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Recent Advances in Human Physiology

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Abstract: The study of human physiology provides important insight into the complex nature of the human body, increasing our understanding of the various systems and processes that occur to keep us alive. Developments in this field provide the basis for the development of novel treatments and therapies that are crucial for the advancement of medicine and improving the health and well-being of people around the world. Recent research into the pathogenesis of SAR-CoV-2 and the discovery of novel treatments for its symptoms have bought this field of science to the forefront. Yet there have also been several other recent advances that have increased our understanding of the human body and provided opportunities for the development of new medicines and therapies. Here we discuss the latest advances in this field, highlighting recent progression in our understanding of cancer metastasis, the development of the brain, and the use of organoids in the study of the human body. Finally, we examine the work of two physiologists that received the Nobel Prize in 2021 for their work in understanding the mechanism behind how humans feel the heat, cold, and mechanical force.

Keywords: physiology, SAR-CoV-2, organoids

Introduction
Antimicrobial Human physiology is a branch of science dedicated to the study of the mechanical, physical, and biochemical functions of the human body. This involves studying the normal function and vital processes of the human body by exploring the functions of its organs and the cells that they are composed of. The study of this sub-section of biology can be traced back as far as 420 BC and covers a range of subjects such as cellular physiology, organ physiology, biological compounds, and anatomy, and the interactions between them that make life possible. Advances in the study of human physiology have uncovered several mysteries about how the body functions, from understanding homeostasis to discovering how signals are sent between different tissues and cells to enable to body to function normally (Kholodenko, 2006; Modell et al., 2015). These discoveries have also increased our understanding of the pathogenesis of many human diseases and subsequently resulted in the advancement of medicine through the development of various treatments and therapies.

In recent years, SARS-CoV-2 has been the focus of many areas of research in human physiology, as researchers have explored the pathogenesis of the virus and developed therapies and treatments to combat the negative health effects that the virus has caused (Cao et al., 2021). In addition, several advances have been made our understanding of how the brain functions, in cancer research, and in the successful use of organoids in replacing animal models (Pun et al., 2021). In 2021, the Nobel Prize in physiology and medicine was awarded to two researchers for their work on understanding how humans feel pain and temperature (Caterina et al., 1997; Coste et al., 2010; McKemy et al., 2002). Here we present a review of these latest advances in human physiology, focusing on how they have advanced our understanding of the human body and where applicable, what this means for the treatment of human diseases.

SARS-CoV-2
Not surprisingly, a lot of the recent work in human physiology has centred around understanding the impacts that SARS-CoV-2, also known as COVID-19, has on the human body. Physiologically, COVID-19 is known to damage several organs and affect some patients’ sense of smell and taste as well as causing respiratory disorders (Wu et al., 2020; Wu et al., 2021). Recent results in this field support the hypothesis that COVID-19 infection causes
variation in heart rate during the recovery period (Solinski et al, 2022). Further, studies into so-called long covid which look at the long-term impacts of COVID-19 infection have found several mechanisms that could help to explain the COVID-19 linked pathophysiology leading to multiorgan systemic disorder. For example, several studies have established that COVID-19 can cause direct viral tissue damage through entry into cells in the body via an entry receptor called angiotensin-converting enzyme 2 (ACE2). ACE2 is expressed in a variety of cells including epithelial cells, nasal goblet cells, gastrointestinal epithelial cells, and pancreatic β cells (Gupta et al, 2020; Hoffmann et al, 2020). In addition, COVID-19 infection can cause endothelial injury, disrupt the regulation of the immune system, and cause hypercoagulability which can lead to thrombosis (Nalbandian et al, 2021). Research has also shown that COVID-19 can alter the epigenetic age of those infected. Researchers discovered this by comparing the DNA methylation in blood samples taken from COVID-19 patients with those from a healthy population and applying epigenetic clocks and telomere length estimator to the methylation profile of each individual (Cao et al., 2022). Taken together, these recent advances give an insight into some of the long-term impacts that COVID-19 has on the human body and advance our understanding of how this virus operates which can pave the way for novel treatments.

Organoids

Most of the foundational knowledge that we have on human physiology has come from animal experiments but, while animal models have been useful in a lot of studies to date, they are not always suitable, and this is the reason that a lot of clinical trials fail. As a result, organoids are emerging as a good in-vitro model for studying specific viral infections such as COVID-19. Organoids are 3D structures grown from stem cells and under optimal culture conditions these stem cells differentiate to produce a complete arsenal of cell types found in the specific tissue of interest. This has revolutionised physiological studies, providing a model that produces results that are more likely to be indicative of what is happening in reality. For example, Mahalingam et al (2020) used single-cell RNA sequencing data to examine the expression of the entry receptors angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 (TMPRSS2) to understand their role in SARS-CoV-2 pathogenesis. Their study demonstrates that organoids can be effectively used to study the pathogenesis of SARS-CoV-2 and to screen drugs that could be used to treat it. Building on this, Pei et al (2021) and Tiwari et al (2021) showed that SARS-CoV-2 can infect PSC-derived lung organoids and that the high expression of ACE2 and TMPRSS2 in the cells facilitated both infection and replication of the virus. In addition to studying viral infection and replication, organoids have also been used to examine what conditions predispose people to more severe COVID-19 infection. A recent study using brain organoids showed that those that have ApoE4, a known genetic risk factor for Alzheimer’s, are more likely to have a more severe case of COVID-19 (Wang et al, 2021).

Developments in our understanding of the brain

The human brain is responsible for quite a large number of functions that take place within our body and in recent years there have been several significant advances in our understanding of how the brain works. There have been several advances made in understanding the development of the brain. For example, differences in the visual processing areas of infants diagnosed with autism spectrum disorder are apparent at 6 months of age. From this finding, researchers hypothesize that this disruption in visual processing could interfere with how the world appears to these children, affecting both their interactions and the way they learn (Girault et al, 2022). Another study looked to understand how stress, and more specifically the hormone cortisol, impacts the brain during childhood. Blankenship et al (2019) demonstrated that an increase in cortisol in response to a stressor during preschooal (age 3 – 5) predicted increased anterior and posterior hippocampal connectivity with parts of the cerebral cortex called the precuneus and cingulate gyrus at school-age (age 5 – 9). This suggests that there are lasting impacts of stress on the brain from a young age. Previous research had led researchers to believe that the brain could adapt to changing situations. However, a recent study has shown that the brain is not as plastic as we once thought. Ortiz-Catalan et al (2020) used electrical stimulation of tactile nerve fibres to restore touch through a bionic hand. Often, attachment of a bionic hand results in misplaced nerve signals and as such the patient feels sensations in a different part of the hand that they
are using. The researchers found that over time this could not be repaired, demonstrating that the brain is not as adaptable as we once thought.

**Cancer Research**

Recent progress in cancer research has identified several mechanisms involved in cancer metastasis. Exosomes are released by all cells as part of their normal physiology, and they can contain microRNAs; mRNAs; and proteins and can carry messages between cells allowing them to communicate. Recently, researchers have shown that exosomes are also released from cancer cells and contribute to cancer growth. For example, exosomes released from cancer cells can destroy the blood-brain barrier which enables it to metastasize to the brain (Cai et al., 2018; Lu et al., 2020). In addition, researchers have found that cancer exosomes can cause new blood vessels to develop in the tumour microenvironment by modulating gene expression which also contributes to metastasis (Yang et al., 2018). Exosomes released by normal cells can also have an impact on how cancer progresses. For example, exosomes released from mesenchymal cells that originated in the bone marrow can cause some cancer cells to become dormant. This is problematic because these dormant cells become resistant to chemotherapy and can be responsible for the disease reoccurring (Ono et al., 2014). Together, this research demonstrates how important exosomes are in the development, metastasis, and reoccurrence of cancer. Exosomes are not the only molecule that is involved in cancer metastasis. Baksh and Finley (2022) recently identified an important early step in the metastasis of a tumour. They found that the variable expression of an enzyme was key to whether a tumour spread.

![Figure 1](Image by Guido4 via Wikimedia Commons)
Nobel Prize 2021
The Nobel Prize in physiology and medicine was awarded to David Julius and Ardem Patapoutian in 2021 for their work that discovered the receptors for temperature and touch. Their work demonstrated the mechanics involved in how humans perceive hot, cold, touch, and pressure through nerve impulses. This was achieved through several independent projects that together provide fundamental insights into how external stimuli and nerve impulses interact. Julius et al identified the cellular target of capsaicin, which is the molecule found in chilli peppers that causes heat (Caterina et al., 1997). By screening a cDNA library from sensory neurons, the researchers identified a novel ion channel that was named TRPV1. This channel was shown to be activated by temperatures perceived as painful. Julius and Patapoutian also independently found a cold-sensitive receptor now known as TRPM8 (McKemy et al., 2002). Several other TRP receptors have since been identified. Patapoutian and his team discovered ion channels activated by mechanical stimuli by using a functional screen of candidate genes. The researchers identified two ion channels, named PIEZ01 and PIEZ02, that function as mechanical sensors (Coste et al., 2010). Further, PIEZ02 was identified as being the major mechanical transducer in somatic nerves and plays a vital role in our perception of touch and the position and movement of the body. Together, this work by the two laureates has demonstrated for the first time the molecular mechanism involved in sensing heat, cold, and mechanical force.

Conclusions
Recent advances in human physiology have increased our understanding of several processes in the human body and provided the basis for developing novel treatments to combat the symptoms of long covid caused by infection with SARS-CoV-2, for various forms of cancer, and for understanding the impacts that stress can have on a developing brain. In addition, the recent advances in the development of organoids for the study of the human body have shown promise in investigating the development and progression of various diseases and for testing novel treatments, removing the need for animal testing which could reduce the likelihood of treatments failing once they reach clinical trials. Finally, advances in human physiology were recognised in the awarding of the 2021 Nobel Prize to two researchers for their work in understanding the mechanisms behind how humans feel pain, heat, and cold which uncovers the mystery that has baffled researchers for decades.

References


**Conflicts of Interest**

The authors state no conflict of interest.
New insight into phosphoproteome research improves the in-depth understanding of honey bee biology

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Abstract: Protein phosphorylation is essential in a variety of biological activities. Notably, phosphoproteomics has opened new possibilities for honey bee biological study at the molecular and biochemical levels, considering the advancements in LC-MS/MS apparatus and computational analysis. This review extensively evaluated recent advances in honey bee biology utilizing phosphoproteomics methodologies in terms of progressive physiology, age polytheism, and biological changes in some organs, tissues, and cells. Furthermore, a comprehensive phosphoproteomic is necessary for new insight into honey bee biology, a better understanding of the research subject, and determining prospective future research areas.

Keywords: Honey bee, phosphoproteomics, LC-MS/MS, bioinformatics

Introduction
The honey bees (Hymenoptera: Apidae) are well-known eusocial and beneficial insects, which produce valuable products including honey, royal jelly, propolis, and pollen, and these are used in cosmetic and medicinal industries [1, 2]. In addition, honey bees play an essential part in crop pollination worldwide because of their diverse food, more extended forager range than other pollinators, and ability to discover the discrete area of resources in the larger environment efficiently using scouting [3, 4]. At an earlier stage, most studies on honey bee biology did not go beyond describing them, whether at the colony or individual animal level. As a result, compared to other organisms such as Drosophila, bees are poorly investigated at the molecular, biological, and cellular levels due to a vast variety of knowledge and technical limitations [5]. However, scientists start to investigate honey bee biology at the molecular level at the beginning of the 20th century.

Recently, the honey bee has been well studied in the context of general biology and beekeeping. Despite its long history as a typical organism for studying social performance, the honey bee is only studied at the molecular level in developing biology, neuroscience, immunology, and aging [6]. The situation has drastically changed, with the completion of honey bee genome sequencing, scientific breakthroughs in protein separation, identification, mass spectrometry (MS), and computer stages. After the completion of the honey bee genomic sequence in 2006 [7], it opened a new era of honey bee functional genomic research. However, honey bee genomics is essential for underpinning honey bee biology but not enough to investigate all of its complexity. So, studying gene expression by proteome research is necessary to understand bee biology. Due to tremendous technological advances in the recent decade in terms of MS, protein preparation, and computational algorithms, proteomic research on honey bees has significantly expanded [5]. Proteomics has evolved over time to include more sophisticated liquid chromatography coupled tandem MS (LC-MS/MS) technologies that have substantially increased in speed and quality, such as resolution, sensitivity, and mass accuracy, allowing for large-scale protein analysis [8, 9]. Moreover, in the past few years, proteomics has proven to be a more effective tool for studying post-translational modifi-

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This review summarized phosphoproteome research to understand honey bee biology at molecular level.

**Phosphoproteomics uncover the wide range of honey bee biology**

Phosphoproteomics is a large-scale investigation that uses mass spectrometry to identify and quantify phosphorylated proteins as well as map phosphorylation sites in a complicated biological sample [10]. It has undergone considerable change since 2000, thanks to advancements in MS-based phosphoproteome methods [10, 11]. Protein phosphorylation such as serine (S), threonine (T), and tyrosine (Y) is a well-known dynamic post-translational alteration in eukaryotes with amazing regulatory and signaling activities [11]. The human phosphoproteome, for example, contains around 30,000 S/T/Y phosphorylation sites [12]. To date, 15 studies regarding S/T/Y phosphorylation on honey bees have been reported (see Figure 1), and detailed description of these studies are mentioned in Table 1. Moreover, using the phosphoproteome technique, new depths have been reached at the molecular and biological level for a wide variety of honey bee biology, including developing biology, physiology, behavior, neuroscience, and immunology (see Figure 2).

**Figure 1:** Publications frequency per year

**Figure 2:** Workflow of phosphoproteomic experiments for honey bee samples.
**Table 1:** The detailed description of phosphoproteome research to unveil honey bee biology.

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<th>Honey bee species</th>
<th>Honey bee tissue and organ</th>
<th>Peptide enrichment</th>
<th>Phosphoproteomic method</th>
<th>Phosphorylated protein</th>
<th>Phosphorylated peptides</th>
<th>Phosphorylated sites</th>
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<td>[13]</td>
<td><em>Apis mellifera ligustica</em></td>
<td>Salivary glands</td>
<td>CBB stained gels</td>
<td>2DE-ESI-QTOF-MS</td>
<td>36</td>
<td>n. r</td>
<td>n. r</td>
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<tr>
<td>[14]</td>
<td><em>Apis mellifera ligustica</em></td>
<td>Embryo and larvae</td>
<td>CBB stained gels</td>
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<td>111, 102</td>
<td>n. r</td>
<td>n. r</td>
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<tr>
<td>[15]</td>
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<td>Venom</td>
<td>Ti⁺⁺-IMAC</td>
<td>LC-MS/MS</td>
<td>3</td>
<td>4</td>
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<tr>
<td>[16]</td>
<td>RJBs, (<em>Apis mellifera ligustica</em>)</td>
<td>Hypopharyngeal glands</td>
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<td>LC-MS/MS</td>
<td>6</td>
<td>6</td>
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<tr>
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<td><em>Apis mellifera ligustica, A. m. carnica</em></td>
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<td>n. r</td>
<td>LC-MS/MS</td>
<td>2</td>
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<td>[18]</td>
<td><em>A. mellifera ligustica, A. cerana cerana</em></td>
<td>Royal jelly</td>
<td>TiO², Ti⁺⁺-IMAC</td>
<td>Nano-LC-MS/MS</td>
<td>16, 9</td>
<td>93, 113</td>
<td>67, 71</td>
</tr>
<tr>
<td>[19]</td>
<td>RJBs, (<em>Apis mellifera ligustica</em>)</td>
<td>Hypopharyngeal glands</td>
<td>TiO², Ti⁺⁺-IMAC</td>
<td>LC-MS/MS</td>
<td>1967</td>
<td>3559</td>
<td>3469</td>
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<tr>
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<td>ITBs, (<em>Apis mellifera ligustica</em>)</td>
<td>Embryo</td>
<td>TiO², Ti⁺⁺-IMAC</td>
<td>LC-MS/MS</td>
<td>1354</td>
<td>n. r</td>
<td>2829</td>
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<tr>
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<td>ITBs, RJBs (<em>Apis mellifera ligustica</em>)</td>
<td>Brain</td>
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<td>n. r</td>
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<tr>
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<td>LC-MS/MS</td>
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<td>672</td>
<td>577</td>
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<td>[24]</td>
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<td>LC-MS/MS</td>
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<td>n. r</td>
</tr>
<tr>
<td>[25]</td>
<td>ITBs, (<em>Apis mellifera ligustica</em>)</td>
<td>Brain</td>
<td>Ti4⁺-IMAC</td>
<td>Nano-LC-MS/MS</td>
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<td>22,656</td>
<td>n. r</td>
</tr>
<tr>
<td>[26]</td>
<td>ITBs, (<em>Apis mellifera ligustica</em>)</td>
<td>Brain</td>
<td>Ti4⁺-IMAC</td>
<td>Nano-LC-MS/MS</td>
<td>916</td>
<td>n. r</td>
<td>n. r</td>
</tr>
<tr>
<td>[27]</td>
<td><em>A. mellifera ligustica, A. cerana cerana</em></td>
<td>Antennal lobes</td>
<td>Ti4⁺-IMAC</td>
<td>Nano-LC-MS/MS</td>
<td>1265</td>
<td>2812</td>
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**Phosphoproteomics of honey bee glands unveils the castes and age-dependent differences**

Different glands which are required for the honey bee to perform their biological task have been studied through phosphoproteome research such as hypopharyngeal glands (HGS) [16, 19], mandibular glands (MGs) [24] and salivary glands (SGs) [13]. HGS are the unique characteristics of Hymenopteran insects, especially in the honey bee that produces royal jelly in nurse bees and digestive enzymes in forager bees. The HGS have a wide range of genes and proteins that cause age-related physiological changes. According to time-resolved proteome profiling, proteins generated in the HGS of worker bees fit age-dependent function occurrence. Furthermore, the phosphoproteomics analysis of HGS at each worker stage reveals that a considerable fraction of cellular protein is phosphorylated, suggesting that phosphorylation may play an essential role in HGS activity [19]. Both phosphoproteome and non-phosphoproteome are required to support the HGS' distinct age-dependent physiology by complementing biological tasks. In phosphoproteome analysis of HGS across the worker ages, the majority of proteins are regulated by phosphorylation independently to their expression level, and comparable protein and phosphoprotein expression profiles are required for the unique secretory physiology of HGS [19]. Furthermore, MGs are a pair of sack-like glands situated inside the head. The secretions of MGs have crucial caste-specific roles linked to honey bee social evolution [28]. The molecular origins of caste-specific MGs activities have been demonstrated by phosphoproteome research. The phosphoproteome reveals the ontogeny and functionality of MGs at each stage. In MGs, mainly phosphorylated proteins are associated with energy, fatty acid metabolic process and energy metabolism [24].

The salivary system shows functional flexibility across the age of bee workers [29]. The salivary glands (SGs) of the honey bees have two parts: cephalic SG inside the head and thoracic SG inside the thorax [30]. The fatty acid esters that makeup brood pheromones and regulation of juvenile hormones, which regulate young bee behavior and encourage pollen gathering, are stored in the larval SGs [13]. However, the secretion of the adult female is different from the larvae. The primary function of the adult cephalic SG is to produce an oily ingredient which involves wax manipulation, mouth lubrication, and release of the pheromones to locate the food sources [31, 32]. The thoracic SG produces the secretion involved in honey and sugar digestion, and pollen and wax moistening [33]. In addition, the thoracic SG expresses more proteins linked to glucose and energy metabolism, protein metabolism and folding, cellular homeostasis, and the cytoskeleton, to promote the gland's efficient honey processing through synthesis and secretion of saliva into nectar [13]. In short, phosphoproteome research is critical to determine the molecular insight how the honey bee SGs obtain the multiple biological mission for efficient management of bee colonies. Thus, changes in protein and phosphoprotein expression levels among cephalic SG and thoracic SG are the primary motivators for worker bees to carry out their various tasks following their physiological status. Further research is required to understand the development and functionality of bee glands by using new phosphopeptide enrichment techniques and the latest LC-MS/MS technologies.

**Molecular basis neurology underlies the honey bee biology**

The brain performs higher-order mental processes such as processing sensory signals and modifying functioning dynamics. In honey bee workers, the age-related behavior transition is nearly associated with alteration in the brain's structure, gene expression, and protein synthesis [34]. The alteration in gene expression in the bee brain can be utilize to forecast natural behavior [35]. In addition, the honey bee brain proteome is also linked to the age-related change from hive worker to forager [36]. To this end, multiple phosphoproteome studies on honey bee brains contribute to a better knowledge of the molecular foundation of neurology in honey bees that activate various social behaviors. In nurse bees, phosphoproteins are involved in brain maturation, signal transduction, development, and the olfactory learning processes during the initial stage of worker life to increase the nursing activities [25]. For instance, phosphatidylinositol signaling and arachidonic acid metabolism are important in RJB nurses' enhanced olfaction sensation in response to larval pheromone stimulation, and richer signal processing pathways in RJB foragers are to encourage a stronger inclination in pollen gathering [23]. In addition, phosphorylated proteins responsible for wide range of pathways depending on age, for instance, AGE/ RAGE, glycolysis/ gluconeogenesis, phosphorylation in nurse bees while ATP metabolic process, metal ion transport, phototransduction in foraging bees [22]. Thus, phosphorylation
is critical for fine-tuning protein activity to regulate brain function in bees performing nursing and foraging tasks.

The production of phosphoproteins in common biological pathways and kinase activities in the forager brains is critical for maintaining central neural activity during foraging. The phosphoproteins of the forager’s brain are strongly involved in the genetic pathway of inositol phosphate metabolism, phosphatidylinositol signaling system, phototransduction, Wnt and glycerophospholipid metabolism, and activated kinases such as JNK, p38, CLK, and PKA are involved brain maintenance, olfactory learning processes, the signal transduction to increase the foraging activities performance [26].

Furthermore, the majority of phosphoproteins discovered in A. cerana are involved in protein metabolism and transport, with the bulk of phosphoproteins in the mTOR signaling pathway being increased in the antennal lob of A. cerana. In addition, phosphorylated proteins increased A. cerana’s protein synthesis-dependent synaptic plasticity, making it easier for the species to process more complex floral olfactory hints in mountain foraging locations [27]. The phosphoproteome-driven brain modulation of honeybee olfactory activities could be relevant for future neurobiological research in honeybees and other insects.

**Molecular insight into biochemistry and function of honey bee products**

Phosphoproteome study of honey bee products such as royal jelly and venom is critical to exploring their biochemical and functional properties. Royal jelly is queen food for the entire life and is high in defensive and immunological defenses [37]. The phosphoproteome analysis of royal jelly between honey bee species showed diverse biological characteristics. The phosphositers, peptide quantity, and antibacterial action of the phosphorylated royal jelly proteins have evolved significant variations between these two bee species. The abundance level of phosphorylated proteins and phosphorylated peptides from the royal jelly of Apis cerana showed stronger anti-microbial and anti-fungal properties. In both species, MRJP abundance levels are better explained by their biological needs for existence and development. For instance, the abundance level of MRJPs is linked with the support of their large body size in western honey bees. While in eastern honey bees, the large number of phosphorylated peptides balances the low level of MRJPs by maintaining life and development [18].

In addition, honey bee venom is created by the female worker and is also known to contain a variety of compounds such as peptides (apamin, melittin, and adolapin etc.), enzymes (phospholipase A2 and hyaluronidase), proteins, amino acids and volatile ingredients. These chemicals show promise in treating inflammation and central nervous system illnesses such as Alzheimer’s, and amyotrophic lateral sclerosis [38]. Toxins from honey bee venom cause immune, metabolic, and neurological responses in victims.

Phosphoproteins studies of honey bee venom explores the depth of understanding of biochemical ingredients and their functions. A comparative proteome and phosphoproteome analysis of honey bee venom reveal three novel phosphorylated venom proteins, which may trigger diverse immune responses by identifying the antigenic determinants [15]. The discovery of three novel phosphorylation sites on venom proteins adds to our knowledge of the biochemical nature of bee venom. In addition, melittin and icarapin were phosphorylated in Africanized and European honey bees. Melittin is the major toxin of bee venom and, was phosphorylated in all venoms at the 10 T and 18 S residues. The phosphorylation of icarapin occurred at the 205 S residue near its known antigenic location [17]. In short, more research is needed to identify novel phosphorylated proteins in honey bee products such as honey, royal jelly, and venoms, as well as further evaluation in other mechanistic investigations of honeybee biology and health-promoting action in humans.

**Conclusions and future research direction**

Honey bee phosphoproteomics research is progressing in the academic community, and substantial progress has been achieved in understanding the molecular foundation of honeybee biology. In various domains of honey bee biology, including developmental biology, physiology, and neurobiology, for instance, numerous expression-based phosphoproteomic modifications have been reported, reaching new depths at the molecular and biological levels. However, additional research is needed into the specialized involvement of phosphoproteins in related specific biochemical pathways in development and behavioral physiology. Further studies are required to examine the function of
phosphoproteins that regulate the honey bee ontol-
ogy and behavioral physiology using advanced LC-
MS/MS methodologies.

References


Conflicts of Interest

The authors state no conflict of interest.
CRISPR/Cas9, a decade of genome editing tools to fix the DNA

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Abstract: This year marks the 10th anniversary of the CRISPR/Cas9 genome editing discovery. Since its discovery in 2012, the CRISPR/Cas9 system has become an indispensable tool in many research fields. This system has been extensively characterized and further optimized to broaden its editing capabilities. Depending on the DNA modification to make, there are now available several editing agents. In this review, we provide an overview of the CRISPR/Cas9 system and how it can be used to fix the DNA using the traditional repair mechanisms non-homologous end joining (NHEJ) and homology-directed repair (HDR), and the most recent gene editing approaches – base editing and prime editing.

Keywords: microbiology, CRISPR, gene editing, kill switch

1. The CRISPR/Cas9 system

Jennifer Doudna and Emmanuelle Charpentier reported for the first time in 2012 the potential of CRISPR/Cas9 as a genome editing tool, a discovery that yielded the researchers the Nobel Prize in Chemistry in 2020 (1). Since then, the ability of Cas9 nuclease to introduce site-specific changes in the DNA has been extensively studied in many different research fields, from medicine to agriculture. However, the clustered regularly interspaced short palindromic repeats (CRISPR)-associated proteins (CRISPR/Cas) have their origin in the adaptive immune system of archaea and bacteria (2,3). This defense mechanism uses RNA-guided nucleases to cleave foreign genetic elements and consists of three main stages: acquisition, expression, and interference (4). In the acquisition stage, a complex of Cas proteins binds to the invading genetic elements and cleaves a portion of the target DNA, called protospacer (4). Then, at the expression stage, the spacers are transcribed and processed into mature CRISPR RNAs (crRNAs) and, lastly, at the interference stage, the Cas protein is guided by the crRNA to recognize and cleave foreign nucleic acid molecules (4–6).

The CRISPR systems can be classified into two different classes (class I and class II), which are further divided into six different types (type I – VI) (4). The composition of the effector module distinguishes CRISPR/Cas class I from class II (4). The class I effector module has several Cas proteins that work together to bind and process the target, while class II systems have a unique crRNA-binding protein that is equivalent to the whole class I effector module (4). Class I includes types I, III, and IV, and class II the types II, V, and VI (4). The different types of CRISPR/Cas systems recognize and cleave DNA (type I, II, and V), RNA (type VI), or both (type III) (7). The effect of type IV on DNA or RNA is still unknown (7).

1.1 CRISPR-associated protein 9

CRISPR-associated protein 9 (Cas9) belongs to the class II-type II CRISPR system (1). In type II systems, an additional RNA molecule is needed – the transactivating CRISPR RNA (tracrRNA) (4). This RNA molecule is responsible for: i) the pre-crRNA processing by RNase III, forming the mature guide RNA (gRNA) composed of the crRNA:tracrRNA complex, and ii) activation of the crRNA-guided DNA cleavage by Cas9 (1). The crRNA has 42 nucleotides (nt), the first 20-nt at the 5'-end correspond to the spacer sequence, and the other 22-nt pair with the 5'-end of the tracrRNA (1). The remaining nucleotides of the tracrRNA are free to interact with the Cas9 protein (1). The 10-12 nucleotides at the 3'-end of the 20-nt crRNA form the seed sequence that confers DNA targeting specificity (8). While mis-
matches in this sequence impair target DNA binding and cleavage, a high level of homology with other DNA regions lead to off-target effects (i.e. the ability of the gRNA to recognize other than the target DNA sequences) (8). To avoid off-target effects, researchers have engineered different Cas9 proteins to produce high-fidelity Cas9 variants that have reduced non-specific DNA interactions maintaining on-target activity (9–14).

The Cas9 protein has a bilobed structure composed of the recognition (REC) lobe and the nuclease (NUC) lobe. As the names indicate, the REC domain recognizes the gRNA sequence, and the NUC lobe cleavages the double-stranded DNA. In addition, the NUC lobe recognizes the protospacer-adjacent motif (PAM) sequence (8,15). The NUC lobe is further divided into two domains - the HNH and RuvC domains - each responsible to cut one of the DNA strands. The first cuts the strand complementary to the gRNA sequence (target strand) while the second cleaves the DNA containing the PAM sequence (non-target strand) (Fig. 1) (15).

Mutations in these nuclease domains, either HNH (H840A) or RuvC (D10A), produce nickase variants of the Cas9 (nCas9), which induce nicks in only one of the DNA strands (16). Furthermore, when both domains carry these mutations that result in a nuclease deactivated Cas9 variant (dCas9) lacking its catalytic activity (16).

After binding the gRNA, the Cas9 protein becomes catalytically active and searches for a suitable PAM sequence (8). This is a 3-nt sequence downstream of the spacer in the nontarget sequence. The PAM sequence varies according to the organism it derives from (8). Streptococcus pyogenes (SpCas9) is the most used Cas9 nuclease, and it recognizes any 5'-NGG-3' sequence (“N” stands for any nucleotide). Once a suitable PAM sequence is found, the gRNA binds the target DNA sequence, and if there is perfect complementarity between the two, the Cas9 cleavages the double-stranded DNA 3-nt upstream of the PAM sequence (Fig. 1) (8). Even though the occurrence of an “NGG” PAM is relatively common in the human genome, the need for a specific motif for targeting limits the DNA target sites to a subset of sequences. To overcome this limitation, researchers have engineered SpCas9 variants that recognize a wider array of PAM sequences (17–25).

**Figure 1 – CRISPR/Cas9 genome editing.** The gRNA (in green) binds the target region and the Cas9 nuclease cuts the double-stranded DNA 3 bp upstream of the PAM sequence (in red). The DNA can then be repaired by two different mechanisms: non-homologous end joining (NHEJ) or homology-directed repair (HDR). In NHEJ, a mixture of nucleotides can be inserted or deleted at the cut site, forming indels. If a donor DNA template is provided the HDR repair mechanism is triggered, and the DNA is precisely repaired. Key: Inserted nucleotides in yellow; Precise edit in purple.
2. Genome editing tools

2.1 CRISPR/Cas9 genome editing

As mentioned above, following gRNA binding to the complementary DNA sequence, the Cas9 nuclease cleaves the DNA creating a double-stranded break (DSB). The default mechanism by which the DNA can be repaired is called non-homologous end joining (NHEJ) (2). This repair mechanism introduces a mixture of nucleotide insertions and deletions (indels) at the cut site, which can cause gene knockout when occurring at coding regions (Fig. 1) (2). Besides indel formation, DSB is also associated with chromosomal translocations and p53 gene activation (26–28).

Another DNA repair mechanism is homology-directed repair (HDR). This is a high-fidelity repair mechanism; however, it is less efficient than NHEJ as it mainly occurs in the S phase of the cell cycle (29). In the presence of a repair template containing homology arms flanking the desired edit, HDR is triggered and leads to precise repair of the genome (Fig. 1) (2). Nevertheless, as the two repair mechanisms can occur in the same cell in different alleles, even when an exogenous donor DNA template is used there is indel formation.

2.2 Base Editing

To introduce specific single nucleotide changes in the DNA and avoid the unwanted indels created by DSB, researchers have developed base editing. These genome editing tools allow the irreversible conversion of one base into another in a direct and programmable manner. Contrary to CRISPR/Cas9-mediated HDR, base editing does not require a donor DNA template and as a Cas9 nickase variant is used it does not generate DSB and, consequently, very low levels or no indels are produced. To date, base editors enable the introduction of all four transition mutations (C-to-T; T-to-C; A-to-G; G-to-A) and two transversions (C-to-G and G-to-C).

2.2.1 Cytosine base editing

The first base editors to be developed were the cytosine base editors (CBEs), in which a Cas9 (D10A) nickase variant is fused with a cytidine deaminase (30). Cytidine deaminase converts the C:G pair into a U:G mismatch (30) (Fig. 2). Then, Cas9 nickase cuts the non-edited DNA strand (target strand) 3 bp upstream of the PAM sequence (in red), permanently repairing the DNA. Key: Editing window in light blue.

Since the development of the first cytosine base editor version (BE1), many enhancements have been done to improve editing efficiency. Besides the deaminase and the Cas9 nickase, the last base editor version (BE4max) has modified nuclear localization signals (NLS) and codon usage, and two uracyl N-glycosylase inhibitor (UGI) domains were also added (32). These two UGI domains inhibit uracyl N-glycosylase (UNG), part of the base excision repair (BER) pathway, avoiding U:G mismatch recognition and reversion back to C:G pair.

One of the main disadvantages of CBEs and other base editing systems is that deaminases not only change the target nucleotide but also all others present in the editing window. To overcome this problem, base editors with narrower editing windows have been developed (33). Another disadvantage is that not always there is a suitable PAM sequence available that puts the target base in the correct editing window. The use of different Cas9 variants, such as SpRYCas9, can increase the range of target sequences (24,25).

2.2.2 Adenine base editing

The adenine base editors (ABEs) were developed following the same rationale behind CBEs development (34). These base editors deaminate any adenosine in the non-target DNA strand and convert it into inosine (I) (Fig. 2) (34). Inosine pairs with cytosine enabling the conversion of an A:T base pair.
into a G:C pair (Fig. 2) (34). As there are no enzymes in Nature known to deaminate adenosine in DNA, to develop ABEs, Escherichia coli (E. coli) tRNA adenosine deaminase enzyme (TadA) was evolved to function on DNA (34). The ABEs result from the fusion of wild-type non-catalytic TadA monomer and the evolved TadA monomer with a nickase Cas9 (34). Typically, the ABEs can convert any A:T to G:C in positions 4−7 of the protospacer (34–36).

2.2.3 C-to-G base editing

The first base editors developed (CBEs and ABEs) were intended to introduce the four base transitions, however, base transversions have occasionally been observed as byproducts of these base editors (37). To develop the C-to-G base editor (CGBE1), researchers took advantage of these unexpected editing outcomes and engineered BE4max (see section 3.2.1) by removing the two UGI domains and adding an E. coli UNG (eUNG) enzyme to its carboxy-terminal (38). A shorter version, miniCGBE1, lacking the eUNG domain was also developed. These C-to-G editors can edit any cytidine at positions 5−7 in the protospacer, being position 6 the most efficient (Fig. 2) (38).

2.3 Prime editing

As mentioned above CRISPR/Cas9 can cut the double-stranded DNA that can be repaired by the NHEJ mechanism, introducing a mixture of indels at the target site. However, there are occasions, for example, genetic diseases, where a specific gene editing approach is required. The homology-directed repair can be used to install precise DNA changes, but it relies on an exogenous donor DNA template, indel formation is not completely avoided, and it is inefficient in most relevant cell types. As an alternative, researchers developed base editing however, this editing approach is not able to perform most nucleotide transversions nor targeted insertions or deletions. To overcome this problem, researchers came up with a new versatile and precise genome editing method called prime editing (39). Prime editing uses Cas9 (H840A) nickase fused to an engineered M-MLV reverse transcriptase (RT) and a prime editing gRNA (pegRNA) that not only specifies the target site as well as it encodes the desired edit (39). The Cas9 nickase cuts the nontarget strand of the DNA exposing a 3′flap that binds to the primer binding site (PBS) of the RNA template serving as a primer for the RT (Fig. 3) (39,40). Then, the RT extends the 3′ flap and copies the edit sequence of the pegRNA (39,40). The endogenous endonuclease FEN1 excises 5′flaps and the edited 3′flap hybridizes with the unedited complementary strand (Fig. 3) (39,40).

However, as only one strand of DNA is edited there are mismatches formation that can be resolved naturally in favor of the desired editing (40). To further improve prime editing efficiency, researchers co-transfected a standard gRNA targeting the complementary strand allowing the Cas9 nickase to nick the unedited strand. Nicking the unedited strand bias mismatch repair (MMR) in favor of the edited sequence by using the edited DNA strand as a template (39,40). Another strategy for prime editing improvement is the co-expression of a dominant negative MMR protein (MLH1dn) to transiently inhibit MMR and, consequently, enhance editing efficacy (41).

Figure 3 – Prime editing. The pegRNA complex (in green) binds to the target region and the Cas9 (H840A) nickase cuts the non-target DNA strand 3bp upstream of the PAM site (in red). DNA nicking creates a 3′ flap that interacts with the primer binding site (PBS) located at the 3′ end of the pegRNA. The DNA/RNA hybrid serves as a primer site for the new DNA synthesis and RT polymerase uses the RT template (in light purple) to extend the 3′ flap and copy the edit (in dark purple) also present in the pegRNA. The unedited 5′ flap is removed by FEN1 (in black) and the edited 3′ flap hybridises with the unedited complementary strand resulting in precise DNA editing.
3. Conclusion
Since its discovery, CRISPR/Cas9 technology has been evolving at a fast pace. This technology has revolutionized genetic engineering, enabling many advances in medicine (e.g. treatment of human genetic diseases) or in agriculture (e.g. improvement of food crops) (42). It evolved into a precise genome editing tool that allows making nearly any DNA change with almost no undesired editing byproducts (43). However, more efforts are needed to further improve CRISPR/Cas9 editing capabilities and to understand the consequences of editing the genome.

References

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**Conflicts of Interest**

The authors state no conflict of interest.
Recent Developments in Microbiology

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Abstract: Research in microbiology is a dynamic and exciting field, and this review article outlines some recent significant developments within microbiology including using shotgun metagenomics to uncover the connection between diet and lifestyle and superbug infections, the current state of Ebola virus diagnostic tests, and the potential of treating superbug infections via innovative phage virus therapy as an alternative to antibiotics. This review also explores two studies using the CRISPR gene editing technique, which has been a revolution within microbiology. The first study undergoes genetic engineering of potatoes to improve their nutritional and industrial applications, which has important implications in improving food stability as well as bolstering production. The second study utilizes CRISPR to engineer bacteria to kill themselves on command, which is important to prevent the excessive spread bacteria that are used for a variety of applications from eating plastic waste in the environment to treating the diseased human gut. Engineering these so-called ‘kill switch’ allows these bacteria to be utilized for their intended purpose without growing uncontrollably in the environment or in the gut.

Keywords: microbiology, CRISPR, gene editing, kill switch

Diet and lifestyle determine our vulnerability to resistant bugs

Antimicrobial resistance (AMR), that is viruses, bacteria and fungi that are resistant to antibiotics, is a significant cause of morbidity and mortality worldwide and it is projected that the consequences of AMR will continue to worsen in the coming decades. Scientists estimate that in 2019 there were 4.95 million deaths worldwide in which AMR was a causative factor, thus investigating ways to control and combat AMR is an extremely important field within microbiology research. The gut microbiome has garnered attention for researching AMR circulation within the population, since microbes present in the gut carry antibiotic resistant genes (ARGs), of which the total composition is called the human resistome. The human resistome is affect by many factors, such as the living environment, climate change, sanitation, and diet. Recent research by Oliver et al aimed to better understand which dietary and lifestyle factors are predictive of AMR in health U.S. adults. They found that those who had a higher fiber, more diverse diet had lower rates of ARGs, indicating that diet may be a potential method for reducing the future global burden of AMR (Oliver et al., 2022). This study utilized a technique called shotgun metagenomic sequencing, which is an extremely valuable method that has transformed the field of microbiology, as it allows researchers to exhaustively sample all genes in all organisms present in a given diverse sample, such as fecal samples in this case, without the need for traditional culturing (Quince et al., 2017).

Gene editing potatoes for nutritional and industrial applications

Clustered regularly interspaced short palindromic repeat (CRISPR)-Cas systems are well-characterized forms of acquired immunity systems that are found in bacteria and archaea. They were first described in 1987, when it was found that bacteria species Escherichia coli had an unusual repetitive DNA sequence in its genome. Since then, CRISPR-Cas-based tools are widely regarded as the most reliable tools for genome editing and engineering and has created a revolution within the field of microbiology (Ishino et al., 2018), with applications in a wide range of topics such as gene editing to cure cancer (Tiruneh G/Medhin et al., 2021), gene editing crops to improve their resilience and growth (Mallapaty, 2022), to learning more about the functions
of unknown genes (Kwon et al., 2015). A recent study by Toinga-Villafuerte et al utilized CRISPR/Cas9-mediated mutagenesis to modify starches in potatoes. The potato is the third most important food crop in the world after rice and wheat, and is energy-dense, yielding four times more calories per hectare compared to grain crops, thus for many countries it is considered a food security crop. In addition to its nutritional value, potato starch is utilized in producing processed foods, adhesives, paper, and textiles. Thus, the team identified the importance of the potato as a target crop for genome editing for modifications to improve either its nutritional qualities or for industrial applications. Starch is composed of two types of polysaccharides: amyllose and amylopectin, and the ratio of these will affect the physical and chemical properties of the starch as well as the nutritional properties. Although amyllose starches are currently used more in products that target health-related nutritional applications due to their lower glycemic index, starches higher in amylopectin have more applications in relation to processed food and other industrial applications, and in fact amylopectin-based starches show much more promise for ethanol production than those starches found in normal potatoes. Thus, this team used the CRISPR-Cas9 system to generate an amylose-free potato by targeting the GBSS alleles in the potato to eliminate amylose. They found that the Yukon Gold variety of potato yielded the best results and they successfully created them amylose-free. The CRISPR-Cas system has been a revolution in crop breeding, as conventional breeding processes could take 10 to 15 years, and gene editing has made that process much shorter. It can prove to be indispensable in improving industry and securing the global food chain in a world with a growing population (Toinga-Villafuerte et al., 2022).

![Figure 1: Schematic of the overall process of genetic engineering to create amylose-free potatoes.](Created with BioRender.com)

**Treating superbug infections with viruses**

As mentioned previously, antimicrobial resistance (AMR) represents a major public health challenge for the future and is associated with high mortality rates and combined with the fact that there has been a significant reduction in the discovery of new antibiotics to treat multi-drug resistant (MDR) bacterial infections, researchers are examining various alternatives to combat the problem. One of such alternatives being explored are bacteriophages, or phages for short, which are viruses that infect bacteria (Delattre et al., 2022). Phage therapy is the use of phages to treat bacterial infections and has been receiving growing support especially over the past 15 years, and although they are not as popular as traditional antibiotics, they are used in Europe in cases of therapeutic failure (i.e.: when there are no other antibiotic options left to treat a bacterial infection. Phages must attach to the bacterium, inject its genetic material which gets replicated, virions assemble and the bacterium is lysed, releasing new phages which can again attach to new bacteria and continue the cycle (Brives and Pourraz, 2020).
Figure 2: Schematic of the bactericidal lytic phage cycle that is utilized in phage therapy.

The attachment stage of the phage is highly specific, which is great news in considering the therapeutic uses of phage therapy for AMR cases, since it can be adapted to target only the infection-causing bacterial species, while leaving the commensal or mutualistic bacteria (i.e.: the ‘good’ bacteria) of the microbiota untouched (Brives and Pourraz, 2020). Recent research by Delattre et al from INSERM in France focused on using phage therapy to treat AMR pneumonia by Escherichia coli, where they created a mouse model to characterize the interactions between phage and bacteria during the course of infection. This was a valuable study because before phage therapy can be administered to humans, the optimal dose, route of administration, and treatment duration must be elucidated, which is notoriously complex in this field. This is because the standardized pharmacology assessments that help to dictate processes of administration, distribution, metabolism, and excretion (i.e.: ADME) of drugs like antibiotics are not designed to be adapted to phage therapy. Their replication in the human body differs between each specific phage-bacteria pairing and their routes of elimination don’t follow the standard metabolic pathways through the kidney or liver as pharmaceutical drugs do. The model created by this team incorporated pre-existing in vitro and in vivo data as well as mathematical models and found that the route of administration determines the success of phage therapy. The mice had better survival when the phages were more quickly administered to their target bacteria, thus the intratracheal route was more favorable than the intravenous route. Interestingly, the model revealed that the dosage given didn’t have much bearing on the overall efficacy. The model also incorporated aspects of the animal’s immune system, which is imperative in phage therapies since phages work in synergy with the immune system of the host, which aids in the overall process of eradicating the pathogenic bacteria. This study is extremely important in the field of microbiology since it lays out a new approach to create a more well-organized approach to clinical development of phage therapies to treat serious AMR-related infections (Delattre et al., 2022).

Engineering bacteria to follow our orders
Throughout the years, humans have found ways to harness the power of microbes, from the production of fermented foods and alcohol to the production of antibiotics and vaccines, there are many benefits to microbial processes which can be availed of. One
such example is the possible uses of microbes that degrade plastic wastes in the environment (Ru et al., 2020). Probiotic microbes have also become important resources in genetic engineering diagnostic and therapeutic technologies, for example *Escherichia coli* Nissle 1917 (EcN), which has been engineered to successfully diagnose and treat bacterial infections, cancers, gastrointestinal bleeding, obesity, and inflammatory disorders. Thus, EcN strains have garnered attention for further research in medical applications, however, as is the case with the plastic-eating bacteria, these are living organisms, and thus releasing them into the environment or the human body, respectively, will have consequences such as environmental contamination and competitive exclusion of native microbes. Any uncontrolled release of microorganisms poses a biosecurity risk, thus a study conducted by Rottinghaus et al examined the concept of biocontainment circuit designs, focused on preventing uncontrolled proliferation of these microbes in the wild. Such a mechanism could be considered a microbial ‘kill switch’, preventing the release of such genetically modified organisms into the environment by engineering them to self-destruct under certain circumstances. The researchers inserted multiple kill switches into the EcN genome via CRISPR technology, allowing the EcN to grow under normal gut conditions in the mouse, but to die on the consumption of an inducer, and subsequently get excreted from the body. This research will be important as it offers the opportunity for on-demand, selective removal of engineered microbes from the gut. This mechanism could also be engineered and subsequently applied to many other microbes, with the possibility of altering the kill switch conditions to environmental or chemical conditions. Therefore it offers a novel mechanism for self-regulated biocontainment, with far researching applications in therapeutics and industry (Rottinghaus et al., 2022).

**A step in the right direction to develop field tests for Ebola virus**

Ebola virus disease (EVD) is a rare but severe disease in humans with one of the highest viral death rates in the world, at an average case fatality rate of 50%.

![Figure 3: Case fatality rate (CFR) of Ebola virus disease compared to other viral diseases.](https://app.biorender.com/biorender-templates)
The incubation period before symptoms appear is 2 to 21 days and symptoms include fever, fatigue, muscle pain and headache followed by vomiting, diarrhea, bleeding and death (World Health Organisation, 2021). Several severe outbreaks have happened across the African continent in the past few decades resulting in thousands of deaths. Good outbreak control heavily relies on early intervention and treatment; however, this can be difficult in remote settings. This is because the gold standard test for diagnosis is reverse transcriptase polymerase chain reaction (RT-PCR) which requires good laboratory capacity, trained personnel, and transport infrastructure, as well as being expensive and time-consuming with results taking at least 2 days. In an outbreak setting with such a serious viral infection, this time can be too late for some patients. Therefore, much research has been focused on creating rapid diagnostic tests (RDT) that can be used in the field, however this hasn’t been an easy feat in terms of reaching the required sensitivity and specificity to compete with RT-PCR (Nouvellet et al., 2015). A recent observational study conducted by Mukadi-Bamuleka et al aimed to address this research gap by evaluating the field performance of three Ebola RDTs during the 2018-2020 outbreak in the Democratic Republic of the Congo. They compared their performance directly with the gold standard RT-PCR and estimated the sensitivity and specificity of each RDT. Unfortunately, the three RDTs did not achieve the desired levels as mapped out by the WHO, however this does not discount their importance in helping to triage people with suspected cases of EVD into high and low risk groups while they await their RT-PCR result, which is extremely valuable in busy and low-resource emergency hospital settings (Mukadi-Bamuleka et al., 2022).

References


**Conflicts of Interest**

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Recent insights into the use of invertebrates as indicators of habitat quality

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Abstract: Invertebrates are an important part of aquatic and terrestrial systems, and are, thus, useful as indicators of environmental changes. Habitat quality can be assessed by noting changes in species composition, and relative abundances, using various indices, and by alterations in physiology and morphology of what are known as indicator species. Research shows that invertebrates can be used to indicate if habitat conditions have changed, either worsened or improved. This is why identifying indicator species is crucial. This article covers some recent findings of how invertebrate organisms can be helpful in aquatic and terrestrial ecosystems in indicating ecosystem and habitat deterioration. We also discuss what makes a good indicator species and the types of taxa that have been used in this way.

Keywords: invertebrates, indicators, habitat, aquatic, terrestrial

Introduction
Conservationists have been concerned over habitat degradation on land and in water, for many years now. Habitat quality is important for both plants and animals in the environment but it is also significant for humans. Pollutants in water and soil can enter the human food chain compromising the food supply, drinking water, and inevitably, human health. Modified ecosystems also have implications for species diversity and may threaten the survival of species that have specialized requirements and cannot easily adapt to changes. Aquatic ecosystems where indicators are used include freshwater and marine environments. Terrestrial ecosystem indicators are useful for assessing soil and vegetation quality.

Why aquatic system health is important
The degradation of both freshwater and marine systems has ramifications for biodiversity and also for humans. People depend on water for irrigation in agricultural systems and also consume fish and macroinvertebrates harvested from water systems. There are multiple recreational uses of water such as swimming, water skiing, and scuba diving, to name a few. Polluted waters, thus, pose a threat to humans and also species that may be sensitive to environmental perturbations.

Species that make good aquatic indicators
Indicator species have been found for both marine and freshwater systems. Species that make good indicators are sensitive to environmental change. Changes in water parameters such as pH, water hardness, turbidity, and chemical concentrations can alter the relative abundances of some species. Besides these abiotic factors, biotic factors also impact species. Changes to food sources like algae, phytoplankton, and aquatic macrophytes can all affect aquatic species. Trophic interactions can also change as a result of altered predator and prey interactions and this can have a cascading effect on an ecosystem (Gallindo et al, 2021). Knowing which species of animals to use to indicate such changes is useful for conservationists.

Aquatic indicators and climate change
Even the impact of global warming can likely be evaluated using invertebrates by knowing what temperature thresholds different species have (Sunderman et al., 2022). Water temperatures influence oxygen levels so it is not surprising that changes in environmental temperature could impact invertebrates in an aquatic system. Sessile organisms such as those making up coral reefs are notable for being sensitive to environmental changes. The corals are, therefore, good as a warning sign that conditions
are worsening in an area (Carriger et al., 2021). Bleaching of corals is an indication of a deterioration in conditions including unusual changes in water temperatures.

**Marine indicators of pollution**

Macroinvertebrates in the ocean are highly sensitive to chemical pollutants and are, therefore, considered useful bioindicators (Deidda et al., 2021). Marine benthic organisms are used to show the impact of pollutants such as sewerage discharge into water (Culhane et al., 2019). A discharge of sewerage into the ocean may increase the abundance of species that do well in such conditions while decreasing the abundance of other species. There was such a change in species assemblages off the coast of Scotland due to such sewerage effluent (Culhane et al., 2019). Sudden nutrient enrichment due to effluent can also trigger algal blooms. An overgrowth of algae has cascading effects through a food web and can increase anoxic conditions making it difficult for the survival of certain species. There are few insects in the oceans, so invertebrates such as mollusks and crustaceans are used as indicators. The impact of effluent discharged into marine waters does influence communities of crustaceans, nematodes, and annelids (Andrew-Priestley et al., 2022). The abundance of these taxa along with traditional indices should be used to monitor environmental impacts in specific regions. Traditional indices include the species richness and Shannon diversity index; these are both useful measures of biodiversity. In the oceans, bivalves are valuable indicators that are sensitive to chemical pollutants; the bivalves often show a change in their endocrine system in the presence of chemicals (Fernandez, 2019). This is significant because mussels are harvested as human food, meaning that the presence of chemical pollutants could compromise the safety of this and other marine food items for human consumption since chemicals bioaccumulate and biomagnify through the food chain.

![Biomagnification in the Arctic Marine Food Web](https://commons.wikimedia.org/wiki/File:Biomagnification_in_the_Arctic_Marine_Food_Web.png)

**Figure 1:** An example of biomagnification of methylmercury in the Arctic marine food web; image is from Wikimedia commons.

**Freshwater indicator invertebrates and insects**

In freshwater systems, invertebrates have been considered good indicators of habitat quality. There are many more insects found in freshwater compared with saltwater, which is why it is not surprising that insects have been used in the past as bioindicators. An assortment of insects occurs in fresh water, including dragonflies, mayflies, stoneflies, bugs, and beetles. Many insect groups, specifically mayflies, stoneflies, and dragonflies have commonly been used as indicators of water quality. Recent studies have tried to determine if traits of invertebrates rather than individual taxa will work better to show agricultural chemicals in water bodies. The results still indicated that the taxa level is most useful (Collins and Fahrig, 2020). For instance, the bugs in the family Corixidae were associated with lower nitrate levels in the water.
Aquatic species and restoration efforts

Aquatic invertebrates, particularly, insects can be used to monitor the effectiveness of restoration efforts in wetlands and streams. For instance, a restored stream in China showed almost 50% of the taxa found in a natural undisturbed stream after about 4 years also occurred in a restored stream. Specific species of damselfly and biting midges were found to be good indicators of the restoration success (Lu et al., 2021). In other cases, crustaceans have been used along with fish, to assess restoration efforts. This was the case in the Florida everglades (Trexler and Goss, 2009). Often it takes some time before restored aquatic ecosystems show substantial recovery of species. This was the case for restored rivers and wetlands in Germany, where it was noted that taxa such as dragonflies did not immediately recover (Schulz-Zunker et al., 2022). Another idea is that more than one indicator should be used and that biotic variables along with measures of abiotic factors like water chemistry be measured to monitor the recovery of degraded habitats.

Species that make good terrestrial indicators

Pollinator species are useful as indicators of terrestrial systems. One reason is that these species indirectly indicate changes in vegetation. Some arthropods are thought of as good indicators in terrestrial systems because they are abundant enough, relatively easy to sample, and in some instances, also easy to identify to species level. It is also valuable if a species is breeding in the habitat, for instance, a leaf beetle which is also found to have larval and pupal stages. This provides more insight into the habitat and its suitability to support some types of invertebrate biodiversity. Myriapods are not thought of as useful when it comes to indicator species on land because they are not normally very abundant and may be harder to sample.

Terrestrial indicators for chemical pollutants

The quality of terrestrial habitats is also relevant and impacts biodiversity and humans. Invertebrate species’ presence, absence, and relative abundances are useful to use as evidence that conditions are deteriorating in an area. In time a decrease in biodiversity or radical change in species composition can signal problems in an area. Changes in pollinator species may impact agriculture since many crops and orchards have plants that rely on insect pollination. The decrease in bees is already a concern globally and one factor may be the use of pesticides in agriculture which could have both lethal and non-lethal, yet detrimental effects (Belsky and Joshi, 2020). Pollutants may also impact insects in ways other than reducing the numbers of individuals. For instance, the morphology and physiology of caterpillars and aphids are altered by the presence of heavy metals (Skaldina and Sorvari, 2019). The problem with pollutants is that these chemicals don’t only impact invertebrates. They end up in the tissues of vertebrates that prey on insects and other invertebrates in the environment. For example, polychlorinated biphenyls and polybrominated diphenyl ethers bioaccumulated in songbirds that were feeding on contaminated invertebrates (Wu et al., 2022).

Soil invertebrate indicators

Invertebrates living in the soil of a terrestrial habitat play a crucial role in breaking down material, recycling nutrients, and aerating the soil. When most people think of soil invertebrates they focus on earthworms, which are known to be helpful for the soil. However, there are also arthropods that are an important part of soil fauna. The mites, Acari, and springtails, Collembola are often the most numerous of the soil arthropods (Menta and Remeli, 2020). Beetles and flies can be useful as indicators of soil condition, especially where larvae occur in the soil. One of the more useful indicator groups for soils is ants. Ants that nest and live in the soil respond to changes in parameters such as humidity. The number of ant nests can even be used to show the quality of the soils (Menta and Remeli, 2020). Orthoptera are indicators of terrestrial conditions, but are better at indicating conditions in grasslands. They are sensitive to industrial pollution and often the populations are greatly reduced in such areas.

Terrestrial arthropods as indicators of forest condition

Using indicators of forest condition is important in countries in Europe where the plan is for sustainable forest management (Oettel and Lapin, 2021). Butterflies are good bioindicators for land disturbance, including for showing the degradation of forests. Species changes often occur among butterflies in response to a modified habitat (Kyeremateng et al., 2018). Species assemblages change so that the presence and relative abundances of Lepidoptera change as the landscape changes. Scientists found this in areas being mined in Ghana. Mining activity
and forest degradation resulted in an increase in savanna and open-area species of butterflies while there was a drop in typical forest species. An advantage of using butterflies as indicators is also that they are relatively easy to identify and sample. This makes it a feasible option for assessing ecological change.

**Terrestrial insects and restoration efforts**

Invertebrates can also be used to show the effects of ecological restoration efforts (Borges et al., 2021). Certain insect groups are known to be sensitive to changes and are, therefore, good indicators of deterioration but also restoration of habitat. Such groups include the Hymenoptera (bees, wasps, and ants), Coleoptera (beetles), and Lepidoptera (butterflies and moths)(Parikh et al., 2021). These insects are also important pollinators, and a reduction in the numbers of these insects may be detrimental to local vegetation. A restored habitat should show healthy biodiversity including the groups already mentioned, but also an increase in general species diversity.

**Conclusions**

Invertebrates are useful as bioindicators of habitat conditions in both aquatic and terrestrial systems. Climate change can also alter species assemblages as shown by coral reefs. Pollutants in water and in soils can alter population numbers, species diversity, and also morphology and taxonomy of indicator species. Chemical pollutants can enter the human food chain when people feed on contaminated marine invertebrates such as mussels and crabs. Similarly, freshwater fish may bioaccumulate toxins from invertebrates they feed on. Changes in terrestrial systems indirectly threaten human health and the food supply by affecting important pollinators like honey bees. Invertebrates can also be used to check on the progress of ecological restoration efforts, both in aquatic and terrestrial systems.

**References**


Conflicts of Interest

The authors state no conflict of interest.
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