目标进行监测,就需要特定的序列参考数据库。唯有借助经过严格质量检查的参考数据库予以适当校准,才能够挖掘经典分类条形码、元条形码以及eDNA监测的巨大潜能(Lim et al., 2016)。水生生物eDNA监测已有专门的数据库(Giroux et al., 2023),目前农业害虫eDNA尚未构建监测专门的数据库(Burian et al., 2021)。

4 存在的问题与对策

综上所述,农业害虫eDNA技术标准化与水生生物eDNA技术标准化在某些环节存在差异(表1)。与水生生物eDNA技术类似,农业害虫eDNA技术流程缺乏统一标准,不同研究结果难以比较,这也影响了该技术在农业害虫监测中的应用。农业害虫生存环境复杂,如涉及不同的气候状况、土壤类别和农作物品种等,采集样品往往痕量,采集样品类型、DNA提取方法等则具有多样性,这些因素都会对eDNA技术的监测结果造成影响,极易出现假阳性、假阴性等问题,导致结果出现偏差(Hassan et al., 2024; Poyntz-Wright et al., 2024)。因此,在野外采样、样品处理以及分子试验阶段应加强以下3个方面的研究。

首先,相对于水生生物而言,农业害虫生存环境更具多样性,这就意味着进行农业害虫eDNA监测时的样品类型选择更丰富,而不同的样品类型往往会导致采样量、采样工具不同。因此,在野外采样时应重点关注以下4个方面:一是需根据监测目标(如存在性验证、种群动态等)及环境异质性(如农田类型、作物布局等)确定样品重复数,采用单株混合样本或多个重复采样;二是需结合害虫生物学特性(如活动规律、发育周期等)、作物物候期(如开花期、收获期等)及监测目的(如多样性评估、防控预警)确定采样时间;三是需兼顾栖息地斑块化特征(如巢穴密度等)与环境变量(土壤湿度、植被覆盖等)来确定采样点,可采用网格化布点提升空间代表性;四是需突破对水体样品的依赖,开发针对土壤、粪便、空气等多种类型样品的采样方法。

表 1 农业害虫与水生生物 eDNA 监测技术各个环节中相关内容比较

Table 1 Comparison of eDNA research across key phases between agricultural pests and aquatic organisms

研究阶段与环节 Research phase and step		水生生物 Aquatic organism	农业害虫 Agricultural pest insect
野外采样 阶段 Field sampling stage	采样时间 Sampling time	水生生物 eDNA 监测中的采样时间因研究对象、研究目的及水体环境等因素而有所不同 (Rishan et al., 2023); 按季节 (Carvalho et al., 2024)、特定时期 (Biggs et al., 2015) 等确定采用时间 The sampling time in aquatic organism eDNA monitoring varies depending on factors such as the research subject, research objectives, and aquatic environmental conditions (Rishan et al., 2023). It is determined based on seasonal variations (Carvalho et al., 2024), specific biological periods (Biggs et al., 2015), or other temporal frameworks relevant to the study	样品采样时间多根据试验需求及目的确定。在开花、收获期 (Rasmussen et al., 2016)、湿季、旱季 (Zenker et al., 2020) 以及害虫暴发期 (Crisol-Martínez et al., 2016) 采样 The sampling timing is predominantly determined by experimental requirements and objectives. Sampling is typically conducted during critical biological phases such as flowering and harvest periods (Rasmussen et al., 2016), wet and dry seasons (Zenker et al., 2020), as well as pest outbreak intervals (Crisol-Martínez et al., 2016)
	采样点 Sampling point	需要综合考虑研究目的、水体类型、水生生物分布特点等多种因素 (Xu et al., 2018) It requires a holistic consideration of multiple factors including research objectives, water-body types, and distribution characteristics of aquatic organisms (Xu et al., 2018)	依据不同的研究目的、研究对象及环境条件等因素综合考量 (Crisol-Martínez et al., 2016; Todd et al., 2020) A comprehensive analytical framework should be established through the holistic integration of multiple determinants, including research objectives, target organisms, and environmental parameters (Crisol-Martínez et al., 2016; Todd et al., 2020)
	样品类型 Sample type	类型较单一, 如水体 (Lu et al., 2024)、沉积物 (Kusanke et al., 2020) 等 Types remain relatively limited, such as water bodies (Lu et al., 2024) and sediments (Kusanke et al., 2020) etc.	类型十分丰富, 如水体 (Valentin et al., 2020)、土壤 (Yasashimoto et al., 2021)、寄主植物 (Pirtle et al., 2021)、诱捕器 (Butterworth et al., 2022)、空气 (Lynggaard et al., 2024)、花粉 (Thomsen & Sigsgaard, 2019)、灰尘 (Madden et al., 2016)、捕食者粪便 (Montauban et al., 2021)、蜘蛛网 (Xu et al., 2015)、猪笼草 (Bittleston et al., 2016)、茶包 (Krehenwinkel et al., 2022) 等 Types are highly diverse, including water (Valentin et al., 2020), soil (Yasashimoto et al., 2021), host plants (Pirtle et al., 2021), traps (Butterworth et al., 2022), air (Lynggaard et al., 2024), pollen (Thomsen & Sigsgaard, 2019), dust (Madden et al., 2016), predator feces (Montauban et al., 2021), spider webs (Xu et al., 2015), pitcher plants (Bittleston et al., 2016), and tea bags (Krehenwinkel et al., 2022) etc.
	样品 采集量 Sample amount	水样的采样量差异较大但相关研究较全面, 范围从 15 mL 到 10 L 不等, 其中, 1~2 L 的采样量应用最广 (Rees et al., 2014) Sampling volumes of water samples vary significantly with relatively comprehensive studies, ranging from 15 mL to 10 L, with 1~2 L being most commonly applied (Rees et al., 2014)	采集量还没有统一标准且相关研究较少, 同一类型样品的采样量差异较大。同样的土壤样品有 25 g 也有 1 L (Todd et al., 2020; Yasashimoto et al., 2021) Sampling volumes lack unified standards and the same type of sample may vary greatly. For example, soil samples may range from 25 g to 1 L (Todd et al., 2020; Yasashimoto et al., 2021)
	样品采集 方式 Sampling method	直接取样 (水体) (Evans et al., 2017)、简单的工具或者各种水收集装置抽取富集 (Mahon et al., 2013)、其他机械辅助采集 (Shelare et al., 2021; Lu et al., 2024) Direct sampling (water bodies) (Evans et al., 2017), simple tools or various water collection devices for extraction and enrichment (Mahon et al., 2013), and other mechanical-assisted sampling (Shelare et al., 2021; Lu et al., 2024)	直接取样 (任何有害虫活动痕迹的材料) (Pirtle et al., 2021)、利用各种工具抽取的空气、富集的水体、淋洗液等 (Valentin et al., 2020; Jackman et al., 2021) 以及自然收集的雨水 (Ladin et al., 2021) 或擦拭叶片 (Lynggaard et al., 2023)、其他机械辅助采集 (Zenker et al., 2020) 等 Direct sampling of materials with pest activity traces (Pirtle et al., 2021), tools for air, enriched water, leaching solutions (Valentin et al., 2020; Jackman et al., 2021), rainwater collection (Ladin et al., 2021), leaf wiping (Lynggaard et al., 2023), and mechanical-assisted sampling (Zenker et al., 2020)

续表 1 Continued

研究阶段与环节 Research phase and step		水生生物 Aquatic organism	农业害虫 Agricultural pest insect
样品重复数 Replication		实验室样方通常设3个重复,而野外监测需根据环境差异调整,河口采样点建议3个重复,海洋采样点建议4个重复(Goldberg et al., 2013; How et al., 2024) Laboratory quadrats typically use three replicates; field monitoring requires adjustment based on environmental variations; estuarine sampling recommends three, marine sampling recommends four (Goldberg et al., 2013; How et al., 2024)	样品重复数由于检测目的、面积等不同而有所不同,鉴别某种特定害虫是否存在时重复数为1个(Valentin et al., 2018);调查一定区域内某种害虫的地理分布范围以及寄主范围样品重复数为15个(Pirtle et al., 2021) Replication varies: one replicate for species presence detection (Valentin et al., 2018); 15 replicates for distribution or host range studies (Pirtle et al., 2021)
样品处理阶段 Sample processing stage		过滤(Hendricks et al., 2022),但滤膜的材质与孔径大小均会对结果造成影响(Capo et al., 2020)。可低温保存、冰浴或-20℃保存(Bizzozzero et al., 2024) Filtration (Hendricks et al., 2022); membrane material and pore size affect results (Capo et al., 2020). Storage via low temperature, ice bath, or at -20℃ (Bizzozzero et al., 2024)	大部分样品如土壤、沉积物、固体混合物、痕迹等无需样品前处理(Bell et al., 2024)。-80℃和-20℃保存或干冰浴冷冻保存(Bell et al., 2024)、干燥保存(Krehenwinkel et al., 2022) Most samples (e.g., solid mixtures) require no pretreatment (Bell et al., 2024). Storage at -80℃, -20℃, dry ice bath (Bell et al., 2024), or desiccation (Krehenwinkel et al., 2022)
分子试验阶段 Molecular experiment stage	eDNA提取 eDNA extraction	DNA提取用水体样品的DNA提取试剂盒(Deiner et al., 2017) Using kits for water sample extraction (Deiner et al., 2017)	DNA提取可用水体、粪便、土壤、空气、微量组织等样品的DNA提取试剂盒(Leempoel et al., 2020; Villacorta-Rath et al., 2023) Kits available for water, feces, soil, air, and trace tissue samples (Leempoel et al., 2020; Villacorta-Rath et al., 2023)
	扩增引物选择 Primer selection	DNA宏条形码(Sato et al., 2021)、物种特异性引物(Rourke et al., 2022) DNA metabarcoding (Sato et al., 2021), species-specific primers (Rourke et al., 2022)	DNA宏条形码(Madden et al., 2016)、物种特异性引物(Valentin et al., 2020) DNA metabarcoding (Madden et al., 2016), species-specific primers (Valentin et al., 2020)
	扩增-测序-分析 Amplification-sequencing-analysis	扩增时PCR、qPCR、ddPCR等各类PCR检测方法始终保持着最高的应用频率 PCR, qPCR, and ddPCR are the most frequently applied methods 在PCR结束后通常将产物交由商业生物技术公司进行测序 PCR products are generally submitted to commercial biotech companies for sequencing	扩增时PCR、qPCR、ddPCR等各类PCR检测方法始终保持着最高的应用频率 Same: PCR, qPCR, and ddPCR are widely used 在PCR结束后通常将产物交由商业生物技术公司进行测序。或仅需运用物种特异性引物开展PCR定性检测以及qPCR或者ddPCR定量分析即可,无需进行测序(Larson et al., 2020) Similar practice. Alternatively, species-specific primers for qualitative PCR or qPCR/ddPCR for quantification without sequencing (Larson et al., 2020)
	序列对比注释 Sequence comparison and annotation	去除错误结果后根据不同目的进行OTU聚类分析等(宋飏和黄原, 2016); ASV降噪分析也逐渐获得关注(杨海乐等, 2023) OTU clustering and (Song and Huang, 2016) and ASV denoising (Yang et al., 2023) after error removal 真菌Unite数据库、Silva数据库、专门的鱼类、浮游生物数据库、Silva或Greengene数据库等(Jackman et al., 2021) Unite, Silva, and plankton-specific databases (Jackman et al., 2021)	目前还没有专门的农业害虫数据库(Jackman et al., 2021) No dedicated database for agricultural pests (Jackman et al., 2021)

其次,在农业害虫eDNA样品的采样处理方面,还需针对不同生物类群和样品类型开展全面的保存方式对比试验,建立详细的保存方式推荐指南;深入

研究不同保存方式的作用机制,通过试验和理论分析、优化组合保存方式,提高eDNA保存效果;同时,开发新型的保存试剂和技术,提高eDNA的稳定性

和回收率。另外,还需研发针对农业害虫监测样本的处理方法,去除或减少土壤、粪便、植物残体等样品中干扰eDNA提取效率的复杂基质。

再次,农业昆虫eDNA样品具有多样性,如潜食叶片、蛀果中粪便、土壤、微量组织等样品,eDNA的提取与分析技术正在不断发展和优化,新的方法与技术不断涌现(Ruppert et al., 2019),使用不同的DNA提取方法、不同的扩增引物及不同的检测手段都可能会产生不同的监测结果(Bell et al., 2024)。因此,在农业害虫eDNA监测的分子试验中,eDNA提取应当根据样本类型来匹配适当提取方法,以避免操作过程中外源DNA的污染,进而防止假阳性结果的产生(Xiong et al., 2024)。目前市场上存在多种商品化的试验材料以供选择,例如针对粪便、土壤等样品可以使用DNA提取试剂盒等,这些能够避免一些干扰(Hermans et al., 2018);另外,还应构建引物筛选平台,联用不同的检测技术高效特异地检测eDNA样品。

5 展望

为有效提升农业害虫监测与防控水平,保障农业生产安全,需从多方面发力,全面强化害虫治理工作。目前,农业害虫eDNA数据库匮乏,严重制约着多物种的监测和分析,现有数据分析方法可能不适用于农业害虫的复杂数据。建议政府、科研机构和相关企业联动,投入资金和人力,建立全面准确的数据库,借鉴其他领域先进的数据处理技术,开发适合农业害虫监测数据的分析算法和软件,构建农业害虫尤其是潜在发生重大农业害虫的eDNA数据库。同时,整合农业害虫相关研究资源,开展多中心联合试验,将其结果与传统监测调查结果进行时间、成本、监测效率上的比较,优化检测技术、流程等(Evans et al., 2017; Allen et al., 2021);并结合实际监测需求,制订涵盖从样本采集到结果分析全流程的标准操作规程(杨海乐等, 2023)。另外,还应将eDNA技术与其他监测手段(如性诱监测、幼虫发生监测、远程智能监测等)结合来构建综合的监测体系(Zenker et al., 2020),可以更全面地掌握农业害虫的发生状况、生活习性以及扩散趋势等,为农业害虫的防控决策提供依据。同时,建立监测结果与防控决策的快速反馈机制,根据监测数据及时调整防控策略(Bell et al., 2024);尤其要加强对新发、突发及潜发农业入侵昆虫的研究,提前储备相关eDNA检测技术和监测方案,提高应对能力。

参考文献 (References)

- Allen MC, Nielsen AL, Peterson DL, Lockwood JL. 2021. Terrestrial eDNA survey outperforms conventional approach for detecting an invasive pest insect within an agricultural ecosystem. *Environmental DNA*, 3(6): 1102–1112
- Aucone E, Kirchgorg S, Valentini A, Pellissier L, Deiner K, Mintchev S. 2023. Drone-assisted collection of environmental DNA from tree branches for biodiversity monitoring. *Science Robotics*, 8(74): eadd5762
- Barnes MA, Turner CR. 2016. The ecology of environmental DNA and implications for conservation genetics. *Conservation Genetics*, 17(1): 1–17
- Bell KL, Campos M, Hoffmann BD, Encinas-Viso F, Hunter GC, Webber BL. 2024. Environmental DNA methods for biosecurity and invasion biology in terrestrial ecosystems: progress, pitfalls, and prospects. *Science of the Total Environment*, 926: 171810
- Biggs J, Ewald N, Valentini A, Gaboriaud C, Dejean T, Griffiths RA, Foster J, Wilkinson JW, Arnell A, Brotherton P, et al. 2015. Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (*Triturus cristatus*). *Biological Conservation*, 183: 19–28
- Bittleston LS, Baker CCM, Strominger LB, Pringle A, Pierce NE. 2016. Metabarcoding as a tool for investigating arthropod diversity in *Nepenthes* pitcher plants. *Austral Ecology*, 41(2): 120–132
- Bizzozzero MR, Altermatt F, Ciccirella R, Walser JC, Willems EP, Krützen M. 2024. Enhancing environmental DNA metabarcoding from marine ecosystems: impact of filter type, storage method, and storage time on the assessment of fish alpha and beta diversity. *Environmental DNA*, 6(3): e570
- Blackman RC, Ling KKS, Harper LR, Shum P, Hänfling B, Lawson-Handley L. 2020. Targeted and passive environmental DNA approaches outperform established methods for detection of quagga mussels, *Dreissena rostriformis bugensis* in flowing water. *Ecology and Evolution*, 10(23): 13248–13259
- Burian A, Mauvisseau Q, Bulling M, Domisch S, Qian S, Sweet M. 2021. Improving the reliability of eDNA data interpretation. *Molecular Ecology Resources*, 21(5): 1422–1433
- Butterworth V, Dansby H, Zink FA, Tembrock LR, Gilligan TM, Godoy A, Braswell WE, Kawahara AY. 2022. A DNA extraction method for insects from sticky traps: targeting a low abundance pest, *Phthorimaea absoluta* (Lepidoptera: Gelechiidae), in mixed species communities. *Journal of Economic Entomology*, 115(3): 844–851
- Capo E, Spong G, Königsson H, Byström P. 2020. Effects of filtration methods and water volume on the quantification of brown trout (*Salmo trutta*) and Arctic char (*Salvelinus alpinus*) eDNA concentrations via droplet digital PCR. *Environmental DNA*, 2(2): 152–160
- Carvalho CO, Gromstad W, Dunthorn M, Karlsen HE, Schröder-Nielsen A, Ready JS, Haugaasen T, Sørnes G, de Boer H, Mauvisseau Q. 2024. Harnessing eDNA metabarcoding to investigate fish

- community composition and its seasonal changes in the Oslo fjord. *Scientific Reports*, 14(1): 10154
- Chen X, Fang JY, Wang M, Shen Q, Sun ZJ, Wang BX. 2021. Monitoring of the invasive species *Pomacea canaliculata* via environmental DNA metabarcoding in Suzhou City. *Plant Protection*, 47(6): 58–65 (in Chinese) [陈晓, 方靖怡, 王萌, 沈晴, 孙振军, 王备新. 2021. 利用环境DNA-宏条形码技术监测苏州地区小管福寿螺的入侵. *植物保护*, 47(6): 58–65]
- Coble AA, Flinders CA, Homyack JA, Penaluna BE, Cronn RC, Weitemier K. 2019. eDNA as a tool for identifying freshwater species in sustainable forestry: a critical review and potential future applications. *Science of the Total Environment*, 649: 1157–1170
- Crisol-Martínez E, Moreno-Moyano LT, Wormington KR, Brown PH, Stanley D. 2016. Using next-generation sequencing to contrast the diet and explore pest-reduction services of sympatric bird species in *Macadamia* orchards in Australia. *PLoS ONE*, 11(3): e0150159
- Deiner K, Bik HM, Mächler E, Seymour M, Lacoursière-Roussel A, Altermatt F, Creer S, Bista I, Lodge DM, de Vere N, et al. 2017. Environmental DNA metabarcoding: transforming how we survey animal and plant communities. *Molecular Ecology*, 26(21): 5872–5895
- Díaz-Ferguson E, Herod J, Galvez J, Moyer G. 2014. Development of molecular markers for eDNA detection of the invasive African jewelfish (*Hemichromis letourneuxi*): a new tool for monitoring aquatic invasive species in National Wildlife Refuges. *Management of Biological Invasions*, 5(2): 121–131
- Espinosa PA, Hardion L, Debortoli N, Bournonville T, Mathot T, Marescaux J, Chanez E, Staentzel C, Beisel JN. 2024. A comparative analysis of eDNA metabarcoding and field surveys: exploring freshwater plant communities in rivers. *Science of the Total Environment*, 954: 176200
- Evans DM, Kitson JJN. 2020. Molecular ecology as a tool for understanding pollination and other plant-insect interactions. *Current Opinion in Insect Science*, 38: 26–33
- Evans NT, Shirey PD, Wieringa JG, Mahon AR, Lamberti GA. 2017. Comparative cost and effort of fish distribution detection via environmental DNA analysis and electrofishing. *Fisheries*, 42(2): 90–99
- Everett MV, Park LK. 2018. Exploring deep-water coral communities using environmental DNA. *Deep Sea Research Part II: Topical Studies in Oceanography*, 150: 229–241
- Feng HQ, Yao Q, Hu C, Huang WJ, Hu XP, Liu J, Zhang YH, Zhang Z, Qiao HB, Liu W. 2023. Recent advances in intelligent techniques for monitoring and prediction of crop diseases and insect pests in China. *Plant Protection*, 49(5): 229–242 (in Chinese) [封洪强, 姚青, 胡程, 黄文江, 胡小平, 刘杰, 张云慧, 张智, 乔红波, 刘伟. 2023. 我国农作物病虫害智能监测预警技术新进展. *植物保护*, 49(5): 229–242]
- Gamage CD, Sato Y, Kimura R, Yamashiro T, Toma C. 2020. Understanding leptospirosis eco-epidemiology by environmental DNA metabarcoding of irrigation water from two agro-ecological regions of Sri Lanka. *PLoS Neglected Tropical Diseases*, 14(7): e0008437
- Giroux MS, Reichman JR, Langknecht T, Burgess RM, Ho KT. 2023. Using eRNA/eDNA metabarcoding to detect community-level impacts of nanoplastic exposure to benthic estuarine ecosystems. *Environmental Pollution*, 338: 122650
- Goldberg CS, Sepulveda A, Ray A, Baumgardt J, Waits LP. 2013. Environmental DNA as a new method for early detection of New Zealand mudsnails (*Potamopyrgus antipodarum*). *Freshwater Science*, 32(3): 792–800
- Goldberg CS, Strickler KM, Pilliod DS. 2015. Moving environmental DNA methods from concept to practice for monitoring aquatic macroorganisms. *Biological Conservation*, 183: 1–3
- Hassan S, Bali BS, Yaseen A, Zaman M, Muneer W, Ahmad Ganicee S, Shah AJ, Ahmad Ganai B. 2024. Bridging the gaps through environmental DNA: a review of critical considerations for interpreting the biodiversity data in coral reef ecosystems. *Marine Pollution Bulletin*, 209: 117242
- Hendricks A, Mackie C, Luy E, Sonnichsen C, Miller L, Wright M, Grundke I, Smith J, Creelman J, Tavasoli M, et al. 2022. A miniaturized and automated eDNA sampler: application to a marine environment//OCEANS 2022, Hampton Roads. Hampton Roads, VA, USA: Institute of Electrical and Electronics Engineers, pp. 1–10
- Hermans SM, Buckley HL, Lear G. 2018. Optimal extraction methods for the simultaneous analysis of DNA from diverse organisms and sample types. *Molecular Ecology Resources*, 18(3): 557–569
- Hinlo R, Gleeson D, Lintermans M, Furlan E. 2017. Methods to maximise recovery of environmental DNA from water samples. *PLoS ONE*, 12(6): e0179251
- How CM, Ip JC, Deconinck D, Zhao MH, Yan M, Cheng JP, Leung KMY, Chan LL, Qiu JW. 2024. Refining sampling efforts for fish diversity assessment in subtropical urban estuarine and oceanic waters using environmental DNA with multiple primers. *Environmental DNA*, 6(5): e70013
- Jackman JM, Benvenuto C, Coscia I, Carvalho CO, Ready JS, Boubli JP, Magnusson WE, McDevitt AD, Sales NG. 2021. eDNA in a bottleneck: obstacles to fish metabarcoding studies in megadiverse freshwater systems. *Environmental DNA*, 3(4): 837–849
- Jo T, Minamoto T. 2021. Complex interactions between environmental DNA (eDNA) state and water chemistries on eDNA persistence suggested by meta-analyses. *Molecular Ecology Resources*, 21(5): 1490–1503
- Kawato M, Yoshida T, Miya M, Tsuchida S, Nagano Y, Nomura M, Yabuki A, Fujiwara Y, Fujikura K. 2021. Optimization of environmental DNA extraction and amplification methods for metabarcoding of deep-sea fish. *MethodsX*, 8: 101238
- Kestel JH, Field DL, Bateman PW, White NE, Allentoft ME, Hopkins AJM, Gibberd M, Nevill P. 2022. Applications of environmental DNA (eDNA) in agricultural systems: current uses, limitations and future prospects. *Science of the Total Environment*, 847: 157556
- Krehenwinkel H, Weber S, Künzel S, Kennedy SR. 2022. The bug in a

- teacup: monitoring arthropod-plant associations with environmental DNA from dried plant material. *Biology Letters*, 18(6): 20220091
- Kudoh A, Minamoto T, Yamamoto S. 2020. Detection of herbivory: eDNA detection from feeding marks on leaves. *Environmental DNA*, 2(4): 627–634
- Kusanke LM, Panteleit J, Stoll S, Korte E, Sünger E, Schulz R, Theissinger K. 2020. Detection of the endangered European weather loach (*Misgurnus fossilis*) via water and sediment samples: Testing multiple eDNA workflows. *Ecology and Evolution*, 10(15): 8331–8344
- Ladin ZS, Ferrell B, Dums JT, Moore RM, Levia DF, Shriver WG, D'Amico V, Trammell TLE, Setubal JC, Wommack KE. 2021. Assessing the efficacy of eDNA metabarcoding for measuring microbial biodiversity within forest ecosystems. *Scientific Reports*, 11(1): 1629
- Lamb PD, Fonseca VG, Maxwell DL, Nnanatu CC. 2022. Systematic review and meta-analysis: water type and temperature affect environmental DNA decay. *Molecular Ecology Resources*, 22(7): 2494–2505
- Lance RF, Guan X. 2020. Variation in inhibitor effects on qPCR assays and implications for eDNA surveys. *Canadian Journal of Fisheries and Aquatic Sciences*, 77(1): 23–33
- Larson ER, Graham BM, Achury R, Coon JJ, Daniels MK, Gambrell DK, Jonasen KL, King GD, LaRacuente N, Perrin-Stowe TI, et al. 2020. From eDNA to citizen science: emerging tools for the early detection of invasive species. *Frontiers in Ecology and the Environment*, 18(4): 194–202
- Leempoel K, Hebert T, Hadly EA. 2020. A comparison of eDNA to camera trapping for assessment of terrestrial mammal diversity. *Proceedings of the Royal Society B: Biological Sciences*, 287(1918): 20192353
- Li HR, Pan HP, Tao YL, Zhang YJ, Chu D. 2017. Population genetics of an alien whitefly in China: implications for its dispersal and invasion success. *Scientific Reports*, 7(1): 2228
- Li HT, Zhang S, Zou KS, Chen ZZ, Chen XL, Jiang PW, Cao YT, Li M. 2022. Establishment and optimization of environmental DNA extraction method from water of Pearl River Estuary. *South China Fisheries Science*, 18(3): 30–37 (in Chinese) [李红婷, 张帅, 邹柯妹, 陈作志, 陈晓雷, 蒋佩文, 曹漪婷, 李敏. 2022. 珠江河口水体环境DNA提取方法的建立及优化. *南方水产科学*, 18(3): 30–37]
- Lim NKM, Tay YC, Srivathsan A, Tan JWT, Kwik JTB, Baloglu B, Meier R, Yeo DCJ. 2016. Next-generation freshwater bioassessment: eDNA metabarcoding with a conserved metazoan primer reveals species-rich and reservoir-specific communities. *Royal Society Open Science*, 3(11): 160635
- Lu SX, Zeng HH, Xiong F, Yao M, He SP. 2024. Advances in environmental DNA monitoring: standardization, automation, and emerging technologies in aquatic ecosystems. *Science China. Life Sciences*, 67(7): 1368–1384
- Lynggaard C, Calvignac-Spencer S, Chapman CA, Kalbitzer U, Leendertz FH, Omeja PA, Opito EA, Sarkar D, Bohmann K, Gogarten JF. 2023. Vertebrate environmental DNA from leaf swabs. *Current Biology*, 33(16): R853–R854
- Lynggaard C, Frøslev TG, Johnson MS, Olsen MT, Bohmann K. 2024. Airborne environmental DNA captures terrestrial vertebrate diversity in nature. *Molecular Ecology Resources*, 24(1): e13840
- Madden AA, Barberán A, Bertone MA, Menninger HL, Dunn RR, Fierer N. 2016. The diversity of arthropods in homes across the United States as determined by environmental DNA analyses. *Molecular Ecology*, 25(24): 6214–6224
- Mahon AR, Jerde CL, Galaska M, Bergner JL, Chadderton WL, Lodge DM, Hunter ME, Nico LG. 2013. Validation of eDNA surveillance sensitivity for detection of Asian carps in controlled and field experiments. *PLoS ONE*, 8(3): e58316
- Mahon AR, Nathan LR, Jerde CL. 2014. Meta-genomic surveillance of invasive species in the bait trade. *Conservation Genetics Resources*, 6(3): 563–567
- Maslo B, Valentin R, Leu K, Kerwin K, Hamilton GC, Bevan A, Fefferman NH, Fonseca DM. 2017. Chiro-surveillance: the use of native bats to detect invasive agricultural pests. *PLoS ONE*, 12(3): e0173321
- Mauvisseau Q, Harper LR, Sander M, Hanner RH, Kleyer H, Deiner K. 2022. The multiple states of environmental DNA and what is known about their persistence in aquatic environments. *Environmental Science & Technology*, 56(9): 5322–5333
- McPherson C, Avanesyan A, Lamp WO. 2022. Diverse host plants of the first instars of the invasive *Lycorma delicatula*: insights from eDNA metabarcoding. *Insects*, 13(6): 534
- Milla L, Schmidt-Lebuhn A, Bovill J, Encinas-Viso F. 2022. Monitoring of honey bee floral resources with pollen DNA metabarcoding as a complementary tool to vegetation surveys. *Ecological Solutions and Evidence*, 3(1): e12120
- Montauban C, Mas M, Wangenstein OS, Sarto i Monteys V, Fornós DG, Mola XF, López-Baucells A. 2021. Bats as natural samplers: first record of the invasive pest rice water weevil *Lissorhoptrus oryzophilus* in the Iberian Peninsula. *Crop Protection*, 141: 105427
- Nagler M, Podmirseg SM, Ascher-Jenull J, Sint D, Traugott M. 2022. Why eDNA fractions need consideration in biomonitoring. *Molecular Ecology Resources*, 22(7): 2458–2470
- Pawlowski J, Bruce K, Panksep K, Aguirre FI, Amalfitano S, Apothéoz-Perret-Gentil L, Baussant T, Bouchez A, Carugati L, Cermakova K, et al. 2022. Environmental DNA metabarcoding for benthic monitoring: a review of sediment sampling and DNA extraction methods. *Science of the Total Environment*, 818: 151783
- Pirtle EI, van Rooyen AR, Maino J, Weeks AR, Umina PA. 2021. A molecular method for biomonitoring of an exotic plant-pest: leafmining for environmental DNA. *Molecular Ecology*, 30(19): 4913–4925
- Poyntz-Wright IP, Harrison XA, Pedersen S, Tyler CR. 2024. Effectiveness of eDNA for monitoring riverine macroinvertebrates. *Science of the Total Environment*, 941: 173621

- Rasmussen JA, Nielsen M, Mak SST, Döring J, Klincke F, Gopalakrishnan S, Dunn RR, Kauer R, Gilbert MTP. 2021. eDNA-based bio-monitoring at an experimental German vineyard to characterize how management regimes shape ecosystem diversity. *Environmental DNA*, 3(1): 70–82
- Rees HC, Maddison BC, Middleditch DJ, Patmore JRM, Gough KC. 2014. The detection of aquatic animal species using environmental DNA: a review of eDNA as a survey tool in ecology. *Journal of Applied Ecology*, 51(5): 1450–1459
- Rehill T, Millard-Martin B, Lemay M, Sheridan K, Mueller A, Morien E, Clemente-Carvalho RBG, Hunt BPV, Sunday JM. 2024. Detection differences between eDNA and mid-water trawls are driven by fish biomass and habitat preferences. *Environmental DNA*, 6(4): e586
- Rishan ST, Kline RJ, Rahman MS. 2023. Applications of environmental DNA (eDNA) to detect subterranean and aquatic invasive species: a critical review on the challenges and limitations of eDNA metabarcoding. *Environmental Advances*, 12: 100370
- Rondon MR, August PR, Bettermann AD, Brady SF, Grossman TH, Liles MR, Loiacono KA, Lynch BA, MacNeil IA, Minor C, et al. 2000. Cloning the soil metagenome: a strategy for accessing the genetic and functional diversity of uncultured microorganisms. *Applied and Environmental Microbiology*, 66(6): 2541–2547
- Rourke ML, Walburn JW, Broadhurst MK, Fowler AM, Hughes JM, Fielder DS, DiBattista JD, Furlan EM. 2023. Poor utility of environmental DNA for estimating the biomass of a threatened freshwater teleost; but clear direction for future candidate assessments. *Fisheries Research*, 258: 106545
- Ruppert KM, Kline RJ, Rahman MS. 2019. Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: a systematic review in methods, monitoring, and applications of global eDNA. *Global Ecology and Conservation*, 17: e00547
- Saccò M, Guzik MT, van der Heyde M, Nevill P, Cooper SJB, Austin AD, Coates PJ, Allentoft ME, White NE. 2022. eDNA in subterranean ecosystems: applications, technical aspects, and future prospects. *Science of the Total Environment*, 820: 153223
- Sato M, Inoue N, Nambu R, Furuichi N, Imaizumi T, Ushio M. 2021. Quantitative assessment of multiple fish species around artificial reefs combining environmental DNA metabarcoding and acoustic survey. *Scientific Reports*, 11(1): 19477
- Senapati D, Bhattacharya M, Kar A, Chini DS, Das BK, Patra BC. 2019. Environmental DNA (eDNA): a promising biological survey tool for aquatic species detection. *Proceedings of the Zoological Society*, 72(3): 211–228
- Shelare SD, Aglawe KR, Waghmare SN, Belkhode PN. 2021. Advances in water sample collections with a drone: a review. *Materials Today*, 47: 4490–4494
- Shi XJ, Jiang YH, Cao L, Zeng C. 2024. Development of environmental DNA metabarcoding primers for marine mollusks and comparison with published primers. *BMC Ecology and Evolution*, 24(1): 73
- Shu L, Ludwig A, Peng ZG. 2020. Standards for methods utilizing environmental DNA for detection of fish species. *Genes*, 11(3): 296
- Skendzić S, Zovko M, Živković IP, Lešić V, Lemić D. 2021. The impact of climate change on agricultural insect pests. *Insects*, 12(5): 440
- Song Y, Huang Y. 2016. The application of DNA metabarcoding in the study of soil animal diversity in Taibai Mountain. *Acta Ecologica Sinica*, 36(14): 4531–4539 (in Chinese) [宋颢, 黄原. 2016. DNA复合条形码在太白山土壤动物多样性研究中的应用. *生态学报*, 36(14): 4531–4539]
- Sternhagen EC, Black KL, Hartmann EDL, Shivega WG, Johnson PG, McGlynn RD, Schmaltz LC, Asheim Keller RJ, Vink SN, Aldrich-Wolfe L. 2020. Contrasting patterns of functional diversity in coffee root fungal communities associated with organic and conventionally managed fields. *Applied and Environmental Microbiology*, 86(11): e00052–20
- Thamke V, Bezabhe YH, Jass J, Olsson PE. 2024. Preservation of aquatic environmental DNA using cationic detergents. *Environmental DNA*, 6(6): e70038
- Thomsen PF, Sigsgaard EE. 2019. Environmental DNA metabarcoding of wild flowers reveals diverse communities of terrestrial arthropods. *Ecology and Evolution*, 9(4): 1665–1679
- Todd JH, Simpson RM, Poulton J, Barraclough EI, Villsen K, Brooks A, Richards K, Jones D. 2020. Detecting invertebrate ecosystem service providers in orchards: traditional methods versus barcoding of environmental DNA in soil. *Agricultural and Forest Entomology*, 22(3): 212–223
- Tordoni E, Ametrano CG, Banchi E, Ongaro S, Pallavicini A, Bacaro G, Muggia L. 2021. Integrated eDNA metabarcoding and morphological analyses assess spatio-temporal patterns of airborne fungal spores. *Ecological Indicators*, 121: 107032
- Trujillo-González A, Thuo DN, Divi U, Sparks K, Wallenius T, Gleeson D. 2022. Detection of Khapra beetle environmental DNA using portable technologies in Australian biosecurity. *Frontiers in Insect Science*, 2: 795379
- Valentin RE, Fonseca DM, Gable S, Kyle KE, Hamilton GC, Nielsen AL, Lockwood JL. 2020. Moving eDNA surveys onto land: strategies for active eDNA aggregation to detect invasive forest insects. *Molecular Ecology Resources*, 20(3): 746–755
- Valentin RE, Fonseca DM, Nielsen AL, Leskey TC, Lockwood JL. 2018. Early detection of invasive exotic insect infestations using eDNA from crop surfaces. *Frontiers in Ecology and the Environment*, 16(5): 265–270
- van der Heyde M, Bunce M, Wardell-Johnson G, Fernandes K, White NE, Nevill P. 2020. Testing multiple substrates for terrestrial biodiversity monitoring using environmental DNA metabarcoding. *Molecular Ecology Resources*, 20(3): 732–745
- Villacorta-Rath C, Lach L, Andrade-Rodriguez N, Burrows D, Gleeson D, Trujillo-González A. 2023. Invasive terrestrial invertebrate detection in water and soil using a targeted eDNA approach. *Neobiota*, 83: 71–89
- Weldon L, O'Leary C, Steer M, Newton L, MacDonald H, Sargeant

- SL. 2020. A comparison of European eel *Anguilla anguilla* eDNA concentrations to fyke net catches in five Irish lakes. *Environmental DNA*, 2(4): 587–600
- Xiong W, MacIsaac HJ, Zhan AB. 2024. An overlooked source of false positives in eDNA-based biodiversity assessment and management. *Journal of Environmental Management*, 358: 120949
- Xu CCY, Yen IJ, Bowman D, Turner CR. 2015. Spider web DNA: a new spin on noninvasive genetics of predator and prey. *PLoS ONE*, 10(11): e0142503
- Xu N, Chang JB. 2016. Preliminary study on fish species detection in the middle and lower Yangtze River using environmental DNA. *Journal of Hydroecology*, 37(5): 49–55 (in Chinese) [徐念, 常剑波. 2016. 长江中下游干流环境DNA样本鱼类物种检测的初步研究. *水生生态学杂志*, 37(5): 49–55]
- Xu N, Zhu B, Shi F, Shao K, Que YF, Li WT, Li W, Jiao WJ, Tian H, Xu DM, et al. 2018. Monitoring seasonal distribution of an endangered anadromous sturgeon in a large river using environmental DNA. *Die Naturwissenschaften*, 105(11/12): 62
- Yan ZL, Luo Y, Chen XY, Yang LY, Yao M. 2024. Angling and trolling for eDNA: a novel and effective approach for passive eDNA capture in natural waters. *Environment International*, 194: 109175
- Yang HL, Zhang H, Du H. 2023. A framework for standardizing the processes of eDNA monitoring and an accessible vision of the future. *Journal of Lake Sciences*, 35(1): 12–31 (in Chinese) [杨海乐, 张辉, 杜浩. 2023. eDNA监测方法标准化框架及未来图景. *湖泊科学*, 35(1): 12–31]
- Yang LF, Liu YG, Tao YL, Zhang WM, Li JY, Chi SQ, Zhang GF, Chu D. 2024. Development of an on-site diagnostic LAMP assay for rapid differentiation of the invasive pest *Phthorimaea absoluta* (Meyrick) using insect tissues. *Pest Management Science*, 80(8): 4069–4073
- Yang LF, Yang N, Fu HB, Chu D. 2023. Research advances in the application of environmental DNA (eDNA) technique in biological invasions. *Journal of Plant Protection*, 50(1): 1–10 (in Chinese) [杨力凤, 杨楠, 付海滨, 褚栋. 2023. 环境DNA技术在生物入侵研究中的应用进展. *植物保护学报*, 50(1): 1–10]
- Yasashimoto T, Sakata MK, Sakita T, Nakajima S, Ozaki M, Minamoto T. 2021. Environmental DNA detection of an invasive ant species (*Linepithema humile*) from soil samples. *Scientific Reports*, 11(1): 10712
- Young RG, Milián-García Y, Yu J, Bullas-Appleton E, Hanner RH. 2021. Biosurveillance for invasive insect pest species using an environmental DNA metabarcoding approach and a high salt trap collection fluid. *Ecology and Evolution*, 11(4): 1558–1569
- Zenker MM, Specht A, Fonseca VG. 2020. Assessing insect biodiversity with automatic light traps in Brazil: pearls and pitfalls of metabarcoding samples in preservative ethanol. *Ecology and Evolution*, 10(5): 2352–2366
- Zhang CZ, Cai JH, Xiao DQ, Ye YW, Chehelamirani M. 2018. Research on vegetable pest warning system based on multidimensional big data. *Insects*, 9(2): 66
- Zhang S, Zhao JD, Yao M. 2020. A comprehensive and comparative evaluation of primers for metabarcoding eDNA from fish. *Methods in Ecology and Evolution*, 11(12): 1609–1625

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