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Research Trends in Animal Science

Editors:

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PREFACE

Animals have been our companions and providers for millennia, and their significance in our lives cannot be overstated. The profound impact of animals on agriculture, human health, environmental conservation, and society at large makes animal science an indispensable discipline that continuously evolves to address new challenges and opportunities. As researchers, scientists, educators, and enthusiasts, we have come together to explore the frontiers of animal science and to share our knowledge with you.

In this book, we have compiled a wide array of research topics covering diverse aspects of animal science. From animal behavior and welfare to livestock production and aquaculture, from veterinary medicine to conservation biology, our aim is to present a holistic view of the dynamic and multidisciplinary nature of this field. The chapters within are authored by esteemed experts who have dedicated their careers to advancing animal science, and we are honored to showcase their valuable contributions.

In the following pages, you will find a blend of theoretical insights, practical applications, and evidence-based solutions that address contemporary issues in animal agriculture, wildlife management, biotechnology, genetics, and more. We hope this compilation will serve as a reference for students, academics, industry professionals, policymakers, and anyone interested in understanding the latest trends and discoveries in the realm of animal science.

We extend our heartfelt gratitude to all the authors who have graciously shared their expertise, the reviewers who diligently evaluated the content, and the editorial team that worked tirelessly to bring this book to fruition. Without their dedication and passion, this endeavor would not have been possible.

In conclusion, we invite you to immerse yourself in the rich and diverse landscape of animal science presented in this book. As we continue to explore and unlock the mysteries of the animal world, may our efforts be guided by compassion, respect, and a commitment to nurturing a harmonious coexistence with all living beings.

Editors

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FISH BUSINESS – AN EMERGING TRENDS AND DEVELOPMENTS

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Abstract:

The fish industry is a crucial part of the world's food systems and is constantly evolving to meet the challenges of overfishing, climate change, and changing consumer preferences. With an increasing demand for traceable and certified seafood products, the industry is witnessing new trends that are transforming the market. According to FAO, an excess of 40 million more tonnes of seafood will be required by 2030 to meet the expanding demand. India, one of the world's top fish producers, has seen a significant increase in fish exports and domestic consumption. The Indian fish market size reached 19.0 million tons in 2022. The growth in exports, increasing per capita consumption, and the growth of organized food retail, among other factors, are driving the growth of the Indian fish market. The development of the domestic market being critical to the growth of the Indian fisheries industry. Therefore, the present article gives insight into trends in and developments in fish business.

Introduction:

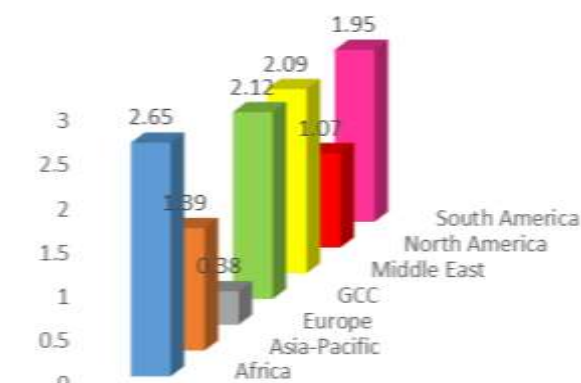
Millions of people worldwide rely on the fishing industry as a substantial supply of protein and important nutrients, making it a key part of the world's food systems. Overfishing, climate change, and changing consumer preferences are the difficulties the sector faces. The fisheries business is constantly changing to meet these problems, and new trends are emerging that are transforming the industry. For stakeholders to adjust to shifting market dynamics and ensure the industry's long-term vitality, they must understand these evolving trends. The fisheries and aquaculture business is dynamic and endlessly changing, responding to shifting demands, markets, and ecosystems, as underlined by the Food and Agriculture Organization (FAO, 2020). Moreover, the industry is witnessing an increased demand for traceable and certified seafood products, reflecting consumers' growing awareness of sustainability issues (World Economic Forum, 2021). As the world's population grows, each nation has a right to demand better management and utilization of the living resources of the sea (Bouzan *et al.*, 2005). Fish is a valuable source of protein that is essential for the human diet. However, not everyone has access to fishing, and therefore, the market serves as a crucial platform for fishermen to sell their catch.

The fish market has seen significant growth in recent times due to the increasing number of consumers, indicating the immense potential of the fish marketing business. This article emphasizes the importance of sustainable fishing practices and the need for responsible management of marine resources to ensure their availability for future generations.

Global scenario of fish business

The FAO (2020) forecasts that by 2030, an excess of 40 million more tonnes of seafood, or an increase of about 30%, will be needed to fulfil the expanding demand. The demand for seafood is likely to increase dramatically. As the increase in world population is a factor in this rising demand, it is also expected that the demand for imported seafood items from wealthy countries will increase along with rising per capita consumption (Gilbert, 2007). China is the largest producer of fish, with an annual production of approximately 63.8 million metric tons. In addition, China's expanding demand of fish in international markets and trade has increased, creating a complex supply chain for the area. China's annual per capita consumption of fish surged to 33.1 kilogrammes in 2010 and has continued to rise, according to FAO. A notable increase in aquaculture production, in particular, has allowed the nation to keep up with population demand for food while also experiencing revenue growth.

CAGR (%) has calculated for seafood market in different regions in the world. Africa has the highest CAGR of 2.65%, indicating that it has experienced the highest average annual growth rate among all the regions listed. South America has the second-highest CAGR of 1.95%, followed by GCC (Gulf Corporation Council) at 2.12%, and the Middle East at 2.09%. Europe has the lowest CAGR of 0.38%,



Source: <https://www.mordorintelligence.com/industry-reports/global-seafood-market>

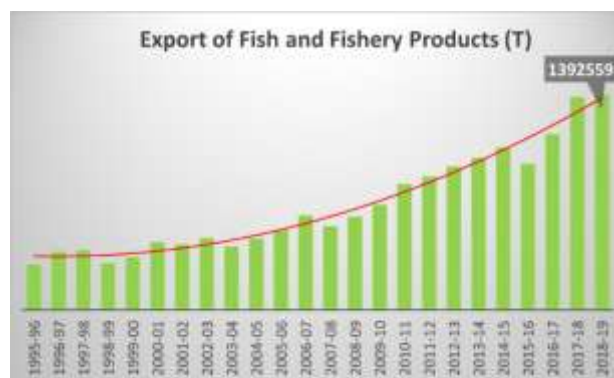
indicating that it has experienced the lowest average annual growth rate among all the regions listed. Asia-Pacific and North America also have relatively low CAGR values of 1.39% and 1.07%, respectively. These CAGR values suggest that Africa, South America, GCC, and the Middle East are regions where the investment or growth opportunities have been relatively high in recent years, while Europe, Asia-Pacific, and North America are regions where the growth opportunities have been relatively lower. However, it is important to note that CAGR values should be interpreted with caution as they do not provide a complete picture of the growth or investment opportunities in a region.

Fish business trends in India

The Indian fish market size reached 19.0 million tons in 2022 (IMRAC, 2022). As of 2021, India ranks second globally in fish production, with a total production of around 14.5 million metric tons per year. India's fish production industry is a significant contributor to the country's economy and employs over 14 million people. In India, fish exports and domestic consumption have both increased significantly in recent years. Over the past few years, there has also been a steady increase in the amount of fish consumed per person. Fish consumption is now being driven by a number of variables in India. Changes in lifestyle, rising meat prices, and the view of fish as a nutritious diet with high levels of digestible protein, PUFA, and cholesterol-lowering properties are some of these (Chhachhar and Omar, 2012).

Indian fish business: The drivers

- **Increasing per capita consumption:** The per capita consumption of fish has been continuously increasing over the past few decades. In the upcoming years, we anticipate that fish consumption will rise even more as a result of rising disposable incomes and evolving consumer preferences.
- **Growth of organized food retail:** It is anticipated that as the organised food retail market expands, customers will have greater access to processed fish products, particularly canned and frozen fish. This is expected to have a favourable effect on market expansion.
- **Increasing awareness on the health benefits of fishes:** India's market for foods that promote health and wellbeing is now expanding quickly. As was previously said, fish is regarded as a healthy diet due to its high levels of digestible protein, polyunsaturated fatty acids, and cholesterol-lowering properties. In the upcoming years, it is anticipated that rising consumer knowledge of fish as a food that promotes health and wellness would have a favourable effect on fish consumption.
- **Growth in exports:** With double digit growth rates in export values, India is also quickly becoming a major fish exporter. Currently, the nation is a major supplier of frozen fish and prawns to a number of foreign markets (Kanchana & Rajamohan, 2022). The export production of fish and fishery products started at 296,277 tonnes in 1995-96 and experienced some fluctuations before reaching its peak in 2018-19 at 1,392,559 tonnes. There were some dips and rises throughout the years, but the trend overall has been one of growth, with the



exception of some years such as 1998-99 and 2003-04. It is also worth noting that there was a significant increase in export production between 2006-07 and 2017-18, where it almost quadrupled in size (MPEDA, 2019).

Categorization of fish done by Indian fish markets:

1. According to the type of fish, the market has been divided into sections for inland fish, marine fish, shrimp and scampi. At present, inland fishes control the market and account for the largest part.
2. The market has been divided into fresh, frozen, canned, dried and other categories based on product type.
3. The market has been divided into retail and institutional segments based on end-user.
4. The market has been divided into organised and unorganised sectors based on distribution route.
5. The market has also been divided into different localities (i.e. South India, North India, North East India, East and West India) (Devi *et al.*, 2023).

Development of domestic fish business system in India

The development of the nation's fisheries industry depends heavily on the creation of domestic markets. The fact that only 15% of all fish landings are used to produce fishery products for export and the remaining 85% are sold in domestic markets shows the significance of domestic marketing. The remaining fish catch is processed, dried, smoked, converted to fishmeal, etc. to make up the remaining 30%, which is marketed fresh. With an average per capita consumption of 5-8 kg (Hand Book of Fisheries Statistics, 2022), fish is consumed by the majority of the nation's population. However, due to a shortage of transportation and the absence of suitable storage facilities, the collected fish is not evenly dispersed to interior locations. Therefore, there is a need for balanced system of distribution to make fish available in the interior areas at reasonable rates.

Through a network of wholesale, major, minor retail, roadside markets, etc., the nation's domestically produced fish, both from the marine and inland sectors, is sold. The bulk of domestic markets lack proper fish handling and storage facilities, and they are also unsanitary. A proper transportation system, with highways, refrigerated vehicles, etc., is also lacking. There is insufficient access to drinkable water, high-quality ice, energy, a proper waste disposal system, etc. The transportation of fish from the landing point to the interior markets takes a long time, resulting in poor material quality, which increases the risk of food borne illnesses, nutritional deficiencies, and post-harvest loss (Kumar *et al.*, 2008).

Rural fish business status of inland fish:

Inland fishing is done in specific places where there are water bodies in landlocked areas. These places could be dispersed across the interior, far from the towns and cities where there are consumer markets. Due of its extreme perishability, fish marketing is particularly important. It needs-



Source: <https://gudipet-fish-market.business.site/>

- i. Proper roads and quick transport facilities
- ii. Suitable container, ice, cold storage to keep fish fresh for longer time
- iii. Suitable agency (or agencies) as fishermen are poor, unorganized and cannot reach consumers.

These facilities are generally not available up to desired expectations and the fishermen face more problems than the fishermen are engaged in marine fishery. Because marine fishery is carried out in relatively concentrated or localised area where infrastructure facilities are provided in a better manner.

Recent developments in fish business

Mobile fish business

The Tamil Nadu Fisheries Development Corporation (TNFDC) sells fresh fish and seafood through a network of retail fish stands and mobile units in the cities of Chennai, Madurai, Coimbatore, Tiruppur, Pollachi, Ooty, Thoothukudi, Kanniyakumari, and Thiruvannamalai. In order to provide clients with a variety of tasty sea foods at a reasonable price, the Corporation operates 3 contemporary mobile sea food restaurants in Chennai (Marina and Neelangarai).



Source: TNFDC
<https://www.fisheries.tn.gov.in/TNFDC>

TNFDC is making efforts to raise awareness about the benefits of eating seafood for good health. For the convenience of its consumers in Chennai, TNFDC has set up an online fish marketing service named meengal.com on a trial basis. This service will eventually be expanded to other regions of Tamil Nadu.

E – fish business

Electronic fish business is the practise of marketing fish by using technology to target specific customer markets. It includes all of the actions a company does on the World Wide Web

(WWW) to draw in new customers, keep existing ones, and establish its brand. 60% of people use the internet. The country with the largest percentage of online shoppers is South Korea, where 99% of individuals with access to the Internet have done so, followed by the UK (97%) and Germany (97%). Asia's online retail sales will total US\$168.7 billion. (Alkufahy *et al.*, 2023).

E-fish businesses in India

1. **Freshtohome:** One of India's biggest online marketplaces for meat and seafood. To ensure quality and freshness, they buy their products directly from farmers and fishermen.
2. **BigBasket:** It is a well-known online grocery delivery service in India that provides a large variety of seafood selections, albeit it is not solely a fish marketing platform.
3. **SeaToHome:** This website specialises in sending consumers in India fresh fish and seafood. Fresh and frozen seafood are among the many options they provide.
4. **Fishvish:** This online marketplace focuses in delivering seafood. Fish, prawns, crabs, and other fresh and frozen seafood goods are available from them.
5. **FreshFishBasket:** This is an online seafood delivery service that operates in several cities across India. They provide a selection of fresh and frozen seafood selections and source their products directly from fisherman (Venkatrayulu *et al.*, 2023).

Conclusion:

The fisheries sector is developing rapidly worldwide, including in India, with the marketing business system evolving day by day, however, some constraints persist in rural and domestic marketing systems. Advanced technologies such as E-marketing, mobile marketing, and marketing through mobile apps are improving the marketing of fish, making it easier for fish farmers and fishermen to sell their fish, and for consumers to purchase it. To ensure success, the government should create awareness about these marketing systems among the people.

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DIVERSITY OF DISEASES CAUSING VIRUS IN FISHERIES AND AQUACULTURE

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Abstract:

Wild and farmed fish are both affected by viral infections. In rare instances, outbreaks of these illnesses in fish populations can result in such severe losses that commercial aquaculture endeavours are forced out of business or bring a specific race of sockeye salmon dangerously close to extinction. The number of reported new virus isolations and the host range of previously characterised viruses have expanded due to the significance of fish viruses to the aquaculture industry. Fish viruses are now represented in 14 of the vertebrate viral families identified by the International Committee on the Taxonomy of Viruses. Iridoviridae, Adenoviridae, and Herpesviridae are the families for fish viruses with DNA genomes, whereas Picornaviridae, Birnaviridae, Reoviridae, Rhabdoviridae, and Birnaviridae are the families for fish viruses with RNA genomes. Orthomyxoviridae, Paramyxoviridae, Caliciviridae, Togaviridae, Nodaviridae, Retroviridae, and Coronaviridae are the several types of myxovirids. The temperature of the host (8–15 °C) has allowed several of these viruses to proliferate most effectively. A handful of the viruses can infect people, cattle, pigs, and other fish species by overcoming the barrier of species. There will undoubtedly be new virus isolations, some of which will ultimately be categorised as new genera or even families, as the demand for aquacultured fish increases and the industry introduces more diverse species of fish into culture.

Keywords: Fish virus, Nervous necrosis virus

Introduction:

The aquaculture industry has seen growth in recent years. China is the largest contributor to aquaculture output, accounting for 89% of the global industry's volume and 77% of its value. China is followed by other emerging nations in the Asia-Pacific area. The production of farmed fish has greatly grown qualitatively and quantitatively in both freshwater and marine water regimes to fulfil the growing demand. Viral aquatic infections, in particular, are linked to sporadic morbidities and mortalities in their natural hosts. Viruses exhibit their ubiquitous nature and have frequently been effective in eluding non-natural hosts due to their ability to endure and

adapt to changing environmental conditions. This characteristic of virus prevalence and subsequent development has been a significant barrier to sustainable aquaculture production. The inability to detect viruses due to their latent state and the lack of understanding of viral aetiology. The following article provides in-depth analysis of some of the important viral infections that are known to impact freshwater fish in India.

1. Rhabdoviridae

Single-stranded RNA viruses called rhabdoviruses are significant fish diseases in North America, Asia, and Europe [1]. Fish are hosts to a number of rhabdovirus species including: Infectious hematopoietic necrosis virus (IHNV), Viral hemorrhagic septicemia virus (VHSV), Hiram rhabdovirus (HIRRV), Snakehead rhabdovirus (SHRV) Spring viremia of carp virus (SVCV), Pike fry rhabdovirus (PRV), Starry flounder virus, and Ulcerative disease rhabdovirus (UDRV) [2,3]. IHNV, VHSV, and SVCV are three of these viruses that need to be reported to The World Organisation for Animal Health (OIE). While SVCV mostly affects cyprinid fishes [5] and IHNV primarily affects salmon and trout [4], the range of VHSV vulnerable hosts is continuously expanding [6]. Both wild and cultivated fish are significantly affected by these viruses in terms of mortality and morbidity. As a result, numerous rhabdoviruses are extensively researched in a few species of fish, and it is becoming obvious how fish defend themselves against rhabdoviruses.

As our expertise of the teleost immune system deepens, more specific understanding of fish rhabdovirus interactions will become clearer. Salmon and trout are the main targets of fish rhabdoviruses, which are among the most dangerous viral diseases of aquacultured fish. The genomes of these viruses have been cloned, sequenced, and produced in tissue culture cells. The Infectious hematopoietic necrosis virus (IHNV), the type species for a new genus of Novirhabdovirus in the Rhabdoviridae, is now known as the salmon virus. The Viral Hemorrhagic Septicemia Virus (VHSV), Hiram rhabdovirus (HIRRV), and Snakehead rhabdovirus (SHRV) are among the additional fish rhabdovirus species included in this genus. These viruses all have negative-sense ssRNA genomes with the following physical map, starting at the 3'-end: N stands for the nucleoprotein gene, P for phosphoprotein, M stands for matrix protein, G stands for glycoprotein, NV stands for nonvirion protein, and L stands for virion RNA polymerase. The NV gene, which stands for nonvirion, is what defines the rhabdoviruses of the genus Novirhabdovirus.

The IHNV, SHRV, and VHSV NV genes have been subjected to reverse genetic study, with varying degrees of success. Deletion of the NV gene for IHNV and VHSV lowered fish pathogenicity and improved virus-induced cytopathic effect (CPE) in tissue culture cells. The

SHRV NV gene was deleted, but this had no impact on the generation of the virus, virus-induced CPE in tissue culture cells, or pathogenicity in live fish challenges. IHNV is a salmonid fish virus that has had disastrous economic effects on fish farmers in outbreaks of masou salmon (*O. masou*), sockeye salmon (*O. nerka*), Chinook salmon (*O. tshawytscha*), Atlantic salmon (*Salmo salar*), and rainbow trout (*Oncorhynchus mykiss*). The ideal growing range for this virus is between 8 and 15 °C. It has been demonstrated that VHSV kills European sea bass (*Dicentrarchus labrax*), turbot (*Scophthalmus maximus*), whitefish (*Coregonus* etc.), Pacific herring (*Clupea pallasii*), and Pacific cod (*Gadus macrocephalus*). VHSV is also a significant virus of salmonid fish. However, its host range is greater. [7].

Unlike the fish rhabdoviruses mentioned above, the spring viremia of carp virus (SVCV) lacks an intervening gene between its glycoprotein and L genes. The classification of the six genera—Lyssavirus, Vesiculovirus, Ephemerovirus, Cytorhabdovirus, Nucleorhabdovirus, and Novirhabdovirus—has been supported by phylogenetic analyses that compare aligned data for the N and G genes of members of the family Rhabdoviridae. Fish rhabdoviruses only exist in the genera Novirhabdovirus and Vesiculovirus at this time. The aquatic vesiculoviruses and the arthropod-borne vertebrate vesiculoviruses constitute a different cluster, according to analyses employing the viral P gene sequences.

2. Paramyxoviridae

In fish, a virus resembling a paramyxovirus was originally described in 1985. Tissue culture cells implanted with a cell-free homogenate of organ tissue demonstrated syncytia development while being used to examine the health of young Chinook salmon in Oregon. [8]. The infected cell line's electron micrographs revealed enveloped, pleomorphic virus particles with diameters ranging from 125 to 250 nm and a single helical nucleocapsid with a diameter of 18 nm and a length of 100 nm. In further infectivity tests with the tissue grown virus, no illness symptom was seen in fingerling trout or salmon. By using electron microscopy, a second fish paramyxovirus that induced epidermal necrosis in young black sea bream was discovered in Japan. This virus has never been in vitro grown. most recently

3. Orthomyxoviridae

The only fish orthomyxovirus that has been completely described up to this point is the infectious salmon anaemia virus (ISAV). The ISAV genome consists of eight RNA segments. The nucleocapsid protein NP, the RNA polymerase PA, the acetylcholinesterase P3 or fusion protein, the hemagglutinin, the proteins P4 and P5, the proteins P6 and P7, and the PB2 component of the virion RNA polymerase are all encoded in segments 1, 2, and 8. The membrane proteins M1 and M2 of the influenza A virus may have ISAV counterparts in the

proteins P4 and P5, while the nonstructural proteins NS1 and NEP of the influenza A virus may be connected to the proteins P6 and P7. Fish red blood cells are not agglutinated by the ISAV hemagglutinin, or mammalian red blood cells.

ISAV was designated as the type species of a new genus of viruses called Isavirus after a comparative sequence analysis of the PB1 gene of ISAV and other Orthomyxoviridae members. The fusion protein gene (segment 5) and the hemagglutinin gene (segment 7) have undergone more recent comparative analysis, and the results show that ISAV isolates can be classified into two subtypes, a North American subtype and a European subtype. Farmed Atlantic salmon that have been infected by ISAV exhibit severe anaemia, leucopenia, ascetic fluids, hemorrhagic liver necrosis, and visceral petechiae. Additionally, rainbow trout, Atlantic herring, and sea trout (*Salmo trutta*) are all affected by the virus.[9].

4. Picornaviridae

In 1988, researchers from New Brunswick, Canada, produced the first known detection of picorna-like viruses in fish, which were found in rainbow smelt (*Osmerus mordax*). Since then, picornaviruses have been discovered in salmonid fish, grouper (*Epinephelus lustauvina*), Japanese parrotfish (*Oplegnathus fasciatus*), turbot, sea bass, grass carp, and barramundi. The majority of these descriptions presumptively identified the etiologic agent as a picornavirus based on the etiologic agent's growth in tissue culture cells and the detection of tiny virus particles with size and morphology-consistent crystalline arrays in the cytoplasm. It has been determined through analysis that the purified blue gill virus is a single-stranded RNA virus. Although the viral genomes' sequences have not been characterised, some evidence. In many cases, sick fish harbour picorna-like virus particles in the brain and medulla, and the victims swim in a corkscrew pattern before passing away.

5. Nodaviridae

The genus Betanodavirus, whose type species is Striped jack nervous necrosis virus (SJNNV), contains Nodaviridae members that infect fish. These viruses have icosahedral symmetry, are nonenveloped, and have virion sizes of about 30 nm. Two positive-sense ssRNA molecules make up the viral genome. The viral polymerase is encoded in RNA1, the biggest portion of the RNA genome. The virion capsid protein is encoded by RNA2. The 75 amino acid protein that is encoded by a third RNA, which is transcribed from the 3' terminal region of RNA1, differs little from the B2 and B1 proteins that are encoded by an analogous RNA3 in the alphanodaviruses. Despite this, both the SJNNV B2 protein and the B2 protein of alphanodaviruses that infect insects have the ability to decrease RNA silencing.

A variety of cultured marine fish suffer from viral nerve necrosis, viral encephalopathy, and viral retinopathy due to the betanodaviruses. In most cases, the condition results in a necrosis and vacuolation of the brain, spinal cord, and retina in young fish. It has been reported in striped jack (*Pseudocaranx dentex*), grouper (*Epinephelus* spp.), red drum (*Sciaenops ocellatus*), guppy (*Poecilia reticulata*), barfin flounder (*Vera spermosei*), red sea bream, tiger puffer (*Takifugu rubripes*), Japanese flounder, Atlantic halibut (*Hippoglossus hippoglossus*), amberjack (*Seriola dumerili*), sea bass, and barramundi. Concerns about the disease's potential spread through the trade in aquarium fish, particularly those from Southeast Asia, have been raised in light of the recent discovery of betanodaviruses in otherwise healthy aquarium fish and invertebrates. 25 isolates' coat protein genes' comparative sequencing studies lead to the following conclusions. striped jack nervous necrosis virus (SJNNV), the red-spotted grouper nervous necrosis virus (RGNNV), the barfin flounder nervous necrosis virus (BFNNV), and the tiger puffer nervous necrosis virus (TPNNV) have four genotypic variations each.[10].

6. Nidovirales

The family Coronaviridae, which also includes the families Arteriviridae and Roniviridae, is made up of the two genera Coronavirus and Torovirus. This family is a part of the order Nidovirales. All members of the Coronaviridae are pleomorphic, enclosed virions with diameters of 126–160 nm and observable surface projections. The helical nucleocapsid contains a single linear, positive-sense ssRNA molecule. It was discovered that a common carp from Japan had coronavirus-like particles on its skin and abdomen as well as petechial haemorrhages. A similar virus has also been discovered to be present in the moribund coloured carp (*Cyprinus carpio*) with ulcerative skin lesions. The virus was cultivated by the researchers in Epithelioma papulosum cyprini (EPC) cells, and when the fish were injected with the virus produced, it led to the identical illness in carp.

7. Togaviridae

Among the viruses that infect vertebrates, the family Togaviridae includes the genera Alphavirus and Rubivirus. These viruses feature spherical, 70 nm-diameter virions with a lipid envelope comprising glycoprotein peplomers and a short-stranded RNA genome that is polyadenylated at both the 3' and 5' ends. Salmon and trout in Europe (Norway, France, the United Kingdom, and Ireland) are killed by salmonid alphaviruses (SAVs). There are at least three different subtypes of SAV: Norwegian salmonid alphavirus (NSAV/SAV-3), sleeping disease virus (SDV/SAV-2), and salmon pancreas disease virus (SPDV/SAV-1) in Atlantic salmon and rainbow trout. SAVs are thought to be a distinct and remote group within the genus

Alphavirus, according to an early investigation on the evolutionary relationships of the alphaviruses.

The first case of pancreas illness in Atlantic salmon was reported in Scotland due to SPDV (SAV-1) infection. After baby fish are transferred from freshwater aquariums to the sea, it happens throughout the first year at sea. The fish develop anorexia, swim slowly, and experience mortality rates of 10–50%. The damaged fish's histological testing revealed cardiac and skeletal myopathy as well as pancreatic acinar necrosis. SDV (SAV-2) infection in rainbow trout is distinguished by the peculiar behaviour of fish lying on their sides at the bottom of the tank. Red and white muscle degeneration is the lesion causing this behaviour. Similar histological abnormalities to those seen in SPDV infection, including progressive pancreatic necrosis and atrophy, multifocal cardiomyopathy, and skeletal muscle wasting, are present.

8. Retroviridae

The family Retroviridae consists of two subfamilies, the Orthoretrovirinae, containing six genera, and the Spumaretrovirinae, containing only one genus. The piscine retroviruses constitute the genus Epsilonretrovirus, a genus established within the Orthoretrovirinae to include the piscine retroviruses: walleye dermal sarcoma virus (WDSV), walleye epidermal hyperplasia virus type 1 (WEHV-1), walleye epidermal hyperplasia virus type 2 (WEHV-2), and snakehead retrovirus (SnRV). The genomes of all of these viruses have been sequenced. There are also numerous reports of C-type (retrovirus-like) particles of about 110–150 nm in epidermal papillomas of European smelt (*Osmerus eperlanus*) and in cells cultured from neurofibromas of damselfish (*Pomacentrus partitus*). A retrovirus has also been suggested as the etiological agent of plasmacytoid leukemia in Chinook salmon.

9. Birna virus

The genomes of Birnaviridae members contain two segments of double-stranded RNA and have single-shelled, unenveloped capsids. The Aquabirnavirus, Avibirnavirus, and Entomobirnavirus families each contain three genera. Each genus' names indicate the host specificity. The bigger genome segment A codes for a polyprotein that has the physical sequence 5'-VP2-NS-VP3-3' in the positive sense and contains the virion capsid protein VP2, an autocatalytic protease NS, and an internal capsid protein VP3. At the 5' end of RNA segment A, there is an extra 17 kDa protein that is encoded in a second reading frame and has been identified as a unique Bcl-2 family anti-apoptosis gene. The virus's RNA-dependent RNA polymerase is encoded in segment B. None of the viral mRNAs appear to have been 5'-capped[11].

10. Reoviridae

The family Reoviridae's genus Aquareovirus contains reoviruses that infect aquatic species. These reoviruses are distinguished by a nonenveloped double capsid shell, 11 segments of double-stranded RNA, and seven structural proteins. In 1979, John Plumb discovered the golden shiner virus, often known as GSRV, in golden shiners (*Notemigonus crysoleucas*). Since then, a number of reovirus-like agents have been discovered in hosts that are crustaceans, mollusks, and fish. Each one grows at temperatures that correspond to the host range and has 11 segments of dsRNA. The RNA-RNA hybridization kinetics of the aquareoviruses have been used to classify them into six groups (A-F) and many proposed species. The type species of the genus Aquareovirus, which contains striped bass, is Aquareovirus A.

11. Herpesviridae

Common goldfish (*Carassius auratus*), eels (*Anguilla* spp.), channel catfish (*Ictalurus punctatus*), masou salmon, lake trout (*S. namaycush*), sturgeon, walleye, and Japanese flounder have all been found to harbour herpesviruses.

The sole fish herpesvirus ascribed to the genus Ictalurivirus is the channel catfish virus, and this genus is not attributed to any of the three subfamilies of the family Herpesviridae—Alphaherpesvirinae, Betaherpesvirinae, or Gammaherpesvirinae. The other fish herpesviruses, cyprinid herpesviruses 1 and 2 (CyHV-1 and CyHV-2), koi herpesvirus (CyHV-3), salmonid herpesvirus 1 and 2 (SalHV-1 and -2), eel herpesvirus (*Anguilla* herpesvirus, AngHV-1), and the acipenserid or white sturgeon herpesviruses remain as unassigned members of the family Herpesviridae. Sharks, eels, pike, flounder, perch, and sharks have all been confirmed to have herpesviruses, according to electron micrographs. Grouper, and other fish.[12,13].

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NATURAL MARINE TOXINS

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Introduction:

Marine toxins are chemicals and bacteria that can contaminate certain types of seafood. Eating seafood may result in food poisoning. The seafood may look, smell, and taste normal. Bivalves and molluscs such as mussels, filter-feed on microscopic algae. About 40 of the 5,000 phytoplankton species known to date under specific circumstances about 300 of them have a high proliferation rate, resulting in high-density algae clouds called blooms. The circumstances for bloom development are not fully understood yet, but specific climatic and hydrographic conditions seem to play a role in the formation of blooms. Blooms are sometimes beneficial for aquaculture and marine biology. However, of the 300 phytoplankton species mentioned above, more than 40 species belonging to the classes of dinoflagellates and diatoms are known to produce phycotoxins (marine toxins). The abundance of these toxic phytoplankton species can vary from a thousand to a few million cells per litre. The high abundance blooms of these toxic phytoplankton species are named harmful algae blooms (HABs). It has been suggested that certain phytoplankton species produce toxins to compete for space with other phytoplankton species.

Phycotoxins can accumulate in various marine species such as fish, crabs or filter-feeding bivalves (shellfish) such as mussels, oysters, scallops and clams. In shellfish, toxins mainly accumulate in the digestive glands without causing adverse effects on the shellfish itself. However, when substantial amounts of contaminated shellfish are consumed by humans, this may cause severe intoxication in the consumer. Throughout the world, toxins produced by algae (including freshwater cyanotoxins) are held responsible for approximately 60,000 human intoxications yearly. Shellfish toxins also cause damage to wildlife and have a negative economic impact on recreation, tourism and shellfish industry.

Common types of marine toxins

Based on their chemical properties, marine shellfish toxins can be divided in two different classes: hydrophilic and lipophilic toxins. Toxins associated with the syndromes

amnesic shellfish poisoning (ASP) and paralytic shellfish poisoning (PSP) are hydrophilic and have a molecular weight (MW) below 500 Da. Toxins responsible for neurologic shellfish poisoning (NSP), diarrhetic shellfish poisoning (DSP), azaspir acid shellfish poisoning (AZP) and other toxins such as pectenotoxins, yessotoxins and cyclic imines all have as common denominator a MW above 600 Da (up to 2,000 Da). These toxins have strong lipophilic properties. Therefore, these toxins are generally called lipophilic marine toxins.

1. Paralytic shellfish poisoning

Paralytic shellfish poisoning results from ingesting bivalve molluscs (mussels, clams, oysters, scallops) that have consumed toxigenic dinoflagellates. The toxins are assimilated and temporarily stored by the shellfish. Most disease incidents involve mussels, clams, and scallops gathered and eaten by recreational collectors often from closed areas. PSP is an extremely dangerous disease that can cause death, there is reason to believe that mild cases due to consumption of marginally toxic clams by recreational diggers are never reported to health authorities or are misdiagnosed.

Symptoms are neurological and normally appear within an hour of eating toxic shellfish; in nonlethal cases, they usually subside within a few days. Symptoms include tingling, numbness, and burning of the lips and fingertips; ataxia; giddiness; staggering; drowsiness; dry throat and skin; incoherence; aphasia; rash; and fever. In severe cases, respiratory paralysis occurs, which can cause death usually during the first 24 hours, so the prognosis for recovery is good for patients surviving this period. No antidote is known, but respiratory support is given when paralysis occurs. There are no sequelae, and patients recover completely. Immunity is not conferred by a poisonous episode and multiple incidents can occur.

The cause of PSP is a complex of toxins known as saxitoxins because all can be considered forms or derivatives of saxitoxin. The 12 most commonly encountered include saxitoxin, neosaxitoxin, gonyautoxins (I, II, III, and IV), B1, B2, C1, C2, C3, and C4, which vary in their toxic effects on mice. Saxitoxin, neosaxitoxin, and gonyautoxins II and III are roughly equal in toxicity, whereas the others are somewhat weaker.

2. Neurotoxic shellfish poisoning (NSP)

Neurotoxic shellfish poisoning (sometimes referred to as brevetoxic shellfish poisoning, BSP) is caused by ingesting shellfish that have fed on the red tide organism *Gymnodinium breve* (formerly *Ptychodiscus brevis*). Red tides occur sporadically in the Gulf of Mexico and off the coast of Florida. They may be carried north in the Gulf Stream, occasionally affecting the coastline of adjacent states. The dinoflagellate blooms are easily observed as a red coloration of seawater, and the organisms can be detected microscopically. Red tides usually cause massive

fish kills, and the carcasses are washed ashore. Irritant aerosols are produced by wind and wave action, which may cause respiratory distress. Filter-feeding molluscs ingest the dinoflagellates and retain the toxin in their tissues for some time.

Symptoms resulting from the ingestion of shellfish containing brevetoxins include tingling and numbness of the lips, tongue, throat, and perioral area; muscular aches; gastrointestinal upset; and dizziness. The intoxication is usually not fatal. Onset is rapid and symptoms subside within a few hours or days at most. There is no antidote.

3. Diarrhetic shellfish poisoning (DSP)

Diarrhetic shellfish poisoning is caused by ingestion of mussels, scallops, or clams that have been feeding on *Dinophysisfortii* or *D. acuminata* and other species of *Dinophysis* and possibly *Prorocentrum*. The disease is common in Japan and has become a problem in Europe.

Symptoms include diarrhea, nausea, vomiting, and abdominal pain. Onset occurs from 30 minutes to a few hours after eating toxic shellfish, and the duration is usually short with a maximum of a few days in severe cases. The disease is not life threatening.

At least five toxins have been isolated from dinoflagellates and shellfish. Okadaic acid is most commonly encountered in Europe where *D. acuminata* is the usual agent, and mixtures of okadaic acid, dinophysistoxins, and pectenotoxins are detected in Japanese cases usually involving *D. fortii*.

4. Amnesic shellfish poisoning (ASP)

Amnesic shellfish poisoning has been proposed by Todd (1989) as a name for the syndrome caused by domoic acid. This severe disease has been identified only in a series of outbreaks in Canada in November and December 1988 involving 103 people. The toxin is present in some varieties of the diatom *Nitzschia pungens* and accumulated in mussels and clams in Atlantic Canada during a period of blooms of the diatom. Symptoms included vomiting, abdominal cramps, diarrhea, disorientation, and memory loss. Short-term memory loss was the most persistent symptom and lasted over a year in several cases. Autopsies on three fatalities showed necrosis of the hippocampus. The disease is particularly severe among older people, some of whom died in the Canadian outbreak.

Specific intoxications

1. Ciguatera toxin

Ciguatera is a clinical syndrome caused by eating the flesh of toxic fish caught in tropical reef and island waters. The toxin is believed to originate in a microscopic dinoflagellate alga *Gambierdiscus toxicus* that grows on reefs. However, other benthic algae have also been implicated. Fish eating the algae become toxic, and the effect is magnified through the food

chain so that large predatory fish become the most toxic. The occurrence of toxic fish tends to be localized, but localization is not consistent and toxic fish may occur sporadically anywhere in a reef or island location. More than 400 species have been implicated in ciguatera poisoning but the fish most commonly implicated include amberjack, snapper, grouper, barracuda, goatfish, and reef fish belonging to the Carrangidae.

The disease affects both gastrointestinal and neurological systems. Gastrointestinal symptoms, including diarrhea, nausea, vomiting, and abdominal pain, appear 3-5 hours after ingestion of the fish and are of short duration. Neurological symptoms begin 12-18 hours after consumption and may be moderate to severe; they commonly last for 1-82 days but may persist for several months. In rare cases, symptoms may last for years, with exacerbation associated with fish consumption or possibly alcohol (Halstead, 1967). Symptoms typically include hot-cold inversion (hot coffee tastes cold, ice cream tastes hot); muscular aches; tingling and numbness of lips, tongue, and perioral region; metallic taste; dryness of mouth; anxiety; prostration; dizziness; chills; sweating; dilated eyes, blurred vision, and temporary blindness. Paralysis and death may occur in a few extreme cases. Symptoms may be extremely debilitating, resulting in extended periods of disability. Intravenous mannitol may relieve acute symptoms.

2. Scombroid (Histamine) fish poisoning

Scombroid intoxication results from ingestion of fish containing high levels of free histamine. Initially, the disease was associated with consumption of scombroid fish such as tuna, mackerel, bonito, and saury. More recently, other types of fish have been identified as causing the intoxication, including mahimahi, bluefish, jack, mackerel, amberjack, herring, sardine, and anchovy. Scombroid fish poisoning has been caused dominantly by mahimahi, tuna, and bluefish.

The disease is correctly described as histamine poisoning, it includes gastrointestinal, neurological, hemodynamic, and cutaneous symptoms such as nausea, vomiting, diarrhea, cramping, headache, palpitations, flushing, tingling, burning, itching, hypotension, rash, urticaria, edema, and localized inflammation. The most frequent symptoms are tingling and burning sensations around the mouth ("peppery tasting"), gastrointestinal complaints, and a rash with itching. The illness is generally mild and self-resolving, with rapid onset of symptoms and duration of only a few hours. Normally, treatment is unnecessary but antihistamine drugs will provide relief.

The histamine is produced in the fish flesh by decarboxylation of free histidine, which is naturally present at high levels in species of fish implicated in scombroid fish poisoning. The production of histamine is due to the action of histidine decarboxylase, an enzyme produced by

bacteria growing on the fish. The Food and Drug Administration considers a level of 20 milligrams (mg) of histamine per 100 grams (g) of flesh, or 200 parts per million (ppm), an indication of spoilage in tuna and 50 mg/100 g (500 ppm) an indication of hazard. Fish histamine poisoning is preventable by proper handling of fish at the time of capture and during subsequent storage, processing, and distribution.

3. Puffer fish poisoning (PFP)

Puffer fish poisoning results from ingestion of the flesh of certain species of fish belonging to the Tetraodontidae. The toxin involved is called tetrodotoxin and was originally believed to be a true ichthyosarcotoxin produced by the fish itself. The toxicity of poisonous puffers fluctuates greatly. Recent observations that cultured puffer fish are a toxin has supported a food chain origin for the toxin, but this has not yet been confirmed. It has recently been shown, however, that certain common marine vibrios can produce a form of the toxin and because vibrios occur as part of the microflora of puffer fish, they may be implicated in toxicity development.

The symptoms of puffer fish poisoning are similar to those described for paralytic shellfish poisoning, including initial tingling and numbness of lips, tongue, and fingers leading to paralysis of the extremities; ataxia; difficulty in speaking; and finally, death by asphyxiation due to respiratory paralysis. Nausea and vomiting are common early symptoms. The similarity in symptoms is not surprising because tetrodotoxin, although chemically different from the saxitoxins, also blocks sodium channels. No antidote has been identified for tetrodotoxin and treatment is supportive. The toxicity of tetrodotoxin is similar to that of saxitoxin, and 1-4 mg constitutes a lethal dose for humans.

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ANIMAL COGNITION

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Abstract:

In the field of comparative cognition research, the focus is on understanding which animal behaviours involve cognitive abilities and the cognitive mechanisms that enable those behaviours. Key inquiries include determining the types of mental representations animals require to solve specific tasks, such as whether they possess mental maps, metacognition, or number concepts. Additionally, researchers investigate how animals learn, exploring whether they rely solely on associative learning or also utilize trial-and-error, play, insight, or social imitation. The process by which animals solve problems is also examined, including their engagement in logical reasoning, causal reasoning, and future planning. Philosophers contribute to these discussions by raising their own questions, such as whether animals have beliefs, which animals possess consciousness, and exploring the relationship between language and thought. They also explore the rationality of animals. To address any of these inquiries, a comprehensive approach is necessary, involving both scientific investigation into the phenomenon and conceptual analysis of the underlying psychological properties.

Keywords: Cognition, Behavior, Social imitation

Introduction:

The field of animal cognition is intricate and multifaceted. Prior to the 1960s, the term "animal cognition" was considered contradictory, as animals were perceived as simple organisms that merely reacted to stimuli in predetermined and unchanging ways shaped by evolution. However, as researchers started closely observing animals both in controlled environments and their natural habitats, they realized that this simplistic perspective fell short in explaining the observed behavioural patterns. Nowadays, while there are numerous interpretations of animal cognition among researchers, there is a general consensus that animal cognition, akin to human cognition, primarily involves the processing of information. This entails how individuals, utilizing their species-specific perceptual systems (such as auditory, visual, olfactory, gustatory, and somatosensory), receive information from their environment, including information from

other members of their species. Furthermore, employing their species-specific neurobiology, they employ their brains to process and respond to that information.

The field of animal cognition can be divided into several subtopics. For instance, researchers specializing in comparative psychology focus on examining specific cognitive abilities, such as numerical competence, across different species. On the other hand, those specializing in cognitive ethology investigate how the evolutionary characteristics of a particular species enable it to interact with various aspects of its environment. Many researchers undertake studies that encompass multiple subtopics, as they involve different aspects like attention, categorization, memory, reasoning, and problem-solving. Sometimes, answering scientific inquiries requires combining laboratory experiments with field research. In all cases, scientists conduct carefully controlled experiments or well-designed observational studies. Over the past few decades, the study of animal cognition has expanded extensively, encompassing an impressive range of species, from insects to our closest relatives, nonhuman primates, and employing a wide array of research techniques.

The challenge of explaining how animals adapt to an increasingly urbanized world is a significant task for evolutionary biologists. Urban environments present animals with new and distinct challenges that differ from those encountered in their evolutionary history. To navigate these rapidly changing habitats successfully, animals may need to adjust their behaviour in a flexible manner within short timeframes. These behavioural adaptations are likely supported by the animals' ability to gather, store, and process information from their surroundings. Consequently, there is a growing interest among researchers in understanding how cognitive abilities enable animals to navigate threats, exploit resources, or potentially limit their ability to do so. Numerous studies are now investigating the cognitive and behavioural differences between animals living in urban areas and those in non-urban settings. This review primarily focuses on birds, as they have been the primary subjects of previous research. However, relevant work in other species is also discussed. The review explores the informational challenges faced by animals in urban environments and examines how different cognitive abilities can aid in overcoming these challenges. Additionally, the review addresses the potential consequences of cognitive variation at both the individual and species levels. It raises questions such as whether urban environments select for or influence the development of specific cognitive abilities, whether individuals or species with certain cognitive traits are more likely to thrive in urban habitats, and how social behaviour and individual personality interact with cognition to influence behaviour in urban settings. The ultimate objective of this review is to consolidate existing

knowledge and identify important directions for future research, aiming to enhance our understanding of the ecological and evolutionary implications of urbanization.

Cognitive mechanism:

A noteworthy example of a cognitive mechanism can be observed in crows (*Corvus brachyrhynchos*) that crack walnuts by dropping them from heights of 5 to 10 meters or more onto hard surfaces like rocks, roads, or sidewalks. Interestingly, these birds display the ability to differentiate between black and English walnuts, dropping the harder black walnuts from greater heights. Furthermore, when dropping a nut, a crow takes into consideration the likelihood of other crows stealing the contents before it can retrieve them. If fewer competing crows are present nearby, the crow carries the nut higher into the air before releasing it. Hence, the nut-cracking behaviour of crows involves numerous cognitive processes such as perception, learning, and decision-making.

Each of these processes can be further examined. For instance, how do crows assess the appropriate height from which to drop the nuts? Do they learn to adjust the dropping height based on the specific type of walnut? In situations where a crow encounters the conflicting conditions of possessing a hard-shelled black walnut while observing several other crows nearby, how do they determine the optimal drop height to employ?

Considerable recent research documents many animals' ability to remember the past, to respond effectively in the present, and to plan for the future. Research also suggests that animals may be able to take into account their current state of knowledge to control their own behavior in an adaptive way. Finally, animals can master numerical and abstract concepts, perform basic arithmetic operations, and even exhibit behaviors which suggest that they possess the roots of analogical reasoning. Dumb beasts? Hardly! Animals of many different species are sensitive to the rich mosaic of events and relationships that are woven into the causal fabric of the environment. How could it be otherwise? Animals evolved under most of the same constraints and contingencies as the human species. To study animal cognition is to study the mechanisms and functions of cognition without the complexities of language or the biases of anthropomorphism. Doing so not only enriches our understanding of cognition in animals, but it also places human cognition into a more complete evolutionary perspective.

Conclusion:

Attention is a crucial cognitive ability that allows us to effectively process essential information from our surroundings. This information is typically processed through our senses, stored memories, and other cognitive processes. Cognitive skills play a significant role in an individual's overall development as they encompass essential functions of the brain, such as

thinking, reading, learning, retaining information, and paying attention. These skills are utilized to solve problems, remember tasks, and make decisions, ultimately influencing the quality of our learning and performance.

Among these cognitive skills, attention stands out as one of the most vital. It enables us to selectively focus on relevant information and efficiently process it. Conversely, a lack of attention hampers and diminishes our information processing systems, leading to difficulties in effectively utilizing and responding to the available information.

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AN INSIGHT TOWARDS BEHAVIOUR AND COMMUNICATION OF ANIMALS

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Abstract:

This captivating chapter explores the diverse realms of animal science, delving into key subtopics that offer profound insights into the intricacies of the animal kingdom. Ethology unravels evolutionary adaptations, social structures, and survival strategies shaping animal behaviour. Animal language and communication unveil the intricate systems fostering social bonds and ensuring species survival.

An understanding of animal nutrition illuminates physiological well-being and ecological roles, while the exploration of locomotion reveals remarkable adaptability in traversing environments. Genetics presents the intriguing domain of epigenetics and gene conservation, influencing phenotypic variations and safeguarding biodiversity.

Embracing the future, animal robotics emerges as a promising frontier, integrating biology and cutting-edge technology for research, conservation, and human-technology interactions. Finally, ecology provides profound insights into animal-environment interactions, emphasizing the need for biodiversity preservation. This chapter offers a comprehensive understanding of the complexities and significance of the animal kingdom, inspiring appreciation for the wonders of life on Earth.

Keywords: Animal science, ethology, robotics, nutrition, locomotion, epigenetics, gene conservation, communication, language, ecology.

Introduction:

Animal science is an interdisciplinary field that centers on the study of animals, encompassing their biology, ethology, genomics, nutrition, physiology, reproductive biology, and husbandry. It pertains to diverse facets concerning both domesticated and wild species, as well as certain aspects of zoonotic and human-animal interconnections. Animal science plays a crucial role in promoting sustainable and responsible practices in agriculture, animal husbandry, and wildlife conservation. It contributes significantly to enhancing animal welfare, ensuring food security, and advancing our understanding of the complex interactions between humans and animals within the broader ecosystem. The knowledge acquired from animal science research aids in maintaining the health and productivity of animals, which is essential for supporting

various industries that depend on animals for food, fiber, research, and companionship. The following chapter discusses animal science as an emerging field that addresses new challenges and opportunities. While rooted in traditional methods, it undergoes rejuvenation through the advancements in technology. In the modern era, with a growing global population, the demand for food production is escalating. Animal science offers various solutions to this issue, including the safe modification of animal genes to enhance yields without causing harm to the animals. Improved breeding techniques are being developed and employed, and the preservation of endangered species is also a key focus. This broad topic encompasses several fields and holds immense potential for addressing critical issues in agriculture, conservation, and sustainable resource management.

The topics covered here mainly revolve around animal ethology, ecology, communication and language, nutrition, locomotion, genetics, and robotics. The study of ethology, or animal behavior, is essential as it provides valuable insights into their patterns, responses to sensory input, and reactions to stimuli. This understanding helps us comprehend their interactions with the environment and determine the optimal conditions to cater to their needs. Animal ecology primarily focuses on studying the interactions of animals with each other and their environment and ecosystem. These studies provide valuable knowledge about their habitats, aiding us in understanding their breeding and grazing behaviours. As for animal communication and language encompass topics related to an animal's sociology, behaviour, and cognition. Understanding these aspects provides valuable insights into their emotional stability, mind-set, sexual practices, and responses to external factors. Animal nutrition plays a crucial role in breeding practices, ensuring the health and welfare of animals, and promoting the sustainability of livestock production. Genetics play an essential role here, as conservation genetics and epigenetics provide solutions to the challenges concerning the extinction of animals that are emerging nowadays.

Ethology

Animal behavior, scientifically known as ethology, refers to the observable actions and responses displayed by animals when they are exposed to external or internal stimuli. These stimuli can encompass a wide range of factors, including environmental cues, social interactions, sensory inputs, and physiological changes. The study of animal behavior involves systematic and empirical observation, experimentation, and data analysis to understand the complexities and intricacies of behavioral patterns exhibited by different species. Ethologists and researchers in this field employ rigorous scientific methodologies to investigate how animals perceive and interpret stimuli and how these perceptions lead to specific behavioral responses. Animal behavior is a product of both innate and learned processes, influenced by genetics, evolution, and

the animal's individual experiences. Consequently, the behavioral repertoire of animals can vary significantly based on the type, intensity, and context of the stimuli they encounter. The complex nature of animal behaviour arises from the interplay of various factors, including neural mechanisms, hormonal regulation, cognitive processes, and social dynamics. Studying animal behaviour requires interdisciplinary approaches, integrating principles from biology, neuroscience, psychology, ecology, and other related fields. The scientific study of animal behaviour has far-reaching implications, including insights into animal communication, mating strategies, foraging behaviours, territoriality, and responses to environmental changes. Additionally, understanding animal behaviour is essential for wildlife conservation efforts, managing human-animal interactions, and improving the welfare and husbandry of domesticated animals. In summary, ethology is a scientific discipline that seeks to unravel the intricate mechanisms underlying animal behaviour, contributing to our knowledge of the natural world and fostering harmonious coexistence between humans and other members of the animal kingdom.

Ethology, or animal behaviour, is the scientific study of the innate and learned course of actions that animals perform in response to various stimuli. It involves observing and analysing various behavioral patterns exhibited by animals, which can vary depending on the type and intensity of stimuli they encounter. Studying animal behaviour is a multifaceted and intricate undertaking due to the complexity and diversity of behavioral responses observed across species. The field of applied animal behaviour science encompasses a comprehensive integration of approaches from ethology, psychology, and neuroscience to investigate and address animal behaviour in practical contexts. Through rigorous scientific methodologies, researchers in this discipline seek to gain a deeper understanding of the behaviour of animals in human environments and enhance their welfare and well-being. The emergence of applied animal behaviour science saw significant advancements during the 1950s, largely influenced by pioneering researchers like David Wood-Gush. During this era, the focus of study primarily centred around the behaviour of domesticated animals, exploring their behavioral adaptability to human settings and interactions. However, it is noteworthy that the emphasis during the Wood-Gush era was more oriented towards the scientific investigation of animal behaviour in domestic animals rather than directly addressing broader animal welfare concerns. (Lawrence, 2008)

Clinical animal behaviour is the systematic handling of problematic animal behaviour and established itself as a distinct scientific field approximately half a century ago. (Mills, 10 November 2022). Animal behaviour is intricately shaped by their interactions with con-specifics (members of the same species) and their environment. Research conducted thus far has identified various types of animal behaviour, including instinctual responses, imprinting, and imitation.

These behavioral phenomena can be scientifically elucidated by examining the process of stimulus reception, subsequent neural and muscular reactions, and the orchestration of cellular and tissue movements that lead to the execution of specific actions. The sensory system, a crucial component, plays a pivotal role in mediating these behavioral responses. This ethological study plays a crucial role in investigating the phenotypic adaptations of animals, which subsequently contribute to the study of natural selection and evolutionary processes. By studying this, including animal behavioral responses, we gain a more comprehensive understanding of species-specific behaviors, social structures, and ecological dynamics.

Communication and language

Animal communication encompasses a multifaceted field investigating the diverse modalities through which species interact, including vocalizations, chemical signals, and intricate body language. Fulfilling crucial functions such as risk assessment, mate attraction, and social coordination, these communication systems are honed by evolutionary processes to ensure survival and reproductive success. Some species display language-like traits, exemplified by honeybees' dance language and great apes' gestural communication. Understanding animal communication provides profound insights into cognitive faculties and behavioral propensities, enabling more effective cross-species interactions and fostering a deeper appreciation of the intricacies of the natural world. This knowledge bears implications for ecological dynamics, wildlife conservation, and the preservation of biodiversity. Moreover, the study of animal communication enhances our understanding of evolutionary pathways and the fascinating diversity of life on Earth.



Figure 1: Visual communication by a dog (credit:udemy)

This chapter serves as a comprehensive exploration of animal communication, focusing on the underlying mechanisms, the diversity of languages observed, and the potential implications for our understanding of animal cognition and human evolution. Animal communication encompasses various modalities through which animals convey information and interact with each other. Animal communication encompasses various modalities through which animals convey information to one another, serving critical functions in their social interactions

and survival strategies. Vocal communication involves the production of sounds like calls, songs, or vocalizations, used for purposes such as territorial defence, mating calls, and group coordination. Bird songs, wolf howls, and lion roars are notable examples of this form of communication. Visual communication relies on body postures, gestures, facial expressions, and displays to convey information related to dominance, aggression, courtship, or warnings. Bird courtship dances, primate postures, and peacock displays are fascinating instances of visual communication in the animal kingdom. Chemical communication involves the use of pheromones, chemical signals emitted by animals, which influence behaviour and physiological states. Pheromones play crucial roles in marking territories, attracting mates, signaling alarm, and coordinating social behaviours among species like ants using pheromone trails or female moths emitting sex pheromones. Tactile communication involves touch or physical contact and serves to build social bonds, convey comfort, express submission, or communicate aggression. This form of communication is exemplified by primate grooming rituals and elephants using physical contact for affiliation and reassurance. Lastly, some aquatic species employ electrical communication, where weak electric fields convey information about location, identity, and social interactions. Electric fish, such as electric eels or rays, utilize electrical signals for navigation, prey detection, and communication within their species. The diversity of communication methods across the animal kingdom showcases the complexity of their interactions and the significance of these forms of communication in facilitating successful cooperation, mating, and survival strategies among various species (Dash, 2017).

Locomotion

Locomotion, derived from the Latin word "locus" meaning place or location, and "motion" referring to movement, encompasses a series of coordinated events in animals that lead to their displacement from one location to another. The musculoskeletal system and the nervous system play pivotal roles in orchestrating locomotion. The propulsive force is generated through muscular contraction, while the nervous system controls and coordinates the movements in a rhythmic manner. The locomotion differs from animal to animal.

Locomotion involves a pattern of coordination and rhythmic control of an animal's body. It can generally be classified into four types, including aerial, aquatic, fossorial, and terrestrial locomotion. Aerial locomotion is primarily observed in birds equipped with wings and air sacs, while aquatic locomotion is carried out by animals living in aquatic environments. Fossorial locomotion involves underground movement and is exhibited by a limited number of animals. The most common form of locomotion is terrestrial locomotion, observed in a wide range of animals living on land. Different species perform these types of locomotion based on the specific environmental conditions they inhabit, and all of these movements are executed through the

coordination of the musculoskeletal and nervous systems. The initiation of locomotion necessitates an external stimulus. This stimulus triggers the transmission of signals to the nervous system, setting in motion some internal responses that culminate in observable movement.

Throughout locomotion, the skeletal musculature plays a crucial role, undertaking essential functions like stabilization, acceleration, and deceleration. The myofilaments, integral components of muscle fibers, are responsible for generating movement, facilitating the coordination and execution of diverse loco-motor activities (Lindstedt, 2013).

Territorial animals typically exhibit locomotion through either bipedalism, using two limbs (hands or legs) for movement, or quadrupedalism, using all four limbs. Bipedalism involves the ability to walk or run on two limbs, which is commonly observed in certain primates, such as humans and some apes. Quadrupedalism, on the other hand, refers to movement using all four limbs, which is widespread among various animal groups, including mammals like dogs, lions, and horses. These locomotion strategies are often influenced by the animal's anatomical adaptations. Bipedalism allows for enhanced agility, better visibility, and freeing of hands for tool use or other tasks. Quadrupedalism provides stability, increased speed, and efficient weight distribution. By adopting these distinct locomotion patterns, territorial animals optimize their movement and energy expenditure to defend their territories effectively and navigate their environments with varying degrees of adaptability and efficiency.

Thus, locomotion, a fundamental aspect of motility, is observed across a spectrum of organisms, from prokaryotes to eukaryotes. This dynamic movement is extensively examined in the discipline of animal science, as it provides valuable insights into an animal's behaviour, physiology, and welfare. Understanding locomotion aids in optimizing animal husbandry practices, ensuring their well-being, and fostering responsible care in various contexts.

Nutrition

Animal nutrition refers to the science and study of the dietary requirements, intake, digestion, absorption, and utilization of nutrients by animals for growth, development, reproduction, and overall well-being. It involves understanding the specific nutritional needs of different animal species and formulating appropriate diets to meet those requirements. Proper animal nutrition is essential for maintaining optimal health, maximizing productivity, and ensuring sustainable agricultural practices in various sectors, including livestock farming, aquaculture, and pet care. The field of animal nutrition encompasses the study of essential nutrients, such as proteins, carbohydrates, fats, vitamins, minerals, and water, and their roles in supporting various physiological functions and metabolic processes in animals.



Figure 2: Types of heterotrophs (credits:teachoo)

Animals require essential nutrients to maintain their health and well-being. Among these nutrients are vitamins, which play vital roles in supporting various biochemical processes, immune function, and overall health. Additionally, minerals are necessary for maintaining bone health, fluid balance, nerve function, and enzyme activity in animals. However, perhaps the most fundamental nutrient for animals is water, which is essential for temperature regulation, nutrient transport, and waste removal, making it a crucial component of their daily survival and physiological processes. Together, these essential nutrients ensure the proper functioning and vitality of animals in their respective environments.

Animal nutrition research encompasses various characteristics crucial for optimizing animal health, welfare, and sustainable production in agriculture. Protein quality and digestibility have been extensively studied, revealing their profound impact on animal growth and development. High-quality protein sources with balanced amino acid profiles are essential for improving feed efficiency and reducing nitrogen excretion, contributing to sustainable animal agriculture. Micro nutrient supplementation, including vitamins and minerals, plays a critical role in physiological processes, necessitating appropriate fortification in animal diets to prevent nutritional deficiencies and enhance overall performance. Sustainable production practices have been a focus of research, with the development of eco-friendly feeding strategies aiming to minimize the environmental impact of animal agriculture, including greenhouse gas emissions and resource utilization optimization. Exploring alternative feed ingredients, such as insect-based feeds and algae-based protein sources, offers potential for reducing reliance on traditional feed sources and promoting sustainable practices. Moreover, research delves into understanding the specific nutritional needs of various animal species and production stages, ensuring their well-being and productivity. The emerging field of gut health and microbiota research examines the influence of nutrition on animals' gut microbial communities, offering opportunities to improve animal health and nutrient utilization. Advancements in precision feeding and nutrigenomics have allowed personalized dietary approaches, considering genetic and environmental factors

that influence an animal's response to specific nutrients, leading to more targeted and efficient feeding practices. By continuously exploring and integrating these characteristics, animal nutrition research strives to enhance animal welfare, optimize production practices, and support sustainable agriculture for a healthier and more sustainable future (Shendure, 2008).

Ecology

Animal ecology is a multifaceted field that encompasses the study of population dynamics, community ecology, behavioral ecology, trophic interactions, landscape ecology, and conservation. It delves into the intricate relationships between animals and their environment, examining factors such as population size, species interactions, resource utilization, and ecosystem functioning. For instance, in the African savanna, a dynamic interplay occurs between predators like lions and cheetahs and their prey such as zebras and antelopes. This predator-prey interaction not only influences population sizes but also drives adaptations in both predators and prey, shaping community dynamics. Additionally, mutualistic relationships are exemplified in coral reef ecosystems, where cleaner fish and client fish engage in symbiotic interactions. The cleaner fish removes parasites from the client fish, benefiting both parties. This intricate dance of mutualism highlights the inter-dependency of species within ecosystems.

In behavioral ecology, researchers study how animals adapt their behaviour in response to ecological factors. For example, in bird populations, individuals may adjust their foraging strategies and breeding behaviour in accordance with resource availability and environmental conditions. These adaptations optimize their reproductive success and survival. Furthermore, trophic interactions, which encompass food webs and energy flow, play a crucial role in ecosystem functioning. Primary producers, such as plants, capture energy from the sun and convert it into biomass. This energy is then transferred through various trophic levels as consumers feed on other organisms. Decomposers, such as fungi and bacteria, break down organic matter and recycle nutrients back into the ecosystem. Satellite tracking data and climate modelling can be used to assess shifts in migration routes and timing. The studies revealed that rising temperatures have caused alterations in the timing of bird migration, with some species arriving at breeding grounds earlier or delaying their departure for wintering areas. These changes could have significant consequences for bird populations, breeding success, and interactions with other species in their ecosystem (Brown, 2016).

Landscape ecology examines the spatial patterns and processes of habitats, investigating how habitat fragmentation, connectivity, and land-use changes impact animal populations and their movements. By understanding the effects of landscape structure on animal dispersal and gene flow, scientists can contribute to conservation strategies that maintain ecological connectivity and preserve biodiversity.

Overall, the study of animal ecology provides valuable insights into the intricate dynamics of populations, communities, and ecosystems. It aids in our understanding of how animals interact with their environment, adapt to ecological pressures, and shape the functioning of natural systems. This knowledge is crucial for effective conservation and management practices to sustain the Earth's biodiversity and ensure the health and resilience of ecosystems.

Conserving genetics

Conservation genetics, an integral component of animal science focusing on animal breeding, encompasses two primary facets: safeguarding endangered animal species and enhancing breeding outcomes. Protecting endangered species is a pressing necessity, given their limited numbers, and the potential consequences of their declining populations. In the realm of animal breeding, improving methods is imperative to achieve both qualitative and quantitative enhancements in production. Utilizing genetic techniques allows for advancements in animal health and productivity, ultimately resulting in improved overall yield. In genetic conservation, two methods are employed: in situ and ex situ conservation approaches, aiming to preserve and sustain the species in their natural habitats or controlled environments, respectively. These approaches aid in securing the genetic diversity and long-term survival of endangered species.

Conservation genetics focuses on factors responsible for extinction and endangerment of rare animals. Absolutely, conservation genetics utilizes molecular tools and concepts of population genetics to address the challenges arising from the endangerment of species. The application of techniques such as DNA fingerprinting, assessing the extent of genetic variation, and studying population structure aids in resolving issues related to endangered species. These methods facilitate the identification of unique alleles and alleles under selection, thus aiding in developing effective conservation strategies and mitigating inbreeding depression. Conservation genetics helps to preserve the genetic health and evolutionary potential of endangered species populations by analyzing genetic diversity and gene flow patterns. Conservation genetics employs powerful tools like pedigree analysis, peeling, and gene dropping to assess and manage genetic diversity and health within captive populations of endangered species. The focus of conservation genetics lies in understanding the genetic factors that influence population dynamics, adaptation, and long-term survival, especially for species facing threats in their natural habitats. Pedigree analysis involves examining genetic relationships among individuals in captivity, constructing family trees, and identifying potential breeding pairs that maintain genetic diversity and reduce the risk of inbreeding depression caused by closely related mating.

Peeling, a more detailed approach, traces the ancestry of individuals to identify influential ancestors who significantly contributed to the current genetic makeup of the population. By using peeling, conservation geneticists can pinpoint crucial founders carrying unique genetic

variations. Emphasizing breeding of these genetically valuable individuals helps maintain species' genetic diversity and increases the likelihood of producing offspring with advantageous traits, enhancing the species' resilience to environmental changes.

Gene dropping, or Monte Carlo simulations, is a computational method used to predict genetic outcomes of different breeding scenarios. By randomly simulating the inheritance of genetic material from known ancestors, gene dropping models the genetic diversity of future populations. Conservation geneticists use these simulations to explore various breeding strategies and identify the most effective ways to maximize genetic diversity and minimize the risk of inbreeding depression within captive populations (Miller, 1992).

In summary, conservation genetics employs pedigree analysis, peeling, and gene dropping as critical tools to manage captive populations of endangered species. Through understanding genetic relatedness, diversity, and the risks of inbreeding, conservationists can make informed decisions to ensure the long-term survival and health of these species. By strategically managing genetic resources within captive populations, conservationists contribute to the broader goal of preserving biodiversity and supporting the eventual reintroduction and recovery of these species in their natural habitats.

Epigenetics:

Epigenetics is an emerging and interdisciplinary field in animal science that encompasses genetics, livestock nutrition, and animal breeding. It involves modifications to gene activity without altering the underlying DNA sequence. The study of epigenetics allows us to gain insights into how behaviours and environmental factors influence gene function. Notably, epigenetics changes can be reversible since they do not involve altering the DNA sequence itself; this offers the possibility of nullification. An essential aspect to consider is that while only gene activity is altered, it impacts how the body interprets the DNA sequence. DNA methylation is the most prevalent epigenetic mechanism observed in animals. The study of epigenetics holds great promise in advancing our understanding of animal biology and the intricate interplay between genes and the environment. By exploring these epigenetic processes, scientists can uncover the underlying molecular mechanisms that regulate gene expression and potentially influence various aspects of animal health, behaviour, and performance. As this field continues to evolve, it will likely contribute significantly to improving livestock breeding strategies, enhancing animal nutrition, and addressing various challenges in animal science and agriculture.

Numerous investigations have provided compelling evidence linking environmental exposures and the aging process to enduring epigenetic alterations that impact the phenotype. These studies have highlighted the remarkable role of epigenetics in mediating the effects of environmental factors and age-related changes on the long-term expression of genes, ultimately

influencing observable traits and characteristics. Also The stipulation that epigenetic traits must be heritable during mitosis or meiosis possesses the advantage of precision but can also be a limitation.

There are two main epigenetic systems: the Polycomb/Trithorax systems and DNA methylation. These systems play important roles in controlling the activity of specific genes during development. The Polycomb and Trithorax systems are named after fruit fly mutants and work to either keep certain genes turned off (Polycomb) or turned on (Trithorax) during development. Without these systems, the correct gene expression patterns in different segments of the fruit fly cannot be maintained. This suggests that the Polycomb/Trithorax systems "remember" and maintain gene-expression patterns established by other cellular mechanisms. There is even evidence that this gene silencing can be passed down to the next generation in fruit flies, although at a low frequency. DNA methylation is another epigenetic mechanism observed in vertebrates. It involves adding a chemical modification to specific sequences of DNA, particularly the CG sequence. These modified sequences can influence gene activity. After DNA replication, the newly synthesized DNA strand is temporarily "unmethylated" on one side (hemimethylated), but it is later completed with methylation by a DNA methyltransferase called DNMT1. This copying process helps maintain the methylation pattern through cell generations. These are crucial for regulating gene activity during development and play a role in passing on specific gene expression patterns to future generations. These mechanisms add a layer of complexity to understanding how genes are controlled and how cells remember and pass on information about which genes should be active or inactive (Bird, 2007).

Robotics

Animal robotics, a captivating interdisciplinary field at the intersection of biology and robotics, aims to create machines inspired by the remarkable capabilities of animals. By closely studying the behaviour, structure, and functionalities of diverse species, researchers develop robotic systems that mimic the form and function of animals. These bio-inspired robots exhibit animal-like locomotion, sensory abilities, and behaviour, providing valuable insights into the principles underlying animal physiology and behaviour. By integrating advanced technologies such as artificial intelligence, computer vision, and sensor integration, animal robots can perform complex tasks in domains like search and rescue operations, environmental monitoring, and exploration of hazardous environments. Furthermore, animal robotics contributes to the development of prosthetics and assistive devices for humans, leveraging knowledge of animal locomotion and sensory systems to create more natural and efficient robotic limbs and exoskeletons, improving mobility and enhancing the quality of life for individuals with impairments. With its potential for advancing scientific understanding and enhancing practical

applications, animal robotics holds promise for a future where machines seamlessly interact with the natural world.

Animal robotics relies on the principles of biomimicry, artificial intelligence (AI), computer vision, sensor integration, actuation systems, bio-inspired materials, and human-robot interaction to create machines that closely resemble and replicate the unique capabilities of animals. Researchers study the biological systems and behaviors of animals to mimic their structures, movements, and sensory abilities in robotic designs. AI enables robots to perceive, reason, and make intelligent decisions, while computer vision provides visual perception capabilities. Various sensors, including vision, auditory, tactile, and chemical, allow robots to gather information from their environment. Actuation systems are designed based on animal locomotion to facilitate complex and agile movements. Bio-inspired materials mimic animal tissues or structures for enhanced functionalities. Moreover, human-robot interaction technologies like natural language processing and haptic feedback enable seamless communication and collaboration between humans and animal robots. This integration of technologies in animal robotics opens up possibilities for advancements in locomotion, perception, and intelligent behavior, as well as practical applications in search and rescue, environmental monitoring, and human assistance (RobotYinquan Zeng, 2018).

Animal-inspired robots offer several distinct advantages across various domains. First and foremost, their bio-mimetic design allows for more natural interactions with humans and other living organisms, fostering improved collaboration and integration in different settings. This natural interaction enhances the acceptance and ease of working with these robots in diverse fields. One of the significant applications of animal robotics lies in environmental monitoring. These robots can be deployed in delicate ecosystems or hazardous environments where human presence might disturb wildlife. By mimicking the behaviours of animals, they can collect valuable data for environmental conservation and research, aiding in the understanding and preservation of our planet's ecosystems. In search and rescue operations, animal-inspired robots prove to be invaluable assets. Their ability to traverse challenging terrains and replicate animal behaviours enables them to access areas that traditional robots may struggle to reach. These robots can assist in locating survivors in disaster-stricken regions, potentially saving lives in critical situations. Moreover, animal robotics fosters bio-inspired innovation. By studying animal behaviors and structures, researchers and engineers can develop creative and groundbreaking robotic designs. This cross-disciplinary approach has the potential to revolutionize various industries, from healthcare and transportation to manufacturing and beyond. Another essential advantage of animal-inspired robots lies in education and inspiration. These robots captivate the interest and curiosity of students and the general public, sparking enthusiasm for robotics,

biology, and technology. As people observe these machines replicating the wonders of nature, they gain a greater appreciation for both the animal kingdom and the potential of robotics in addressing real-world challenges. In conclusion, animal robotics brings forth a range of advantages that extend from promoting natural interaction and aiding in environmental monitoring to facilitating search and rescue missions and inspiring innovation. As technology advances further, animal-inspired robots will continue to contribute significantly to society in numerous beneficial ways.

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STUDY OF BIRD FAUNA OF MALANGAON DAM, SAKRI DISTRICT DHULE MAHARASHTRA, INDIA

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Abstract:

Malangaon Dam Sakri is a perennial Dam. It provided a good habitat for residential and migratory birds. The present study deals with avifauna of Malangaon Dam which reveals the presence of 18 species of bird species with 10 different orders. The order Passeriformes is dominant over other bird species. Due to encroachment and anthropogenic disturbances, the resident and local migratory birds are threatened.

Keywords: Avifauna, Perennial, Wetland, Malangaon, Poaching etc.

Introduction:

Birds are the best Known and most easily recognized of all animals. Birds have mixed with mankind in every aspect of life. They are unique in having feathers for flying which also clothe and insulate their bodies to make possible a regulated body temperature. They have easily avoided all kinds of enemies on land adopting an aerial mode of life. There are distinctive colorations and voices of birds were finding near Malangaon Dam Sakri. Many birds are economic importance because of their food habits.

Fresh water wetlands are fragile ecosystems which are fast deteriorating and shrinking due to manmade activities. India has 65,000 Wetlands covering an area of 4.5 million hectares (Anon, 1990). Indian Subcontinent represents 2094 forms belonging to 1200 species of avifauna (Ali and Ripley, 1983; Ripley, 1992). The abundance of high ecological diversity in the country and the diverse aquatic ecosystems of India represent 417 forms belonging to 318 species and 146 genera of the avifauna of the Subcontinent (Vijayan, 1991). A good number of works in relation to birds have been done in India (Sampath and Krishnamurthy, 1993; Pentewar, 2018). The present study is carried out to asses Avianfauna of Malangaon Dam Sakri.

Material and Methods:

Malangaon Dam is an ideal habitat for wetland birds. The Survey of the avifauna of this Dam was undertaken during the period from November-2007 to December-2009. The birds were identified by available guideline given by Ali (1996); Sonobe and Usui (1993) and with a pair of

binoculars 7x and 8 x magnifications while walking over the bund of the dam. The birds enumerated were classified and represented in the form of checklist.

Birds sighted during the surveys have been categorized based on their migratory nature as follows: R =Resident, R/PM=Resident with Partial movements, R/SM=Resident with summer influx, SM=Summer Migrant, WM=Winter Migrant, WM/PM=Winter Migrant with Partial Movements.

Results and Discussion:

Table 1: Systematic list of Avianfauna of Malangaon Dam, Sakri District Dhule Maharashtra (India)

Sr. No	Scientific Names	Order	Family	Common Name	Migratory Status
1	<i>Columba livia</i>	Columbiformes	Columbidae	Pigeon	R
2	<i>Psittacula eupatria</i>	Psittaciformes	Cacatuidae	Parrot	R
3	<i>Bubo bubo</i>	Strigiformes	Strigidae	Horne owl	R
4	<i>Pava cristatus</i>	Galliformes	Phasianidae	Peacock	R/PM
5	<i>Centrocercus minimus</i>	Galliformes	Phasianidae	Pheasant	R/PM
6	<i>Gallus gallus</i>	Galliformes	Phasinidae	Red jungle fowl	R
7	<i>Eudynamis scolopaceus</i>	Cuculiformes	Cuculidae	Cuckoo	R
8	<i>Dendrocopus mahrattensis</i>	Perciformes	Picidae	Woodpecker	R
9	<i>Passer domesticus</i>	Passeriformes	Passerdae	House sparrow	R
10	<i>Corvus splendens</i>	Passeriformes	Corvidae	House crow	R
11	<i>Acridotheres tristis</i>	Passeriformes	Strurnidae	Myna	R
12	<i>Quelea cardinalis</i>	Passeriformes	Ploceidae	Weaver bird	R/PM
13	<i>Pseudogyps bengalensis</i>	Accipitriformes	Accipitridae	Bengal Vulture	WM/PM
14	<i>Milvus migrans</i>	Accipitriformes	Accipitridae	Black kite	R
15	<i>Ardea cinerea</i>	Pelicaniformes	Ardeidae	Grey heron	R
16	<i>Ardea cinerea</i>	Pelicaniformes	Ardeidae	Grey heron	R
17	<i>Anas creca</i>	Anseriformes	Anatidae	Duck	SM/
18	<i>Alcedo atthis</i>	Caraciformes	Alcedinidae	Kingfisher	R

Abbreviations: R =Resident, R/PM=Resident with Partial movements, R/SM=Resident with summer influx, SM=Summer Migrant, WM=Winter Migrant, WM/PM=Winter Migrant with Partial Movements.

The observed birds are listed in Table 1 on the basis of their scientific names, common names, orders and family. A total 18 species of birds were identified in the area of the Malangaon Dam. Some are resident some are resident cum local movements, while other are winter migrants. We observed total 18 birds belonging to 11 orders and 14 families. The order

Passeriformes is dominant over other orders presented 4 species, followed by order Galliformes (3 species). Order Accipitriformes and Pelicaniformes represented 2 species each, order Columbiformes, Psittaciformes, Strigiformes, Cuculiformes, Perciformes, Anseriformes and Caraciformes with each single species. One species Bengal vulture (*Pseudogyps bengalensis*) out of 18 species of birds recorded has been under the category critically endangered. The major populations of species in the dam are Pigeon (*Columba livia*), Parrot (*Psittacula eupatria*), Sparrows (*Passer domesticus*), crows, (*Corvus splendens*), Grey herons (*Ardea cinerea*) and others were seen in small flocks in wet land of dam. The species like grey heron were locally migratory found in and around the dam depending upon the water conditions.

The major species of the dam viz. Pigeon, parrot, sparrows and crows were more in the month of October. This number gradually increased in the month of February respectively. The uncommon bird pheasant make its presence only when the dam was with major amount of water. The present study is corroborative with the earlier works viz. Islam and Rahmani (2004); Donar et al (2012); Singh and Banyal (2013); Kumar and Paliwal (2015); Pentewar, (2018) and Pawar et al (2019). Kumar and Paliwal (2015) studied the bird of Pond dam and recorded over 415 bird species and 65 families. Sharief et al 2018 study avian faunal diversity of Pong Dam revealed presence of 169 bird species, 16 orders and 48 families of birds.

Conclusion:

The bird species are abundant in Malangaon area but protect them from encroachment and poaching by constant patrolling in the dam to minimize poaching of birds. Reduce the fishing, agricultural and anthropogenic activities near the dam. Create a awareness among the people about this ecosystem.

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AQUACULTURE INDUSTRY-THEIR PRESENT AND FUTURE PROSPECTS

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Abstract:

The aquaculture industry is experiencing significant growth and offers a sustainable solution to meet the increasing global demand for seafood. It has diversified to include various species and is driven by technological advancements and a focus on sustainability. Integrated systems and market expansion into new regions are also contributing to its success. However, the industry faces challenges related to disease management, water quality, feed sustainability, social and economic concerns. Addressing these challenges is essential for ensuring long-term success and sustainability in the aquaculture sector. Overall, the future prospects of the aquaculture industry are promising, with continued innovation and responsible practices driving its growth and contribution to global food security.

Introduction:

Aquaculture is one of the fastest growing industries for food production worldwide, and its products constitute a major source of protein for human consumption (Ahmad *et al.*, 2021). It is defined as 'the farming of aquatic organisms including fish, molluscs, crustaceans and aquatic plants in selected or controlled environments (Bouelet *et al.*, 2018). It is currently one of the fastest growing food producing sectors in the world. To meet the growing demand for fish products, aquaculture is expanding quickly. According to estimates from the Food and Agriculture Organization (2018), it accounted for 46% of all fish production in 2018, with 52% of that value going toward human consumption. Annual growth in capture production was predicted to reach 62% by 2030 (Kibenge, 2016; Freitas *et al.*, 2020). The increase in global population and the decline in capture output both contribute to this demand (Rahman *et al.*, 2019). There is a fall in or close to stasis in the increase of supply from catch fisheries as a result of the aquaculture sector's rapid expansion. It follows that aquaculture will continue to contribute more and more to fish supplies. In the official aquaculture industry, the farm is sizable, expertly run, and employs cutting-edge technology. It is widespread in industrialized nations including the United States, Japan, Norway, and France, which account for around 8.6% of the world's

aquaculture production (WHO, 1999). However, informal aquaculture is made up of semi-organized, low-tech, and underdeveloped infrastructure units, particularly in low-income nations with a food deficit. Even the main aquaculture-producing nations, such as China, India, the Philippines, Indonesia, and Thailand, which account for roughly 80% of world aquaculture production (FAO, 1995), include members of this category. Furthermore, as labor would be required in all related businesses, it has the potential to provide employment. Aquaculture has several facets and offers a variety of environments, from freshwater to marine, and from low-tech, computerized closed indoor water recirculation systems to basic, still ponds.

Aquaculture industry

As aquaculture industry is most often linked with the farming of fish, besides it also includes the cultivation of other plants and animals present in the water. A variety of species, such as fish, crabs, mollusks, seaweed, and others, are frequently chosen for aquaculture. In this business, fish or other aquatic animals are cultured either in freshwater or in marine environments (Ahmed *et al.*, 2021). Grass carp was the primary fish produced for the last eight years having a share percentage of 10.5% i.e 5.7 million tons among the finfish group. However, Silver carp and Nile tilapia came in second and third, with a share percentage of 8.8% (4.8 million tons) and 8.3% (4.5 million tons), respectively.

The molluscs production in 2018 saw significant contributions from cupped oysters (29.5% or 5.2 million tons) and Japanese carpet shells (23.6% or 4.1 million tons). Meanwhile, white leg shrimp dominated the crustaceans group, making up 52.9% (5.0 million tons). The increase in overall production, particularly in finfish species, was attributed to China's policy modifications and the influence of local and international consumers and markets on the production value chain, according to the Food and Agriculture Organization (FAO, 2018).

Types of aquaculture

Aquaculture, as defined by El-Sayed (2020), as it is the farming of aquatic creatures such as fish, molluscs, crustaceans and aquatic plants.

Freshwater aquaculture

Farmers using best culture system like ponds, recirculation aquaculture systems (RAS), or other inland waterways such as biofloc system, which are built based on economic reasons for better production in aquaculture system. This culture system mostly raising fish and other aquatic speices like crab, aquatic plants and shrimp etc. (Li and Liu, 2019).

According to Goddard and Delghandi (2020) species including tilapia, carps and crustaceans contributed around 64% of the worldwide fish production in 2016; however, in 2018

the ratio dropped to 62.5%. Because of their consistency and quality, freshwater aquatics are heavily dependent on climatic and hydrologic regimes (Huang *et al.*, 2020).

Marine culture

Marine culture, or mariculture, is a practice conducted either in the sea or along the coast, depending on the salinity of the water. Ahmed and Thompson (2019) reported that freshwater, brackish water, and sea water have salinity concentrations of 30 ppt, indicating the various types of water used in mariculture. Mariculture involves raising fish in cages or ponds in various ways, such as conventional, extensive, semi-intensive, or intensive methods (Joseph and Augustine, 2019). It currently occupies one-third of marine waters, and its expansion is encroaching further and deeper into the ocean. In 2018, mariculture produced 30.8 million tons globally, with Asia contributing 88.69% (72.8 million tons) of the total worldwide aquaculture production. High-value fish species like salmon, seabass, seabream, barramundi, and trout, as well as bivalve molluscs and seaweed, are the dominant species in mariculture. Experts predict a significant increase in the proportion of aquaculture taking place in marine environments, as indicated by various studies (Davies *et al.*, 2019).

Culture systems for aquaculture

During starting or expanding an aquaculture project, selecting the most suitable culture system is crucial, as different systems are designed for either freshwater or marine environments, and they vary in setup and species compatibility. The categorization of culture systems into open (cages, net pens, floats, trays, and rafts), semi-closed (raceways and ponds), and closed systems (RAS) helps determine the level of control and intervention required for maintaining appropriate temperature, oxygen levels, and waste collection (Tidwell, 2012). However, the descriptions of these systems can be complex and confusing due to blurred or unclear demarcations.

Aquaculture systems can be classified based on three fundamental factors: the type of rearing facilities (ponds, cages, raceways, pens, enclosures, tanks, etc.), the water exchange method (static, open, semi-closed, closed), and the degree of culture intensity (extensive, semi-intensive, intensive, high-intensive). Combining any of these criteria creates a fourth criterion in fish farming methods (Lopez, 2011). Among the various options, structural culture (using ponds, raceways, or RAS) is the simplest to define as fish are frequently reared or farmed in these facilities. Similarly, according to Goddard and Delghandi (2020) aquaculture systems can be classified into open, closed, and semi-closed systems based on the volume of water used during the culture phase. Open systems use natural flow to clean waste and rely on existing water bodies like lakes, rivers, and seas, making them cost-effective and requiring less management. Closed systems recirculate and recondition water, requiring minimal water exchange. Semi-closed

systems, known as once-through or flow-through systems, discharge water after passing through the system.

The intensity of culture is determined by two factors: the number of aquatic organisms per unit area or volume and the capacity for natural productivity. This classification leads to three categories: intensive, semi-intensive, and extensive systems (Tidwell and Bright, 2018). Intensive systems require a large number of fish and a significant amount of feed to achieve higher output due to the provision of abundant protein (Oddsson, 2020). Aquaculture practices have different implications on the environment and production. Estimates show that feed input energy losses are minimal, but waste indirectly affects the environment's water supply. Extensive systems, with less human interference, have a lower production level and a stocking density of 500 kg/ha. Semi-intensive systems utilize a mix of natural food sources and additional feeding, allowing for higher stocking densities within the culture system.

Fish farming practices are categorized as polyculture (multiple species) or monoculture (single species). Polyculture is more efficient in utilizing food for fish production compared to monoculture, leading to better resource utilization (El-Sayed, 2020). These practices have implications for sustainable and efficient aquaculture management, considering environmental impacts and production outcomes.

Similar to the fish farming approach, integrated fish farming is a method that involves the co-production of fish and livestock species, such as rice, ducks, or chickens. This approach aims to utilize byproducts efficiently, benefiting both the fish crop and the livestock subsystem. By integrating fish and livestock farming, farmers can optimize land and water usage, leading to improved farm yields with reduced labor and financial expenditures. Aquaponic systems are another innovative approach that combines plants and aquatic creatures. In these systems, animal waste undergoes nitrification, converting it into nutrients for plants, which are then recycled back into the culture system. This process reduces water pollution and creates a sustainable and balanced ecosystem. Aquaponic systems can be implemented both indoors and outdoors, and they come in various sizes to suit different needs. The three most popular culture units used by farmers in aquaculture are ponds, flow-through/raceways, and RAS (Recirculating Aquaculture Systems). These units offer different advantages and characteristics, providing diverse options for sustainable and efficient aquaculture practices (Zambri *et al.*, 2018).

Ponds

Pond culture is a prevalent method in aquaculture production, classified into three types: levee ponds, watershed ponds, and depression ponds. These ponds hold a specific amount of water and rely on natural internal processes to purify the water, eliminating the need for constant

water exchange. Typically, water inflow occurs until it reaches a certain level before being discharged. After two or more fish harvests, fresh water is introduced to replace the contaminated water. This method is effective in maintaining water quality and sustaining fish growth (Ngo *et al.*, 2017).

According to El-Sayed (2020), the rate of fish production in pond culture is influenced by the daily feed input and the maintenance of water quality. To enhance the waste removal process and increase oxygen content, the system can be controlled by adding fresh water or using an aeration system. The size of the ponds can vary depending on factors such as production volume, environmental conditions at the site, and the type of fish species being cultured. Pond sizes typically range from 100 to 100,000 square meters with an average depth of 1.2 to 1.5 meters. Proper control and management of these factors are essential for optimizing fish production in pond culture systems. Pond systems in aquaculture offer several advantages, such as their simplicity, low labor, and energy requirements. However, they are also subject to external factors like weather conditions and face intense competition in the fish market. On the other hand, flow-through systems are commonly employed for salmonids. These systems consist of a series of raceways or tanks where water flows and eventually gets washed out into an external water body. Flow-through systems are convenient to use when a sufficient supply of clean water is available. They are best situated downstream of redirected rivers or streams.

Recirculating system/RAS

Recirculating aquaculture systems (RAS) employ mechanical filtration and biofiltration to treat waste and recycle water. These systems require additional make-up water to compensate for water lost through evaporation (Egloff *et al.*, 2018). RAS systems offer advantages over other methods as they have less negative impact on the environment and use water more efficiently (Davidson *et al.*, 2016). However, their main drawbacks are the high initial and ongoing energy costs associated with construction and operation. The installation of biofiltration to improve water quality for recycling back into the culture system contributes to an estimated cost of USD 500,000 to produce 100,000 pounds of fish annually, excluding land or building expenses.

Best practices in aquaculture

Aquaculture development raises concerns about its impact on the environment, economy, and society. To ensure sustainable and responsible production, various practices and protocols have been developed. Best Management Practices (BMPs) serve as standards to guide fish producers toward more sustainable methods. By following BMPs, aquaculture operations can minimize their ecological footprint and negative impacts, promoting long-term viability and responsible seafood production. According to Sivaraman *et al.* (2019) and Giri (2017), Best

Management Practices (BMPs) offer an efficient approach to mitigate environmental consequences while achieving resource management objectives in aquaculture. BMPs involve technically sound and workable techniques to reduce environmental pollution and control costs in aquaculture facilities. Adequate soil conditions, sufficient water supply, and careful management of feed rates and fish density based on location can help minimize solid and water-related issues in aquaculture operations (Ozbay *et al.*, 2014). Implementing BMPs is crucial for maintaining environmental sustainability and economic viability in aquaculture.

Practical techniques such as agricultural irrigation, wetlands, settling basins, coagulation processes, and biological filters can effectively enhance the effluent quality from aquaculture ponds (Adhikari and Fedler, 2020). In countries with established regulations, guidelines and permits for effluent standards should be implemented. In nations without such rules, Best Management Practices (BMPs) are recommended to mitigate environmental impacts. Independent organizations and stakeholders in aquaculture and fisheries may offer codes of conduct as ethical guidelines for fish farms aiming to promote sustainable and responsible aquaculture (Vince and Haward, 2019). These measures contribute to reducing environmental consequences and ensuring the sustainable development of the aquaculture industry.

In the past, the aquaculture sector utilized a variety of chemicals for fish growth, disease prevention, and facility upkeep in order to make sure that production ran smoothly and produced goods of high quality. The use of some compounds, including those used in chemotherapy, anesthesia, ectoparasiticides, endoparasiticides, vaccinations, and microbial infection, in medications, disinfectants, and antifoulants has recently been restricted. The environmental impact of these pesticides is dependent on the chemical concentration, farm size, and size of the surrounding water bodies, despite the fact that they are effective at boosting aquaculture productivity (Dauda *et al.*, 2019). For instance, disease is a major concern and potential barrier to salmon production (Gallo *et al.*, 2020); as a result, some recommendations are made to lessen or prevent disease outbreaks, such as appropriate site selection, high-quality diets, rotating rearing sites, and biosecurity (Love *et al.*, 2020).

Future prospects:

Despite significant efforts to promote inland aquaculture in India, only one-third of the potential freshwater area suitable for ponds and tanks has been utilized for scientific fish culture. To fully realize the production potential, it is crucial to employ current technologies effectively, disseminate technical knowledge, and provide necessary material inputs. Freshwater aquaculture in India has emerged as a sustainable industry due to its operational flexibility, compatibility with other agricultural methods, and its potential for eco-restoration. Recognizing its potential

and impressive annual growth rate of over 6%, the Indian government is actively promoting the growth of aquaculture. "Operation Aqua-Gold" is a national freshwater aquaculture development plan that aims to double the aquaculture area to 1.2 million hectares. This plan seeks to further harness the potential of freshwater aquaculture and contribute to the country's aquaculture sector's continued success.

To achieve the desired output, strategies have been devised to increase the area of freshwater aquaculture by 45.2% and improve productivity by 50.9%. These strategies consider the potential and challenges in different states and involve a combination of horizontal and vertical expansion. To implement these tactics, an additional 0.37 million hectares of water would be required. This increase in fish production would necessitate expanding the area dedicated to ponds and tanks, as well as increasing seed and feed availability. To boost freshwater aquaculture's fish production capacity, a focus on both horizontal and vertical expansion of the industry is essential. Furthermore, the National Aquacultural Development Plan (Gopakumar *et al.*, 1999) emphasizes the need for growth, intensification, and diversification of culture systems. These approaches aim to strengthen and enhance the freshwater aquaculture sector in India, ensuring its continued growth and success.

The success of freshwater aquaculture strategies in India depends on key elements such as increasing the aquaculture-related land area from 0.83 to 1.2 million hectares. Intensification of aquaculture methods aims to maximize output without harming soil-water ecology, potentially doubling fish production. Changes in leasing policies include extending lease terms to over 10 years and linking rent increases to productivity and multiple use. Integration of carp, catfish, and prawn cultivation with agriculture, animal husbandry, horticulture, and forestry is being promoted. Additionally, support for breeding and cultivation of ornamental fish addresses domestic demand and foreign exchange opportunities. These measures collectively strive to enhance freshwater aquaculture, ensure sustainability, and boost economic growth in the sector.

To enhance freshwater aquaculture in India, several crucial steps are being taken. The establishment of hatcheries for carps, catfishes, and freshwater prawns, along with decentralized fish seed production at block levels, ensures a steady supply of fish seeds. Developing fish feed using locally available and cost-effective plant and animal-based ingredients, especially for brackish water aquaculture, is prioritized. Training and educating farmers and business owners on feed manufacturing and distribution are essential. Fishing cooperatives are encouraged to actively participate in input delivery, particularly for fish feed and seed. Budgetary support is provided for freshwater aquaculture research, infrastructure development, education, and extension programs. Moreover, creating a reliable and comprehensive database using

standardized nomenclature and classification for relevant criteria, such as pond size, fish seed quality, and types of fish caught, helps in efficient management and planning. These initiatives collectively aim to strengthen freshwater aquaculture and foster sustainable growth in the sector.

To establish a comprehensive database for freshwater aquaculture, an expert group would be responsible for continuous data collection over time and space. Necessary infrastructure, such as fish and shrimp hatcheries, aquaculture farms, feed mills, and related businesses providing equipment like aerators, feed dispensers, and anti-fish-disease preparations, is crucial for both production and post-harvest activities.

In designated aquaculture zones, it is essential to strengthen the marketing infrastructure, which includes facilities like ice makers, cold chains, storage facilities, well-maintained roads, and efficient transportation. These initiatives are vital for enhancing the efficiency and productivity of freshwater aquaculture, facilitating better market access, and ensuring the sustainable growth of the industry.

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ZOONOSIS

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Abstract:

An infectious disease known as a zoonosis has spread from non-human animals to people. Zoonotic infections can spread to people by direct contact, food, water, the environment, viral, bacterial, parasitic, or other unconventional agents. Due to our strong contact with animals in agriculture, as companions, and in the natural environment, they constitute a significant public health issue globally. Zoonoses can also interfere with the trade and production of animal products used for food and other purposes.

Zoonoses make up a significant portion of all newly discovered infectious diseases as well as many already known ones. Some illnesses, like HIV, start as zoonotic strains before evolving into human-only varieties. Other zoonoses may result in recurrent illness.

Keywords: infectious, pathogens, parasitic, recurring disease

Introduction:

Humans can contract zoonotic pathogens from any point of interaction with domestic, farm, or wild animals. Due to the vast number of novel or previously unidentified diseases that are known to occur in some wild animal populations, markets that sell the meat or byproducts of wild animals are particularly high risk. Agricultural workers in regions where farm animals are frequently given antibiotics may be more likely to contract diseases that are resistant to the existing antimicrobial medications. People who live close to wilderness regions or in semi-urban settings where there are more wild animals are more likely to contract diseases from rodents, foxes, or raccoons. By increasing human-wild animal contact, urbanization and the degradation of natural habitats raise the danger of zoonotic illnesses.

Risk factors

- Having a compromised immune system and handling a pet or other animal. Children under the age of five are more likely to contract zoonotic infections. They frequently ingest objects, including their hands, and they don't always wash their hands thoroughly or frequently.
- Your residence (city, rural, or agricultural).

- Contact with animals at public sandboxes or petting zoos.
- Contact with wildlife or game.
- Poor treatment of animals or their surroundings. Handling a litter box, a sick animal, or bedding that has been contaminated by urine, vomit, or excrement are all prohibited.
- There is a greater danger of infection when young animals (puppy or kitten) are in contact.
- Any interaction with an unusual pet. Dogs and cats are common household pets.

Signs and symptoms of illness

Body aches, headaches, fatigue, swollen lymph nodes, nausea, vomiting, pain, flu-like symptoms, lesions, scratches, or bite marks on the skin.

Diagnosis

The different diseases and infections that might result from zoonoses can be diagnosed using particular testing. Results of tests are typically available in 2 to 14 days. The doctor who will be treating your kid can explain how long each test will take.

Treatment

The type of infection determines the course of treatment for zoonoses.

Exercise and Diet:

Your kid's doctor or nurse will provide you with any specific dietary or exercise recommendations if your child has a zoonoses illness.

Prevention and Care at Home:

Pets can benefit a child in beneficial ways. They can support both physical and mental recovery. The following advice can help you keep your youngster secure around pets:

- Your child should not acquire any additional pets while undergoing bone marrow transplantation, radiation therapy, or chemotherapy.
- Pets should receive routine veterinary care and vaccinations. Pets need to be groomed to maintain healthy skin, coats, and teeth. To reduce the chance of scratching, nails should be filed short. If your pet is sick, take them to the vet and keep them away from your child until they are well.
- Kitchens and other areas where food is produced and consumed should not be visited by pets. Feeding pets raw foods, such as raw meat or eggs, is not advised. They shouldn't be permitted to drink from toilets or bodies of standing water, or hunt or eat in the wild.

Prevention and control

Each pathogen has different prevention strategies for zoonotic diseases, but a number of ways are proven to be successful in lowering risk in both the community and on an individual

level. The possibility of food borne zoonotic disease outbreaks through items like meat, eggs, dairy, or even some vegetables is reduced by safe and adequate recommendations for animal care in the agricultural sector. Standards for the safe disposal of garbage and the preservation of surface water in the natural environment are equally crucial and effective. When zoonotic infections do emerge, community transmission can be slowed down by educational programs that encourage handwashing after interaction with animals and other behavioral modifications.

The control and prevention of zoonoses are complicated by antimicrobial resistance. usage of antibiotics on farm animals.

Conclusion:

An infectious disease called zoonosis can transmit between species, from animals to people or vice versa. Zoonotic infections can spread to people by direct contact, food, drink, or the environment. They can also be bacterial, viral, parasitic, or involve other unconventional agents, according to the World Health Organization. Due to our strong contact with animals in agriculture, as companions, and in the natural environment, they constitute a significant public health issue globally. Zoonoses can interfere with the trade and production of animal products used for food and other purposes.

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PRESERVATIVES IN FISH PROCESSING

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Introduction:

A preservative is a substance or a chemical that is added to products such as food products, beverages, pharmaceutical drugs, paints, biological samples, cosmetics, wood, and many other products to prevent decomposition by microbial growth or by undesirable chemical changes. In general, preservation is implemented in two modes, chemical and physical. Chemical preservation entails adding chemical compounds to the product. Physical preservation entails processes such as refrigeration or drying. Preservative food additives reduce the risk of foodborne, decrease microbial spoilage, and preserve fresh attributes and nutritional quality. Some physical techniques for food preservation include dehydration, UV-C radiation, freeze-drying, and refrigeration. Chemical preservation and physical preservation techniques are sometimes combined.

Classification of preservatives

Preservatives are classified into two classes: Class I and II.

1. Class I preservatives:

These preservatives are those which are not restricted in any food unless otherwise provided in the rules. eg. common salt, sugar, dextrose, glucose syrup, wood smoke, spices, vinegar and honey.

2. Class II preservatives:

These preservatives are those which are restricted to a specified group of foods in concentrations not exceeding limits prescribed for each. e.g. benzoic acid and its salts, ii. sulphurous acid and its salt, iii. nitrites and nitrates and iv. sorbic acid and its salts.

Chemically preservatives are broadly classified into:

1. **Inorganic preservatives:** They are unstable elemental compounds.
2. **Organic preservatives:** They are stable, unstable and volatile compounds.
3. **Anti-mould agents:** They are fatty acids containing 1 to 14 carbon atoms.

Need for Preservatives

1. To extend the shelf life of foods
2. To improve their safety
3. To provide unique organoleptic characteristics

Properties of Preservatives

Preservatives should not

1. Injure the health of the consumer
2. Be irritant
3. Decompose into toxic substances
4. Retard the action of digestive enzymes

Mode of Action of Preservatives

1. Ionic state of preservatives

Preservatives have a wide range of molecular structure and they have a tendency to form ions. Carboxylic acids such as sorbic, benzoic, formic, acetic, lactic and propionic are monobasic. Aqueous solutions of sulphur dioxide behave as solutions of a dibasic acid.



Hydrogen sulphite ion is also in equilibrium with disulphite ion.



In diluted aqueous solution, significant amounts of hydrogen sulfite ions (HSO_3^-) are formed. In concentrated systems, significant amounts of disulphide ions are formed. When sodium or potassium disulphite is used, the salt is hydrolyzed to hydrogen sulphite ion. The nitrites are salts of nitrous acid, which is the source of nitrosating species, N_2O_3 and oxides of nitrogen, NO.

2. pKa values of preservatives

When a preservative is added to food, its ionic state is determined by the pH of the food and the pKa of the acid. The pKa values of the preservatives are given in Table 1.

The fraction, α of an acid which remains undissociated at a given pH is calculated using the formula, $\alpha = (10^{\text{pH} - \text{pKa}} + 1)^{-1}$. Since, significant changes in α occur in the range $(\text{pKa} - 2) < \text{pH} < (\text{pKa} + 2)$, the proportions of ionized and unionized forms of the acids change substantially over the pH range of foods.

Table 1: pKa values of the preservatives

Preservatives	pKa
Sorbic acid	4.76
Benzoic acid	4.18
Sulphur dioxide	1.86
Formic acid	3.75
Nitrous acid	3.40
Acetic acid	4.76
Lactic acid	3.08
Propionic acid	4.88

3. Lipophilic and hydrophilic behaviour

Another essential feature of food preservative is their molecules should have balance of lipophilic and hydrophilic behavior. They should be capable of traversing non-polar membranes and sufficiently soluble in aqueous environment of the microorganisms. Undissociated carboxylic acids tend to partition between the aqueous and oil phase of food. Undissociated acids are soluble in non-aqueous environments, while ionized acids are insoluble in non-aqueous environments. A partition coefficient, P is defined by, $P = C_{oil} / C_{aq}$, where C refers to concentration of undissociated acid in each phase. The P values vary from 0.17 and 2.5 for propionic and sorbic acids, respectively.



Mechanism of antimicrobial action

The microbial inhibition may be brought about by any one of the following mechanisms:

1. Interference with genetic mechanism of cell division, which is mainly caused by denaturation of vital genes and oxidation in autocatalytic synthesis of polypeptides
2. Interference with cell membrane permeability.

3. Interference with the enzyme systems of the microbe by competing with the substrate for place on the active enzyme surface or by interfering with the forward progress of an enzyme-catalyzed reaction by accumulating the end products of the reaction
4. Inhibition of bio-synthesis of vitamins
5. Formation of complexes with heavy metal ions.
6. Fat soluble agents or surface-active agents will cause the cell contents to react with the substrate.

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THE SECRETS OF BEES – MYTHS AND FACTS

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Abstract:

A healthy environment and economy depend on bees, which are essential for pollinating many of the plants and flowers that support a variety of other animals' habitats as well as the food we need to survive. According to the Food and Agricultural Organization, about 80% of all agriculture plants are specialized for pollination by animals, primarily insects like bees. Because many of our fruits, vegetables, and other foods rely on bee pollination, bees are vital to the preservation of our world. Without pollination, we risk going without food because many of our fruits, vegetables, and other crops used to feed our livestock rely on it for fertilization. despite the fact that bees have a much greater capacity for pollination than any other pollinator. Some bee-related myths and realities. Important bee facts include their foraging habits, how honey is made, communication, queen pheromones, honey bee glands, their dancing abilities, and the importance of bees to humankind.

Keywords: Bees, facts, myths and pollination

Introduction:

All honeybees are social and cooperative insects. Honeybees play a crucial role in pollinating flowers, fruits, and vegetables. They gather into a ball to retain heat and survive the winter on stored honey and pollen. There are three different categories of hive members. Workers build and guard the hive, search for nourishment (pollen and nectar from flowers), and clean the air by beating their wings. The eggs that will give rise to the hive's subsequent generation of bees are laid by the queen. A hive typically only has one queen. If the queen passes away, the workers will make a new queen by giving one of the female workers a special delicacy called "royal jelly." The worker can become a fruitful queen thanks to this potion. All honeybees are social creatures that reside in nests or hives with other bees. The honeybee is unique for the dancing manoeuvres it uses inside the hive to convey details to other bees about the position, range, size, and quality of a certain food source in the neighbourhood.

Myths:

Myth 1: Honey bees can sting their victim repeatedly

The workers of honey bees have several stingers. The barbs of their stingers, particularly those used on mammals with thick skin like humans, snag on the skin of the targets they attack. The bee dies after having its stinger removed because doing so is fatal to it.

Myth 2: All bees sting

Not every bee has a stinger. For instance, male bees are unable to sting. The stinger, often known as the sting, is an altered egg-laying tool. As a result, only women possess these. In spite of having a stinger, many bee species' females are actually unable to sting. Most bees won't sting until provoked or threatened because they usually sting to defend their nest.

Myth 3: Wasps are bees

Wasps are not bees, despite belonging to the same order of insects. Bees collect pollen and nectar for their offspring and are vegetarians. Carnivores include wasps. Certain species are notoriously vicious, especially if you destroy their nests. Bees typically don't bite. The exception is a species of bee that is uncommon in the United States called Africanized bees.

Myth 4: Bee stings can be avoided by spraying the nest with water

A bee nest won't be impacted by water. Most likely, you'll only agitate the bees there and raise your risk of being stung.

Myth 5: All bees produce honey

Only 5% of bee species produce honey. Only stingless bees and honey bees generate enough honey for human use. One to two teaspoons at most may be found in bumble bee hives. Bumble bees are not perennial; they are yearly. To survive the winter, they don't need to make a lot of honey.

Myth 6: Bees are hard workers

Worker bees, including honey, bumble, and stingless females, put in a lot of effort. However, a lot of guys don't contribute anything to the nest. The solitary bee species' females may only work for a few weeks.

Myth 7: Adult bees live for a very long time

Solitary bees only have a short lifespan, just long enough to reproduce, construct nests, and generate young. Male and worker honey and bumble bee lifespans are around six weeks. The workers split their time between tending to the hive and collecting pollen and nectar. Queens have longer lives. While honey bee queens can live up to four years, bumble bee queens can survive for up to one year.

Myth 8: Bees won't sting at night

It's a common misconception regarding bees that they don't sting at night, however this is untrue. Bees can sting at any time to defend themselves.

Myth 9: Most bees live in hives

In hives, only social bees reside. Only a small portion of the ten percent of bee species that are sociable build hives. The majority of bees are solitary and construct their own homes in tree trunks or the ground.

Myth 10: Bees are kept away if you rid your lawn of dandelions and flowers

Even though they pollinate plants and flowers, bees will build their nests miles away from the flowers and other plants they visit. It doesn't matter if you have flowers in your yard if a bee scout finds a nice area to build a nest.

Myth 11: Sealing the hole in the wall where the bees are nesting will kill the bees on the inside

You run the risk of upsetting bees if you block a nest's entrance. They might dig up areas you don't want them to, like the inside of your home. Bees have been observed to bore through drywall and wood. Contacting local bee experts is your best option.

Facts

A honey bee colony is made up of 20,000 to 60,000 honey bees and one queen. Female worker honey bees live for around six weeks and do all of the labor. The queen bee can live for up to five years, and her job is to lay eggs in the hive.

Fact 1: Busy pollinating bees

Almost 90% of wild plants and 75% of the world's major crops rely on animal pollination. Pollinators such as bees are responsible for one out of every three mouthfuls of our food. Crops that rely on pollination are worth five times more than those that do not.

Fact 2: Honey i'm home!

Honeybees and bumblebees, for example, reside in hives or nests above or below ground, but most solitary bees nest in the ground. Bees can be found in a surprising number of places. Marshes, shingle, sand dunes, soft cliffs, heathlands, wetlands, chalk grasslands, quarries, gravel pits, sea walls, and even post-industrial terrain are examples of these.

Fact 3: Bringing a bee back to life

If you see a bumblebee that appears to be struggling, it could simply be resting, especially if the bee is a queen in early spring. If you believe the bee is in distress, carefully place the bee on a bee-friendly bloom. If there are no bee-friendly flowers around, make a 50/50 mixture of white sugar and water to provide the bumblebee with a one-time energy boost and the

carbs it requires to fly. Simply apply a drop or two of sugar water to the front end of the bee with a teaspoon or an upturned drinks cap in a covered area and give the bee time to recover.

Fact 4: Anyone, including you, can help a bee out

We can all help bees by planting flowers in our gardens, balconies, and windowsills. You can also tell your friends and family how awesome bees are and encourage them to make their wild spaces bee-friendly. Plant a variety of blooms in your garden from March to October to provide nectar for bees. Bees adore traditional cottage garden flowers as well as native wildflowers such as primrose, foxglove, and marigolds.

Fact 5: Bees actually have four wings

When flying, the two wings on each side hook together to form a bigger pair, and then unhook when not flying.

Fact 6: Bee gees

The 'waggle dance' is a honeybee dance move. It's not a dance motion at all, but rather a sophisticated technique of communicating with their nestmates about where to go to find the best supply of food. The researchers at Sussex University spent two years decoding the waggle dance.

Fact 7: The brainy bunch

The brain of the buff-tailed bumblebee is the size of a poppy seed. That's astonishing given that scientists have trained them to score a goal in 'bee football' in exchange for a sugary treat. Unbelievably incredible!

Fact 8: They have surprisingly smelly feet

Bumblebees may use their 'smelly footsteps' to discriminate between their own fragrance, the scent of a relative, and the scent of a stranger, according to researchers at the University of Bristol. This means they can increase their chances of locating food and avoid blossoms that have already been visited.

Fact 9: A dinner for queens

In a honeybee colony, if the queen bee dies, the workers can produce a new queen bee. They accomplish this by selecting a young larva and giving it a specific meal called "royal jelly," which causes the caterpillar to mature into a productive queen.

Important information about bees

Foraging of bees

Bees fly from their colony to gather propolis, pollen, and nectar, all of which are required for hive maintenance. Because their queen's pheromones may be detected up to three miles away, they may travel that far to forage. Individual bees are flower monogamous; once they've made their choice, they'll stay with one flower for the rest of their life, despite the fact that a hive can

obtain nectar from a variety of flowers. Nobody knows, but if a bee is first seen foraging in season. If the flower is born later in the season, she may develop feelings for someone. As a result, bees are the most effective natural pollinators on the planet. They bring pollen with them as they collect nectar from blossom to flower.



(Source: <https://www.istockphoto.com/photos/bee-pollen>)



(Source: <https://allisonsapiaries.com/how-do-bees-find-nectar/>)



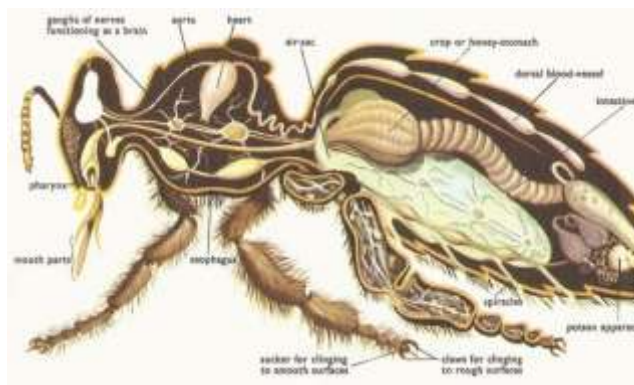
(Source: <https://www.perfectbee.com/learn-about-bees/the-life-of-bees/why-and-how-bees-forage>)

How honey form?

Each foraging bee suckers nectar with its long, tube-like tongue, which it then stores in a secondary honey-sac stomach. A worker can visit up to 1,500 blooms in a single trip to collect her own weight in nectar (about 70 grams). The production of honey then begins. Younger worker bees collect nectar from foragers' honey sacs and mix it with an enzyme called invertase in their stomachs to break down the sucrose in the nectar into glucose and fructose.



(Source: <https://www.libelium.com/libeliumworld/success-stories/temperature-humidity-and-gases-monitoring-in-beehives/>)



(Source: <https://www.quora.com/Do-bees-fart>)

The workers regurgitate the converted nectar into the hive's outer cells after converting the sugars, fanning it with their wings to eliminate moisture. When the moisture level of the nectar falls below 18%, it is covered in its cell with a wax cover, indicating that it has fully

developed into edible honey and is ready to be harvested for human use. This low-moisture honey is resistant to bacterial deterioration and, if stored properly, will keep indefinitely.



(Source:<https://mudsongs.org/honey-bees-fanning/>)



(Source:<https://lychettbayapiaries.com/2018/03/28/how-bees-make-honey/>)



(Source:<https://aponjonjouthosamabay.in/honey-story>)

Communication

The honey bee society cannot function without appropriate communication. The majority of honey bee communication is by aroma and taste. Hormones and pheromones are the names given to the complex system of chemical messengers. A pheromone is a chemical secreted by one individual that, when picked up by another member of the same species, induces that individual to act in a specific way. A pheromone varies from a hormone in that it is transmitted from one person to another.

Queen pheromones

One of the most active and vital chemical communication pheromones in the bee population is the queen's mandibular gland discharge. This mandibular gland secretion has been dubbed "queen substance" for a long time. The pheromone is minimal in newly emerged queens, but by the sixth day, the mandibular glands may have released enough of the chemical to attract drones for mating. Every day, mature queens secrete twice as much.

The chemicals are sprayed all over the queen's body as she is groomed by workers. After receiving the pheromone from the queen via antennal contact, workers distribute it with one another in the way of food transmission. The worker bees become agitated within an hour of the queen being removed from her colony, and four hours later, they begin responding in ways that indicate they are ready to replace the queen. The pheromone known as "queen substance" promotes foraging and brood rearing while suppressing replacement queen rearing, sex attraction, and swarm stabilization.

Scent or nasonov gland

Workers have a scent (Nasonov) gland towards the apex of the abdomen. The gland secretes a mixture of seven terpenoids, the majority of which are employed for navigation.

Workers release the chemical combination by standing high on their hind legs, abdomen elevated, and tilting the last abdominal segment downward while fanning the wings. Sister bees use smell to locate their homes, food sources, and water sources. It collaborates with the queen substance in a pheromone concert to keep the swarm bees together.

Glands of the honey bees

Some of the most significant bee glands are the Dufour's gland, hypopharyngeal gland, mandibular gland, Nasonov gland, wax gland, and venom gland.

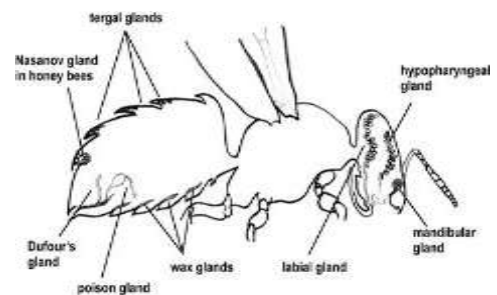
Dufour's gland

It is an abdominal gland that is part of the ovipositor or sting mechanism of female hymenopterans. It drains at the dorsal vaginal wall in bees. The gland is lined by a single layer of epithelial cells, which discharge chemicals into the hollow interior. The bee is shown labelling flowers and hives with scent-marking pheromones generated by the Dufour's gland.



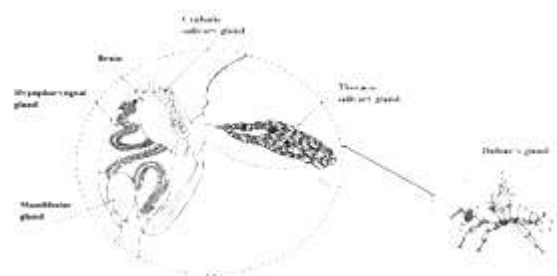
Pharyngeal glands

A gland consists of a long, coiled tube with multiple solid lobules connected to it on each side of the skull. Individual ducts from the glands open near the base of the hypopharynx. It is fully developed in worker bees, vestigial in queens, and non-existent in males. It secretes royal jelly or bee milk in nurse bees. They produce brood food, which is used to feed young larvae, and they are likely involved in caste determination.



Mandibular glands

Small glands form near the base of the mandibles of Hymenoptera. They produce 9-oxodecanoic acid (the "Queen substance"), a pheromone linked to colony control that is identical to the alarm pheromone produced by honey bee queens.



Wax gland

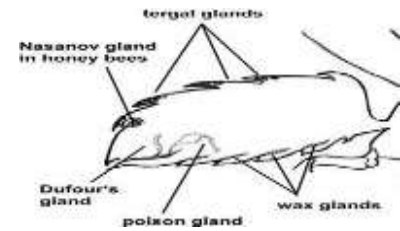
Sternites 4, 5, 6, and 7 have wax glands. They are created up of epidermal cells that have been changed. The wax is released as a liquid, then hardens to form a wax scale. When that fluid comes into touch with air, it transforms into wax. The wax scale is transferred to the mandibles by the hind leg. To produce 1 kilogram of wax, 8.4 kg of honey must be consumed. Only workers who were fed pollen during their first 5-6 days of life had active wax glands.



(Source:<https://beeuorganics.wordpress.com/2012/12/20/how-do-honey-bees-make-beeswax/wax-glands/>)



(Source:https://issuu.com/beesfd/docs/126_bfdj_mar2018/s/12838125)



(Source:
https://www.researchgate.net/figure/Setup-of-experiment-1-The-two-bumble-bee-nest-boxes-are-connected-by-a-glass-tube-air_fig2_10933711)

Venom or poison gland

It is a sting-related modified reproductive accessory gland. The venom gland is made up of two long, slender, convoluted tubules that float freely in the hemolymph on the back of the abdomen. Secretory cells can be seen along the tubules, with their smaller ducts connecting to a larger, chitin-lined duct. Each tubule ends with a little glandular enlargement, and the two tubules unite to form a brief common duct. The duct enters the venom sac at the front end. A modified ovipositor serves as the stinging device. A protein called melittin is a major component of venom, which also contains apimin.



Bee sting

Honey bees can only sting once in their lifetime. The infusion of toxin into the victim by the sting is accompanied by the release of warning pheromones, a process that is expedited if the bee is killed. The emission of alarm pheromones near a hive may attract additional bees, who may also exhibit defensive behaviours until there is no longer a threat, usually because the victim has fled or been killed.



Dancing language of honey bees

A worker bee dances after returning to the honey comb with pollen or nectar. The dances are a method of "telling" other workers where the food is located. The worker bee communicates and guides other bees in pollen and nectar collection by employing unique motions to signal

instance and direction. Honey bees have three sorts of dances: the round dance, the sickle dance and the waggle dance.



Round dance

When a food supply is very close to the hive, usually less than 50 meters away, a bee can dance in a circle.

Sickle dance

The sickle dance identifies food supplies that are 50 to 150 meters away from the hive. This dance is shaped like a crescent and serves as a link between the round and waggle dances.

Waggle dance

Bees communicating at food sources more than 150 meters away from the hive do the waggle dance also known as the wag-tail dance. This dance, as opposed to the circular dance, expresses both distance and direction. When waggle dancing, a bee initially travels straight forward for a short distance, then loops back to the starting point in a semicircle, runs straight again, and finally makes a semicircle in the other direction to complete a full figure '8' circuit.

Bees for man kind

Bees are critical to human and environmental health. Honey and other products have medical benefits, and bees are important pollinators for food supplies. According to trials and studies, honey is antiseptic, antibacterial, antipyretic, anti-inflammatory, antiallergenic, antitoxic, sedative, laxative, antianemic, antioxidant, healing and cleansing (internal and external), moisturising, and blood-purifying. It promotes rehydration, is easy to digest, increases immunity, and is beneficial for a variety of skin disorders (Needham, 2008).



Honey has long been used to treat a variety of diseases. Worm infestation, haemorrhoids, eczema, ulcers, wounds, thirst, hiccups, fatigue, dizziness, hepatitis, and constipation are among the applications that lack scientific evidence.

Bees wax is another important component that has historically been used in waterproofing and fuels. It now has health benefits and is found in many skincare products. Additionally, pharmaceutical businesses employ it in creams. Bee bread, pollen, royal jelly, bee wax, and venom are some more bee products that are beneficial to human health. Bees wax is used to lower cholesterol and relieve discomfort. It is also used to treat ulcers, diarrhoea, hiccups, and swelling (inflammation) (Basa *et al.*, 2016). A 2020 study found that melittin, a chemical found in honeybee venom, could kill cancer cells (Duffy and colleagues, 2020).

In recent years, it has become clear that honey is not the primary reason to safeguard bees. This is due to bees' important function in pollination, in which they use the hairs on their bodies (trichomes) to transport huge grains of pollen between plants. Around 75% of crops produce higher yields when pollinators assist in pollination (Van and Vaage, 2016). Bees are the most important pollinators of both wild and agricultural plants. To put it another way, bees are critical to the growth of many plants, especially food crops.

Conclusion:

Pollinators, particularly bees, are critical to the success of each of the three bites of food consumed globally. Bees are vital to culture and the environment because they pollinate plants and generate honey and other medical products. Furthermore, there is sufficient evidence to support the use of honey in the treatment of illness conditions. As a result, it is critical to protect pollinators such as bees.

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LABORATORY TESTS FOR PREGNANCY DIAGNOSIS

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For the purpose of determining pregnancy in domestic animals, laboratory tests rely on the presence of urinary oestrogens, circulatory hormones (progesterone, oestrogen), or other pregnancy-specific molecules.

Cuboni test:

The Cuboni test was created by Cuboni in 1934 and later updated (Galina and Cox, 1969). Carried out in the mare to assay urine conjugated oestrogens for pregnancy diagnosis. Test is still useful beyond 150 days of pregnancy and forecasts foetal viability. Take a 15 ml urine sample, add 3 ml of conc. HCL, heat it in a water bath for 10 minutes, and then cool it under running water before adding 18 ml. Supernatant (mostly benzene) is collected in another tube after being forcefully shaken with benzene for half a minute. Add 3 to 10 ml of concentrated H₂SO₄ to this, and then let the mixture to cool after being heated in an 80°C water bath for 5 minutes.

Result:

The lower H₂SO₄ layer begins to glow a dark, oily green, signifying a positive (pregnant) test. Presence of a brownish colour without any fluorescence indicates that a test is negative (not pregnant).

Other biochemical tests for the detection of urinary estrogens in mares

1. Mouse test:

The presence of oestrogens in the blood or urine of pregnant mares causes vaginal edoema, the emergence of cornified cells, and mucus discharge when administered to mice or rats with ovariectomies.

2. Phenolsulphonic acid test:

Urinary oestrogens are extracted using ether, and urine pigments are then hydrolyzed after being conjugated with phenolsulphonic acid reagent brings out a pink to cherry red tint. Tests carried out between 120 and 250 days of pregnancy are 70-80% accurate.

3. Mucin test:

Vaginal mucus from a pregnant mare revealed pregnancy cells, which were darkly stained columnar epithelial cells. having a 94% efficacy from day 70 until the conclusion of the pregnancy.

Barium chloride test:

Pregnancy diagnosis in the bovine species. Take 5 ml of urine + few drops 1% barium chloride→ warmed slightly. Positively, in pregnant animals, the urine remains clear. Negatively a white precipitate generated around non-pregnant cows. The test's accuracy was stated to be between 70 and 95 percent. observed only a poor accuracy (64%) with a significant incidence of false positive and false negative outcomes. Between days 50 and 90 of pregnancy, the test in camels was deemed to be 85% accurate.

Two tests on milk can be used to detect pregnancy in cows:

1. Milk-alcohol coagulation test:

It involves mixing milk from pregnant cows with alcohol in equal parts and letting it stand for one to three hours.

2. Copper sulphate test:

It can be used to determine whether an animal is pregnant by combining 1mL of milk with a few drops of 3% copper sulphate. Low accuracy (52.0–64.2%) Frequently, tests are equivocal and complicated by breeds and diseases of the udder.

Costa's test:

It was created by Costa (1927) to screen for pregnancy in female humans. Using a 65.3% accuracy rate, the test was performed on cows and was known to be positive after 38 days of insemination. The underlying theory is based on the haematin sedimenting in novocaine solution. After adding 3 drops of blood and centrifuging the combination of 1.5 mL of 2% novocaine and 5% sodium citrate solution, add 1 drop of formalin. Precipitate of a grey or greyish yellow colour or hue usually emerges within 15 minutes of pregnancy.

Assay of gonadotrophins

Equine chorionic gonadotrophin secretion is adequate to serve as a diagnostic marker molecule for pregnancy. Equine pregnant females' urine and serum can both be used to detect endometrial cups, which can form as early as day 35 and secrete the equine chorionic

gonadotrophin (eCG). The eCG, which serves as the foundation for biologic tests and the tests now used on farms, continues to be secreted from day 40 of pregnancy until day 120.

1. Aschiem Zondek (AZ) test:

Mice are given either 5 ml IP or 0.5 ml sc of test mare's serum every day for two to four days. 2–3 rats (aged 22 days). Rats are slaughtered either 72 hours after IP injection or 96 to 120 hours after SC injection. Multiple corpora haemorrhagica on the ovaries and uterine edoema are signs of a positive test. 90% accuracy was achieved between the first 60 and the last 100 days of the mare's pregnancy.

2. Friedman test (rabbit test):

Rabbits (aged 14 to 20 weeks) housed in isolation receive an IV injection of test mare's serum (2 cc). Laparotomy is carried out 24 hours later and the presence of corpus haemorrhagicum and uterine edoema are signs of a positive test.

Pregnancy associated glycoproteins (PAG)

PAGs are produced in ruminant animals including cattle, buffalo, sheep and goats. From bovine foetal membrane extracts, two pregnancy-specific proteins (PSP) A and B have been discovered. PSP-B was discovered to be exclusive to the placenta, whereas PSP-A was identified as a-fetoprotein. From 29 to 30 days following breeding, it is possible to accurately verify the presence of molecules in the maternal circulation. Continually present in maternal blood throughout pregnancy and for up to 100 days after delivery.

Serum radioimmunoassay is used in the test. The PSPB's sensitivity and specificity at 29–30 days after insemination are known to be 92.0% and 82.6–91.9%, respectively, based on RIA. The PAG molecule can be found in cow serum thanks to ELISA techniques.

Limitations

- Non availability of the protein in milk or urine.
- Presence of PAG up to 100 days postpartum leading to false positive result.
- Non availability of commercially available kits for its detection.

Early pregnancy factor

The Rosette Inhibition Bioassay was initially used to identify the condition in pregnant mice, and afterwards in sheep and cattle. between 24 to 48 hours of fertilisation, was found in the serum of every species examined, and vanished within 24 to 48 hours following embryo death or removal. Early pregnancy factor (EPF), an immunosuppressive substance that manifests as early as 6 to 48 hours after mating and works to reduce the immune response in the mother, enables pregnancy to develop. On days 13–16 and 25 after breeding, pregnant and non-pregnant cows

showed substantial differences in rosette inhibition titers. In the US, there is a commercially available kit (ECF test, Concepto Diagnostics Knoxville, TN). However, it is well recognised to have low reliability and requires significant improvement.

Relaxin assay

Relaxin can be detected in the peripheral circulation of pregnant bitches at 20 to 30 days of gestation while it is missing in non-pregnant bitches at all phases of the reproductive cycle. It is produced by the placenta in cats and dogs, and by reducing uterine activity and aids in maintaining the uterus quiescent. Canine relaxin enzyme-linked immunoassay (ELISA), which is commercially available, was used in a clinical investigation of domestic dogs (ReproCHEK, ®a Synbiotics Corporation, San Diego, CA, USA) as it is only produced during pregnancy. Earlier than 25 days following ovulation, the hormone has been seen in maternal peripheral blood. During the third week of pregnancy in cats, it manifests, with concentrations dropping off soon before delivery.

Progesterone hormone assay

CL formed on the ovary subsequent to ovulation produces progesterone for maintenance of pregnancy for a reasonable time period in some species and for entire gestation in other species like the cow, buffalo, goat and sow. Low levels of progesterone in maternal blood around 18 to 24 days after breeding can indicate that the animal is not pregnant, while high levels can indicate that the animal is most likely pregnant. Tests done between 18 and 24 days after breeding showed a specificity of about 98%. Because early embryonic mortality tampers with the results, the test's accuracy for pregnant animals is limited (only 75% of the time).

Limitation

- Due to their high price and low specificity, commercially available ELISA, plasma, or milk progesterone assay kits have not gained much attraction.
- Both non-pregnant cows who don't enter oestrus again and pregnant cows whose embryonic demise happens later may produce erroneous results.
- Because pregnant bitches do not create placental progesterone and their levels of progesterone are comparable to those of non-pregnant bitches, a progesterone assay cannot be used to determine whether a bitch is pregnant.

Estrone sulfate

Being produced by the conceptus or the feto-maternal axis, its presence in the urine, milk, faeces, or blood is a sign of pregnancy. The availability of a suitable laboratory and of commercial assay kits are prerequisites for detection. Steroids can be detected in urine, serum, or

other bodily fluids using radioimmunoassay, enzyme immunoassay, or other more accurate and specific diagnostic techniques. For zoo and feral species, where faeces are the easiest specimens to collect, evaluation of steroids like oestrogen from faeces is particularly beneficial. Using on-farm kits like Wee-Foal-Checker® or Equitest ES®, which require urine or serum as the test material, commercial kits have been created for pregnancy diagnosis in mares. It is advised to use these commercially available tests only after 120 days of gestation have passed and is advisable for small horses and donkeys as rectal palpation or ultrasonography are exceedingly difficult to use for diagnosing pregnancy in these animals.

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QUORUM SENSING IN AQUACULTURE HEALTH MANAGEMENT

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Introduction:

Today's era is about to use natural inhabitance as economic resources, out of which aquaculture is largest growing sector which not only cope up food but also economic benefit as aquaculture growing day by day and certain technologies used for rapid production of fishes measure outbreaks occurs in aquaculture due to outcome of various diseases hence certain methodologies implemented to checkout disease outcome and hence Quorum sensing will be useful in the field of aquaculture.

Quorum sensing is a method by which bacteria use signal molecules to regulate gene expression in response to population density. Prokaryotes use the widespread phenomena of Quorum sensing to interact with eukaryotes and with one another. Numerous biological processes, including Biofilm formation, the production of virulence factors, swarming motility, bioluminescence, etc., are supported by it, which is driven through Quorum sensing signaling molecules in a density dependent way. Quorum sensing, which is frequently utilized by pathogens (disease-causing organisms) in disease and infection processes, allows populations of bacteria to communicate and coordinate behavior among themselves. Tomasz *et al.*, discovered bacterial activity incorporating Quorum sensing in the middle decades of the 1960s while researching Pneumococcus's after that it been nomenclatures as Streptococcus pneumoniae's) capacity to absorb free genetic material (DNA) from its surroundings.

Bacterial populations, signal molecules, and behavioral genes make up typical Quorum-sensing systems. Bacteria produce signal molecules, designated autoinducers, into the environment, where their concentration increases over time as the quantity of bacteria expands. The compounds are detected by bacterial populations after they reach a certain concentration threshold, at which point the relevant response proteins are activated, controlling a variety of behaviours includes infectiousness, horizontal transfer of genes, creation of biofilm, and virtuosity Quorum sensing is a key take up a note related with protein production in relation with bacterial behavior and the coordination among Quorum sensing particles. Mechanism in many microorganisms since many of these mechanisms is only functional at specific population numbers. Present chapter deals with the study of Quorum sensing in aquaculture health management.

Infection and Quorum sensing

Quorum sensing is one of the many social behaviors and intercellular communication mechanisms that bacteria have developed over the course of evolution. Since Quorum sensing has undergone extensive molecular research, many species' mechanistic features are well understood and frequently contribute to pathogenicity affects the virulence of certain plant pathogenic organisms as well. Despite the part Quorum sensing plays in virulence during animal and plant laboratory-engineered infections, Quorum sensing mutants are frequently isolated from natural infections, indicating that the function of Quorum sensing during infection and its role in pathogenesis are still poorly understood and are promising areas for further research. We talk about the function of Quorum sensing during infection.

The role of Quorum sensing during infection of various organisms has been investigated. Bacteria with a variable variety, particularly gram-negative species viz. (alpha), (beta), and (gamma). *Pseudomonas aeruginosa* is among the pathogenic bacteria, one the greatest comprehension of the factors in Quorum sensing to regulate virulence [2]. N-acyl homoserine lactones (AHL) are used in the majority of gram-negative bacteria Quorum-sensing systems, that has been examined to far [3].

The AHL is also involved in bacterial bioluminescence and Quorum sensing, between other life activities. despite these to it, it performs a crucial part in the susceptibility the the food to spoilage [4].

When presented with AHLs with four to eight carbon acyl side chains, the acetylated homoserine lactone biosensor *Chromobacterium* (CV026) responds effectively, but it responds extremely badly to AHLs that have acyl chains of at least C10 [5].

Environmental factor and Quorum sensing

There are many salty ecosystems, including marine and hypersaline habitats, in the world. These can be salty soils, salty lakes, sun salterns, or seawater. To adapt to the stress circumstances produced by these habitats, the bacteria that live within produce and nurture specific bioactive compounds and physiological pathways. Based on indices , they are capable of producing chemicals with unique characteristics from those present in non-saline environments. Many marine organisms, including bacteria, have demonstrated the ability to tamper with Quorum-sensing intercellular communication networks in recent researches. Quorum quenching (QQ) and Quorum sensing inhibition (QSI) are the two primary mechanisms of Quorum sensing interference. Quorum quenching and Quorum sensing inhibition (QSI) are the two major mechanisms of Quorum sensing interference [6]. Certain bacteria use a method of chemical communication called Quorum sensing to control a variety of crucial for biochemical and habitat specific bacterial behaviors. The interest in Quorum sensing and its function in the ocean has recently grew, despite the fact that it was initially discovered in a marine bacterium nearly four

decades ago. It is now known that Quorum sensing, controlled by signals such as acylated homoserine lactones (AHLs) or furanosyl-borate diesters [autoinducer-2 (AI-2) molecules], participates in critical processes within the marine carbon cycle, the health of coral reef ecosystems, and trophic interactions between a variety of eukaryotes and their bacterial associates. Surface-attached (biofilm) communities are where the most researched QS systems in the ocean are found, and they depend on AHL.

The most well studied Quorum sensing systems in the ocean are surface-attached (biofilm) communities that rely on AHL signaling. AHL-Quorum sensing is very sensitive to the physical, chemical, and biological characteristics of the environment, and it can respond to anthropogenically induced change, such as ocean acidification and rising sea surface temperatures [7]. Beside these in terms of fresh water it may be utilized as to treat waste water, there are certain documents which show some engineered biological treatment processes by emphasizing the role of Quorum sensing signaling and Quorum sensing inhibition, which will also help us develop efficient approaches for controlling microbial adhesion, colonization, and biofilm formation. Recently discovered Quorum sensing mechanisms and their role in the formation of biofilms for different species. The signaling molecules involved in various microbial granulation processes and potential applications of some of their natural and synthetic alternatives in the treatment of membrane biofouling are given special consideration.

Quorum sensing its detection and biosensor

Quorum sensing plays a major role during state of infection in diseased fish. Early detection of pathogen leads to lesser spread of infection hence we have to opt certain methodologies that should be reliable and quick. As soon as the bacterial concentration exceeds a predetermined threshold, Quorum sensing systems produce or passively release different signaling molecules viz. Acylated homoserine lactones, Diffusible Signal Factors, Autoinducer-2, Diketopiperazines, 4-Hydroxy-2-Alkylquinolines. Early signs of pathogenicity can be seen in signaling molecules. The beginning of an infection can be determined by looking for these chemicals *in vitro* or *in vivo*.

A bioreceptor, also known as a monoclonal antibody, RNA, DNA, glycan, lectin, enzyme, tissue, or whole cell, is an immobilized sensing device enabling a biosensor to detect a target biomarker particular to a certain pathogen. This technology can significantly improve the assessment of the health of fish produced for food by providing methods to monitor the health of fish, determine their physiological condition, and detect abnormalities at an early stage.

Commercial cell analyzers have recently been introduced to provide convenient and dependable equipment for life science research and pharmaceutical development in response to the impedance measurement technique's rapid development [8].

Bacteriocin production and Quorum sensing

Through the use of Bacteriocin, bacteria defend themselves from external barriers over other bacteria. Some species seem to conditionally alter their Bacteriocin production in response to their environment.

We looked examined whether variations in the cost and utility of making Bacteriocin in the bacteria *Lactobacillus plantarum* may account for such conditional behavior. they have been discovered that:

- i. either the injection of a synthetic autoinducer peptide or a plasmid that constitutively encodes for the production of this peptide might upregulate the production of bacterial Bacteriocin;
- ii. Bacteriocin production is cost effective and may sometimes leads in higher cost, resulting in reduced growth when grown in poor and, to a lesser extent, in rich media;
- iii. Bacteriocin production offers a fitness advantage when grown in competition with sensitive strains; and
- iv. the fitness advantages offered by Bacteriocin production are greater at higher cell densities. These findings demonstrate how biotic and abiotic factors might influence the costs and advantages of increasing Bacteriocin production. [9]

Additionally, acylases, lactonases, oxidoreductases attack the AIs of pseudomonads. One of the following outcomes can be brought on by enzymes: There are three ways to employ AI degradation:

- i. To fine-tune the endogenous Quorum sensing system;
- ii. To modulate the Quorum sensing system; and
- iii. To use ai degradation as a method to exploit ais as nutrition sources. The Quorum sensing system of pseudomonads is impacted by the inactivation of ai synthases and the alteration or degradation of ais [10].

Bacteriocin production based upon Quorum-sensing

An essential but still poorly understood step in bacterial gene regulation is the down-regulation of Quorum-sensing dependent pathways. In this study, we demonstrate that the gene regulator *plnC* not only functions as an activator gene in *Lactobacillus plantarum* C11's Quorum-sensing based Bacteriocin synthesis, but it also simultaneously codes for shortened versions that have been demonstrated to suppress Bacteriocin production. The shortened species of *PlnC* are thought to be translated from alternate start codons found in the so-called receiver domain of the regulator, according to DNA sequence analysis and amino acid N-terminal sequencing.

They have been carried out a series of systematic truncation mutations, ten in the receiver domain, one in the hinge region, and two in the C-terminal DNA-binding domain, to analyze the structure-function relationship of shortened species of *PlnC*. It was discovered that Bacteriocin

synthesis was suppressed by any truncation mutation having a damaged receiver domain together with an intact DNA-binding domain. In additional Quorum-sensing based Bacteriocin systems (spp in *L. sakei* LTH673 and NC8-pln in *L. plantarum* NC8), a similar gene repression mechanism mediated by truncated regulators was also discovered, raising the possibility that this mode of repression may represent a common strategy used by bacteria to down-regulate specific Quorum-sensing based pathways [11].

Quorum sensing and antibody disruption

Stressful situations can arise on intensively farmed fish, and this may lead to disease outbreaks. Historically, chemotherapeutic drug delivery was used to treat disease in farmed fish. Alternatives have been sought after, though, due to the rise in bacterial strains that are resistant to antibiotics, the expensive expense of chemotherapy, the temporary nature of the protection, and widespread concern over the use of chemicals. Despite the fact that the first study on fish vaccination appeared in the 1940s, it wasn't until the late 1970s that vaccination as a disease prevention strategy became economically viable. The majority of antigens can cause an antibody response in fish. However, protection is not necessarily correlated with a large antibody response to an antigen. On the other hand, protection can be attained in the absence of a substantial antibody response. here I would like to focus upon the nature, mode of expression, and its specificity of antibodies generated by fish pathogens. Quorum sensing been utilized to study various antibodies the study conducted by Junguk Park, *et.al* [12].

Microdiffusible compounds known as self-inducers are used by bacteria to communicate via the Quorum sensing mechanism. The expression of virulence genes is one of many microbial processes that have been shown to be under the control of QS. We provide an immunopharmacotherapeutic strategy for reducing QS in the Gram-positive human pathogen *Staphylococcus aureus* in this article. A rationally created hapten was used to elicit an anti-autoinducer monoclonal antibody, AP4-24 H11, which effectively suppressed QS in vitro by sequestering the autoinducing peptide (AIP)-4 produced by *S. aureus* RN4850. Importantly, AP4-24H11 completely protected against a deadly *S. aureus* challenge and decreased *S. aureus* pathogenicity in an abscess formation animal model. These results lay a solid groundwork for future research on the use of immunopharmacotherapy in the management of bacterial infections. where Quorum sensing regulates the expression of virulence components.

Implications of bacterial Quorum sensing in the future

A novel target for antibacterial therapy is Quorum sensing. Finding new methods to treat bacterial infections has become necessary due to the persistent appearance of numerous drug-resistant bacterial strains. Quorum sensing has been shown to be an intriguing target for the development of new anti-virulence therapies due to the fact that a wide range of organisms use it to regulate the production of virulence factors. If the Quorum sensing mechanisms that regulate

virulence factors can be addressed, the pathogenic organisms can be made non-virulent. The majority of the scientific study has concentrated on AHL-based QS systems and has been on the synthesis and characterization of AI analogs. AHLs can now be accurately referred to as "small molecule toxins" due to their inherent cytotoxic action [13].

The Quorum sensing pathways can be damaged on a variety of levels, including those of AIs and R proteins, which are extremely specialized for one another. Non-cognate AIs often only slightly activate or may completely prevent R protein activation. As a result, analogs that bind to R proteins but do not activate them may function as antagonists to block AI binding, which would then stop the Quorum sensing cascade. Numerous bacteria, including *V. fischeri*, *A. tumefaciens*, *Chromobacterium violaceum*, and *Aeromonas salmonicida*, have already shown that AI analogs can prevent the activation of R proteins [14]. AHLs can be broken down by an enzyme that was recently found in a *Bacillus* isolate. The *aiiA* gene (AI inactivation) encodes this enzyme, which has two domains that are similar to the active sites of the following metalloenzymes: arylsulfatase, metallo-B lactamase, and glyoxalase II. The production of proteolytic enzymes was lowered and AI production was noticeably reduced when *aiiA* was expressed in *E. carotovora* [15]. The biosynthesis pathways of several AHL molecules have been clarified in order to discover another method of interfering with Quorum sensing. One of the most efficient ways to stop the Quorum sensing cascade would be to disrupt the AHL biosynthetic route and stop AHL production, potentially by using analogs of AHL precursors [16–17].

The development of the anti-AHL monoclonal antibody (mAb), RS2-1G9, elicited against a synthetic 3-oxo-C12-HSL analog, recently pioneered an immunotherapeutic strategy for QS quenching. The *in vitro* neutralization of 3-oxo-C12-HSL by the RS2-1G9 was reported to effectively reduce QS signaling in *P. aeruginosa* and provide protection to mammalian cells.

Utilizing a hapten with a more stable lactone component in place of the hydrolytically labile thiolactone, monoclonal antibodies against staphylococcal AIPs have been created. The mAbs created in this way were tested *in vivo* and *in vitro* and were found to have strong Quorum quenching properties, including the ability to protect mice from a potentially fatal *S. aureus* infection [18,19].

Recently, a novel *Bacillus* spp. strain from the fish *Carassius auratus gibelio*'s intestine was isolated and named Quorum sensing Inhibitor-1 (QSI-1) by the researchers. The isolated QSI-1 has at least one type of AHL-degrading enzymes that can break down the AHLs of the fish pathogen *Aeromonas hydrophila*, significantly reducing the production of its extracellular proteases, increasing the survival rate of infected fish. The isolated QSI-1 is also capable of using AHL molecules as the only source of energy. Thus, it's possible that this QSI-1 strain will be utilized in aquaculture as a probiotic [20].

A promising application area for QSIs appears to be the creation of antibacterial coatings based on natural or modified QSIs, in addition to their use as medications or therapeutic agents. It has been investigated how to immobilize furanone derivatives on polymer materials to create antibacterial medical devices, which gives us ideas for how to employ marine bacteria-derived QSIs to create aseptic or antibacterial materials for disease treatments [21].

Conclusion:

Present chapter have designed to understand the acute use of Quorum sensing mechanism not only for the detection of pathogen but also useful in Numerous biological processes, including Biofilm formation, the production of virulence factors, swarming motility, bioluminescence, etc.our suggestion is that to be focus upon Bacteriocin production based upon Quorum-sensing and here we will get an acute identification of antibodies so it will help to design preventive drugs and medications against potential aquaculture pathogen.

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PCR BASED METHODS IN FISH MICROBIOLOGY

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Introduction:

Developments in molecular biology have sped up the development of novel techniques for identifying fish diseases. Molecular diagnostic techniques are quicker, more accurate, and more sensitive than approaches using pathogen culture, serology, histology, and biological chemistry which are typically used to determine the sources of fish sickness. Molecular diagnostic techniques are useful for identifying particular pathogens that are challenging to cultivate in vitro or need a lengthy development period. They are also substantially faster at producing results than culture. It facilitates quicker bacteremia identification, especially for low levels of bacteria in specimens, and sooner informed decision-making.

The most important molecular techniques are PCR-based molecular diagnostic procedures, such as Random Amplified Polymorphic DNA (RAPD), Real-Time Polymerase Chain Reaction (RT-PCR), Multiplex Polymerase Chain Reaction (multiplexPCR), and others. In recent years, these have been used more and more to diagnose fish sickness. Disease can be diagnosed using molecular diagnostic techniques even in asymptomatic fish breakouts might be avoided. As a result, the need for antibiotic therapy can be decreased, and the emergence of germs resistant to antibiotics can be stopped. In this chapter, we make an effort to provide an overview of the potential of PCR-based molecular diagnostic techniques and their use in identifying fish pathogens.

PCR based methods in fish pathogen identification

Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction (PCR) is called as molecular photocopying. This is a quick and low-cost method used to copy brief DNA segments. Studies of isolated fragments of DNA are almost difficult without PCR amplification since considerable volumes of a sample's DNA are required for molecular and genetic analysis. (Mullis *et al.* (1986), who invented PCR, received the 1993 Nobel Prize in Chemistry for his contributions to the field of DNA research.

How and why is PCR used?

The DNA generated by PCR can be used in a variety of laboratory techniques after it has been amplified. For instance, PCR was a key component of most mapping techniques used in the Human Genome Project (HGP). A number of laboratory and clinical methods, such as DNA fingerprinting, the detection of germs or viruses (especially AIDS), and the identification of genetic abnormalities all benefit from the use of PCR.

PCR machine procedures

1. Denaturation

Using a thermal cycler, the solution inside the tube is heated to at least 94°C (201.2°F). Denaturation of double-stranded DNA is the process by which heat causes the hydrogen bonds in the original DNA sample to dissolve, separating the DNA into single strands (Borah, 2011; Sarah Maddocks and Jenkins, 2017).

2. Annealing

The sample mixture is subsequently cooled to a temperature of 50 to 60°C (122 to 140 °F), which enables the DNA polymerase enzyme and DNA primers to attach to the distinct strands of DNA that were caused by the heat (this is known as annealing of the primers). The nucleotides (A, T, C, and G) from the newly added mixed solution will now pair with the variously separated strands of DNA produced by the heating procedure (Borah, 2011; Tymoczko *et al.*, 2011).

3. Extension

They combine to create a new complementary strand of DNA, often known as an extension of the DNA. From each of the single strands of the original sample molecule, a new duplicate double-stranded DNA molecule has been created (Caetano-Anollés, 2013; Drouin *et al.*, 2007). The temperature swings between 95°C and 50–60°C. The thermal cycler, which automatically repeats the heating and cooling cycles of the process, is then used to repeat the cycle approximately 20 to 40 times. Every time the cycler performs a heating/cooling cycle, the resulting DNA sequence is doubled. As a result, after 35 doubling cycles, a single short piece of DNA from one sample can be amplified to generate millions of copies (Baynes and Dominiczak, 2009).

Nested PCR

Two sets of unique primer pairs were used in two subsequent PCR amplifications in order to increase the sensitivity and specificity of the PCR reaction. Low-abundance genes may be amplified by the nested PCR since it uses less target DNA (Liop *et al.*, 2000). In a nutshell, the

amplicons produced by the first amplification are subjected to a second amplification. Kamolvarin *et al.* (1993) provided the initial description of this method.

Due to numerous vessel openings required to complete the two amplification cycles, nested PCR has a significant risk of Eppendorf contamination. This might have led to a sharp increase in false positives. At extremely low bacterial infection densities—3–4 bacteria per gram of fish tissue—Nested PCR allowed the identification of *R. salmoninarum*, *V. vulnificus*, *A. hydrophila*, *E. tarda*, *S. iniae*, and *P. damsela* (Farzadnia and Naeemipour, 2020). In Egypt, mixed infections of *Streptococcus* and *Myxobolus tilapiae* coinfections in moribund Nile tilapia have been successfully diagnosed using nested PCR (Eissa *et al.*, 2021b).

Multiplex PCR

Using several primer pairs, multiplex PCR can concurrently target and amplify many distinct genes from various bacterial pathogens, producing specialized DNA products of various sizes. The first time this method was mentioned as a potential method to identify deletions in the dystrophin gene was in 1988 (Chamberlain *et al.*, 1988). This method is time- and money-efficient, with great accuracy. It also reduces pipette mistakes (Ador *et al.*, 2021). This technique has been used to simultaneously identify five different pathogenic bacteria (*Y. ruckeri*, *A. salmonicida* subsp. *salmonicida*, *A. hydrophila*, *R. salmoninarum*, and *F. columnare*), three different pathogenic bacteria (*A. hydrophila*, *F. columnare*, and *E. ictaluri*), four different pathogenic bacteria (*V. parahaemolyticus*, *V. cholerae*, *V. alginolyticus* and *V. vulnificus*) (Adams and Thompson, 2011).

Three distinct pathogens, namely *A. salmonicida*, *F. psychrophilum*, and *Y. ruckeri*, were simultaneously identified using this technique. The simultaneous recognition of four different pathogens, including *F. psychrophilum*, *L. garvieae*, *Ps. putida* and *Ps. aeruginosa*, was also accomplished using the mPCR. Additionally, *S. parauberis*, *S. iniae*, *L. garvieae*, and *S. difficilis* were all simultaneously identified using multiplex PCR (Mata *et al.*, 2004).

Identification of pathogens

Vibrio, *Aeromonas*, *Streptococcus*, and *Edwardsiella* were identified using multiplex PCR at extremely low bacterial concentrations of 100 CFU in fish tissues and 50 CFU in culture. To prevent reactions between target DNA and primers, a multiplex PCR could not use more than six primer pairs. The main drawback of this approach is that it may require multiple tries to standardize and that primer pair sets may become self-inhibitory. In Egypt, *A. hydrophila* and *Y. ruckeri* were found to co-infect farmed fish using multiplex PCR (El-Hady and Ahmed, 2014).

Real-time PCR

When SYBR Green dyes bind to double-stranded DNA, they release fluorescence through the excited fluorophore, allowing real-time PCR or quantitative PCR to count the amount of amplified products. RT-PCR, as opposed to regular PCR, identifies the targeted DNA amplification while the PCR progresses rather than at the end. Real-time PCR has a very high sensitivity and specificity and can detect extremely little amounts of DNA in various tissue samples. The RT-PCR process comprises a series of temperature changes and is repeated 25–50 times. The length of time and recommended temperature for each cycle of RT-PCR depend on a variety of variables, including the temperature at which the primers anneal, the quantity of divalent ions present in the reaction, the concentration of deoxyribonucleotides (dNTPs), and the DNA synthesis enzyme (Austin, 2019).

This method eliminates the need for gel documentation while allowing for very accurate detection of low-copy DNA targets. The ability to quantify the DNA or RNA template present in the specimen is the main benefit (Tom *et al.*, 2004). For the development of the assay, the RT-PCR technology calls for more technical proficiency. Additionally, there are just a few standardized protocols and no RT-PCR kits for the many genes of the causative agents.

As a result, the assay is a costly and challenging test to scale. Even while this technique might show when pathogenic DNA material is present during an infection, it cannot tell whether the host is infected or not. Pathogenic bacteria in fish, like *A. salmonicida* and *S. parauberis*, are identified and measured using real-time PCR. *Francisella noatunensis* subsp. *orientalis* was detected with 99.2% accuracy and sensitivity in the farmed *O. niloticus*. According to Chapela *et al.* (2018), the multiplex qPCR was successfully used to detect concurrent infections with *A. salmonicida*, *V. anguillarum*, and *T. maritimum*.

The results showed the excellent specificity of this technique to identify coinfection scenarios or issues with natural contamination because they were consistently confirmed in all methodological replicates. Real-time PCR was used in Egypt to identify the co-infection of Nile tilapia with the tilapia lake virus and other *Aeromonas* species (Nicholson *et al.*, 2017).

Loop-mediated isothermal amplification

Nucleic acid amplification can be accomplished with the low-cost, straightforward, quick, and highly specific LAMP technique. To target particular genes, this instrument employs a Bst DNA polymerase and six distinct primer sequences. This polymerase enzyme lacks 5'-3' exonuclease activity but exhibits high 5'-3' DNA polymerase activity and strand displacement. The LAMP amplification reaction uses this enzyme. The amplified products, which produce >

500 mg/mL of PCR products, are stem-loop DNA structures with several inverted repeats of the target and cauliflower-like structures with multiple loops (Nagamine *et al.*, 2001).

Conclusion:

As per the references cited, we conclude that the use of PCR in fishery science, have various horizons as they are useful in acute identification of pathogen. So, as we can diagnose new genetic sequences so as the mild change in fish pathogen may lead in variable modes of transmission.

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MOLTING IN CRUSTACEANS

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Abstract:

Crustaceans are of economic importance and are the targets of aquaculture or commercial fishing activity, contributing positively to food security because they are important sources of food protein. Due to the discontinuous nature of crustacean growth, and the absence of anatomical structures for aging, analysis of crustacean growth and the hormones connected with it is troublesome for aquaculture methods. A crustacean's exoskeleton is also known as its shell or cuticle, which is made primarily of chitin, calcium salts, protein, and fat, which is a rigid component that covers the animal's entire exterior. A new cuticle develops during the molting process, which is followed by periodic shedding (ecdysis) and replacement of the old cuticle. The moult cycle, divided into the four primary stages of intermolt, pre-moult, ecdysis, and post-moult, is the most important and difficult step in the physiology of crustaceans. It includes the time between two successive moults. Calcium (Ca) is necessary because calcium carbonate (CaCO₃) is deposited in the newly formed exoskeleton to harden it after the old exoskeleton has been shed. Since calcium is essential for the production of the exoskeleton, calcium levels fluctuate during the molt cycle. The maturity of crustaceans is tightly controlled by these eyestalk components. Ecdysteroids, also known as methyl farnesoate (MF) and gonad stimulating hormone (GSH) are non-eyestalk hormones that are involved in the control of maturation. They are produced by the Y-organs and mandibular organs, respectively. The molt inhibiting hormone (MIH) prevents the formation and/or secretion of ecdysteroids from the Y-organ, while vitellogenin inhibitory peptide (VIH) prevents reproduction when under stress circumstances like hypoxia, hyper or hypothermia. This present chapter deals with the detail aspects of crustacean moulting.

Keywords: Moulting, Crustaceans, Hormones, Neurocontrol, Calcium level, MIH, MOIH, Y Organ, Mandibular organ.

Introduction:

A crustacean is an invertebrate that includes a variety of species. Whether evaluated by species richness, diversity in anatomy, or diversity in lifestyle, crustaceans represent one of the

animal kingdom's most remarkable evolutionary radiations. Its members inhabit most habitats on Earth, from the deepest ocean trenches to mountaintops and deserts, and range from microscopic plankton mites to terrifying giant crabs to sessile barnacles to amorphous parasites that are almost unrecognizable as animals. Many of these species are of economic importance and are the targets of aquaculture or commercial fishing activity, contributing positively to food security because they are important sources of aquatic food protein (Duffy, 2010).

Due to the discontinuous nature of crustacean growth, and the absence of anatomical structures for aging, analysis of crustacean growth and the hormones connected with it is troublesome for aquaculture methods. The crustacean's exoskeleton protects it but also restricts its growth. The shrimp molts to grow. As a result, unlike fish, the animal's growth occurs in steps or sequential stages rather than continuously.

Molting (Ecdysis)

A crustacean's exoskeleton is also known as its shell or cuticle, which is made primarily of chitin, calcium salts, protein, and fat, which is a rigid component that covers the animal's entire exterior, including the gills and the anterior and posterior regions of the GI tract. A new cuticle develops during the molting process, which is followed by periodic shedding (ecdysis) and replacement of the old cuticle. They first loosen the connective tissues that separate their living tissues from their extracellular cuticles, break free of these cuticles relatively quickly, expand their new, flexible exoskeletons with water or air, and then quickly harden them for defense and locomotion—both of which are necessary for growth, reproduction, and metamorphosis (Hosamani *et al.*, 2017).

The moult cycle, divided into the four primary stages of intermolt, pre-moult, ecdysis, and post-moult, is the most important and difficult step in the physiology of crustaceans. It includes the time between two successive moults. Although it may be difficult to identify between these stages, it is easy to spot the behavioral variations between them (Waddy *et al.*, 1995).

- 1. Inter-moult stage (Anecdysis):** The most extended phase of the molt cycle, the inter molt period, involves muscle regaining and the storing of energy reserves such as glycogen and lipids takes place.
- 2. Pre-moult stage (Proecdysis/ Peeler stage):** Pre-molt is characterized by somatic muscle atrophy, exoskeleton resorption, and the development of a new exoskeleton in anticipation of the commencement of ecdysis.
- 3. Moult stage (ecdysis):** Entails the exoskeleton being shed as a result of a fast uptake of water from the environment that causes the exoskeleton to burst.

- 4. Post-moult stage (Metecdysis/ Soft back stage):** The intake of water during post-molt allows calcium and other minerals to deposit, expanding the new, still-soft exoskeleton, which is crucial for the animal's growth. Sclerotization and mineralization are used to harden the exoskeleton.

Calcium levels in different moulting stages

Calcium (Ca) is necessary because calcium carbonate (CaCO_3) is deposited in the newly formed exoskeleton to harden it after the old exoskeleton has been shed. Since calcium is essential for the production of the exoskeleton, calcium levels fluctuate during the molt cycle. Depending on the moulting stage, calcium requirements vary in intensity. When crustaceans molt, their old exoskeleton removed, and the new one calcified during the post-molt period, these are the critical times for this mineral intake. For aquatic species, whole body Ca absorption occurs mostly through the gills and changes from intermolt to premolt to postmolt in these animals. The body typically has the most calcium cations. The exoskeleton is often fully calcified during intermolt, and the animal is in calcium equilibrium with its surroundings. Calcium is resorbed from the skeleton throughout the premoult stages and may be lost to the environment or stored within the body. Aquatic species typically have significant losses and little calcium storage. Aquatic organisms can only lose soluble calcium through outward transfer over the gills.

There are numerous ways to store calcium; most of them share a common taxonomic pattern. The primary forms are calcium phosphate granules in midgut gland (Brachyura), gastrolith (Astacidea and some Brachyura), haemocoel (some Brachyura), posterior midgut caeca (Amphipoda), and ventral region of body (usually in Isopoda) cells. The skeleton is shed during ecdysis, and the calcium that was still in it is lost from the body. Following ecdysis, recalcification starts using calcium that was mobilized from the reserves. Other calcium sources are used concurrently or when the reserves are depleted.

As a result, both marine and freshwater organisms absorb calcium at comparable rates. These substantial reserves allow the animals to progress to an advanced stage of calcification and resume foraging and feeding, which are required to complete calcification. It is poorly understood how calcium metabolism is regulated during the intermolt cycle. Ecdysone appears to regulate calcium absorption and calcium synthesis during premoult, but it is unclear how it regulates calcium metabolism during postmolt and intermolt.

Most species have significant calcium concentrations in their haemolymph, however a sizable amount of this calcium is non-ionized. Although calcium resorption causes a rise in overall calcium levels in premoult, the concentration of ionized calcium barely changes. Blood

calcium levels typically decrease postmoult, perhaps falling below the premoult range (GREENAWAY, 1985).

Hormonal control on ecdysis

External factors that influence moulting through endocrine regulation include temperature, salinity, pH, the amount of food in the pond, and the presence of heavy metals in the water. Similar to the pituitary-hypothalamus of vertebrates, the eyestalk neural tissue of crustaceans secretes a number of neuroendocrine hormones. The primary secretory byproducts of eyestalk are biogenic amines, opioids, crustacean hyperglycemic hormone (CHH), molt-inhibiting hormone (MIH), crustacean gonad-inhibiting hormone (VIH/GIH), and vitellogenesis/gonad-inhibiting hormone (VIH/GIH). The maturity of crustaceans is tightly controlled by these eyestalk components. Ecdysteroids, methyl farnesoate (MF) and gonad stimulating hormone (GSH), are non-eyestalk hormones that are involved in the control of maturation. They are produced by the Y-organs and mandibular organs, respectively.

The CHH family of neuropeptides has the capacity to influence a variety of regulatory functions in the physiological system of crustaceans: The molt inhibiting hormone (MIH) prevents the formation and/or secretion of ecdysteroids from the Y-organ, while vitellogenin inhibitory peptide (VIH) prevents reproduction when under stress circumstances like hypoxia, hyper or hypothermia. The hormones produced by two endocrine systems, the eyestalk ganglia and Y-organs, are coordinated and controlled by two endocrine systems in the intricate process of molting. The synthesis and secretion of the hormone ecdysteroids (molt hormone, 20 Hydroxy-Ecdysone), one of the key factors known to regulate the process, are some of the influences on it. In decapod crustaceans, the molting gland, or Y-organ (YO), is the main location for ecdysteroid production (Ohira, 2021).

Ecdysteroids' concentrations change throughout the molt cycle and are influenced by the interactions of production, metabolism, and excretion. Based on how they affect the control of molting, the internal elements can be divided into two categories. "Positive regulators" are hormones that stimulate, whereas "negative regulators" are hormones that inhibit activity. MF, ecdysteroids, and GSH are positive regulators, while MOIH and MIH are negative regulators.

Ecdysteroid biosynthesis consists of two steps: the first involves turning cholesterol into 5-diketol, and the second involves turning 5-diketol into released chemicals. Stage 1 includes the "Black Box" reactions involving 3-oxo-(4) intermediates, the conversion of (4)-diketol to 5 - diketol by 5 -[H]-reductase, and the conversion of cholesterol to 7-dehydrocholesterol (7DC) by 7,8-dehydrogenase. According to the species, the stage two reactions yield four main byproducts: ecdysone, 3-dehydroecdysone (3DE), 25-deoxyecdysone (25dE), and 3-dehydro-25-

deoxyecdysone (3D25dE). These substances are transformed by peripheral tissues into the hormones 20-hydroxyecdysone (20E) and ponasterone A (25-deoxy-20-hydroxyecdysone, or 25d20E), which are active (Rowley, 2016).

Molt-inhibiting hormone (MIH), a polypeptide generated and secreted by the X-organ/sinus gland complex of the eyestalks and crustacean hyperglycemic hormone (CHH), regulates the Y-Organ in the cephalothorax. The neuroendocrine axis between the eyestalk ganglia and the Y-organs is a part of the long-loop feedback because MIH inhibits the activity of the Y-organs during the intermolt and post-molt stages. Elevated ecdysteroids at the premolt stage promote MIH production but inhibit its release in the eyestalk ganglia. Additionally, it implies that certain ecdysteroids may have short-loop feedback mechanisms that stimulate and inhibit ecdysteroidogenesis in Y-organs (Corgos *et al.*, 2007).

Throughout the molt cycle, the YO transitions between four physiological states, which are mediated by the signaling pathways for molt-inhibiting hormone (MIH; basal state), mechanistic Target of Rapamycin Complex 1 (mTORC1; activated state), Transforming Growth Factor-(TGF)/Activin (committed state), and ecdysteroid (repressed state). The eyestalk X-organ/sinus gland complex produces MIH, which prevents the production of ecdysteroids.

Due to the high quantities of MIH that inhibit the activity of Y-organs, intermolt stage organisms have low levels of ecdysteroids. A decrease in protein synthesis and cholesterol uptake in Y-organs is observed with the suppression of MIH, which may have an impact on the synthesis of ecdysteroids. The maximal number of binding sites increases dramatically after molting, as does the binding affinity of Y-organ membranes to MIH and the amounts of its second messenger, cGMP.

Ecdysteroids with increasing concentrations in the hemolymph start and direct premolt processes like exoskeleton synthesis, exoskeleton resorption, claw muscle atrophy, and limb regeneration growth. Ecdysis is brought on by a sharp decline in hemolymph ecdysteroids after premolt. The post-molt period's low ecdysteroid titer promotes the development of claw muscles and the completion of exoskeleton synthesis and calcification. In mature decapods, intermolt can last anywhere between weeks and years (Hiruma and Kaneko, 2013).

Ecdysteroids produced by the YO are primarily excreted by the antennal gland. By generating apolar conjugates, the hepatopancreas removes ingested ecdysteroids.

Mandibular organ

Methyl farnesoate (MF), which is produced by the crustacean mandibular organ (MO), causes molting by encouraging the production and release of ecdysteroids from Y-organs. The X-organ-sinus gland complex of the eyestalk produces and secretes MOIH (mandibular organ

inhibiting hormone), which inhibits the production of methyl farnesoate from the mandibular organ and its related regulatory actions (Borst *et al.*, 2001).

Responsiveness of Y-organs to MIH

An increase in cyclic 5' adenosine monophosphate (cAMP) is what causes MIH to take effect; in some species, cGMP is also implicated. The following set of findings suggests that a cyclic nucleotide is a key regulatory element: dibutyryl cAMP, adenylyl cyclase activators, or inhibitors of cyclic nucleotide phosphodiesterase all replicate the inhibitory effects of MIH. By reducing the number of receptor sites for the lipoprotein carrier of cholesterol, cyclic AMP prevents the receptor-mediated uptake of cholesterol (the obligatory ecdysteroid precursor). De novo protein synthesis, which is a factor in ecdysteroidogenesis, is likewise suppressed by MIH via cAMP.

The ability of Ca^{++} (or a Ca^{++} ionophore) to increase ecdysteroid synthesis, counteracting MIH action, suggests a function for cellular free calcium (Ca^{++}). Instead of altering adenylate cyclase activity, the process involves lowering cAMP levels by increasing phosphodiesterase activity via calmodulin. Ca^{++} counteracts the inhibition of protein synthesis caused by MIH or cAMP. Since MIH and Ca^{++} are antagonistic to one another, ^{45}Ca -preloaded cells release more Ca^{++} when MIH is present.

Protein kinase C (PKC), which is present in Y-Organ cells, is activated to produce more ecdysteroids. MIH has not much impact on PKC activity, but Ca^{++} stimulates it. These studies and others show that cAMP synthesis and Ca^{++} -mediated cAMP breakdown are downstream events in the PKC-activated rise in ecdysteroidogenesis. Regarding the latter, ecdysteroid production is dose-dependently inhibited by both selective and non-specific inhibitors of protein tyrosine kinases (PTK) (Corgos *et al.*, 2007).

Impacts of improper moulting in aquaculture

The significance of moulting for growth in farmed shrimp is examined in relation to the physiological cycle, induction triggers, behavioral changes, and compositional changes. Additionally, the components of successful moulting are reviewed, as are potential management options for moulting. Quality of the pond's water, health, and nutritional state are additional important factors in successful ecdysis.

The body of the shrimp is severely disrupted during molting. As its shell is just beginning to develop during the post-molting phase, it is particularly vulnerable. The cuticle does not yet act as a complete physical barrier, and the shrimp must use bodily reserves to mineralize and strengthen its brittle cuticle. At this time, certain illnesses are likely to manifest themselves. For instance, shrimp have shown a high susceptibility to the White Spot Syndrome Virus (WSS) in

stages A and B of the post-molt phase. The shrimp's internal environment is also severely impacted by the osmotic shock brought on by the significant water intake; this significant change severely disrupts the shrimp's cell activities.

Numerous methods of inducing ecdysis in crustaceans have been studied, including cheliped autotomy, which stimulates ecdysis to repair defense organs, eyestalk ablation, which reduces the production of molt-inhibiting hormone (MIH), and injection of hormones, which quickly increases the concentrations of ecdysteroids in the hemolymph.

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FUTURE DIRECTIONS IN FISHERIES AND AQUATIC ANIMAL HEALTH RESEARCH

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Abstract:

This abstract provides an overview of the diverse topics covered in the book chapter on "Future Directions in Fisheries and Aquatic Animal Health Research." The chapter explores the integration of technology in fisheries management and research, highlighting the potential of artificial intelligence, machine learning, and big data analytics in improving monitoring, management, and decision-making processes in aquatic animal systems. Additionally, the chapter delves into the applications of genetic tools and advances in genomics, highlighting the importance of genetic mapping, selective breeding, and gene editing technologies for disease resistance and improved productivity in aquaculture. Furthermore, the chapter discusses the emergence and spread of infectious diseases in aquatic ecosystems, emphasizing the impact of climate change and the importance of early detection, rapid diagnosis, and the One Health approach in combating zoonotic diseases. Moreover, the chapter explores the advances in vaccination and immunostimulants for enhancing the health and disease resistance of aquatic animals, including the potential of oral vaccines and mucosal immunity. Sustainable practices and environmental impacts in fisheries and aquaculture are also addressed, focusing on assessing environmental impacts, promoting sustainable aquaculture, and implementing ecosystem-based approaches for long-term sustainability. Additionally, the chapter examines the application of nanotechnology in aquatic animal health and fisheries, exploring how nanotechnology-enabled sensors, drug delivery systems, and antimicrobial agents can contribute to real-time monitoring, therapeutics, and disease prevention. The challenges and opportunities in marine conservation and restoration are discussed, highlighting conservation strategies, ecological restoration approaches, community engagement, and global cooperation. Furthermore, the chapter examines the impacts of climate change on fisheries and aquatic animal health, addressing the effects on physiology, behavior, and distribution, as well as the identification of adaptation strategies and mitigation measures. Lastly, the chapter considers socioeconomic and policy considerations,

evaluating the socioeconomic implications, promoting sustainable fisheries, and engaging stakeholders and communities in decision-making processes for effective management of fisheries and aquatic animal health. This comprehensive chapter provides insights into future research directions in fisheries and aquatic animal health, aiming to contribute to the advancement of sustainable practices and the conservation of aquatic ecosystems.

Integration of technology in fisheries management and research

Fisheries management and research play a crucial role in ensuring sustainable utilization of aquatic resources. Technology has been rapidly evolving, and its integration in fisheries management and research has revolutionized the way these areas are approached. By embracing technology, fisheries management and research organizations can improve data collection, analysis, monitoring, and decision-making processes. This note aims to explore the various technologies being integrated into fisheries management and research and highlight their benefits and challenges (Kemp *et al.*, 2023).

1. Remote sensing

Remote sensing technology involves the use of satellites, aerial platforms, and drones to collect data on the Earth's surface. This technology has enhanced the understanding of surface temperature, water quality, and the distribution and dynamics of fish and their habitats (Bellec *et al.*, 2019). Remote sensing enables fisheries managers and researchers to monitor and identify changes in fish stocks and habitat conditions, aiding in effective resource management.

2. Acoustic telemetry

Acoustic telemetry involves the use of underwater acoustic signals to track fish movement and behavior. By tagging fish with transmitters and deploying an array of receivers in water bodies, researchers can obtain detailed information on fish migration patterns, habitat utilization, and survival rates (Heupel *et al.*, 2018). This technology enables the identification of critical habitats and aids in the development of effective conservation strategies.

3. Electronic tagging

Electronic tagging technologies, such as archival tags and pop-up satellite tags, provide valuable insights into fish behavior and migration patterns (Schick *et al.*, 2020). These tags offer continuous data collection on fish movement, depth, temperature, and feeding habits. Such information is vital in understanding fish behavior, stock connectivity, and migration routes, enabling fisheries managers to implement adaptive management strategies.

4. Data analytics and modeling

Advances in data analytics and modeling have significantly improved fisheries management and research outcomes. By integrating large datasets, such as catch records, environmental data, and biological information, sophisticated models can be developed to predict fish population dynamics, the impact of fishing practices, and potential effects of climate change

(Punt *et al.*, 2016). These models aid in informed decision-making and the implementation of sustainable fishing practices.

5. DNA barcoding

DNA barcoding technology has emerged as a powerful tool for assessing fish species composition and population genetics. This methodology relies on analyzing specific DNA sequences to identify species and determine genetic diversity (Rubinoff *et al.*, 2006). DNA barcoding enables better species identification and assists in combatting illegal, unreported, and unregulated fishing, contributing to effective fisheries management and conservation efforts.

6. Underwater imaging

Underwater imaging technologies, such as underwater cameras and remotely operated vehicles (ROVs), allow for visual documentation of fish communities and their habitats. These imaging tools provide high-resolution imagery, enabling researchers to assess benthic habitats, fish abundance, and behavior (Williams *et al.*, 2017). Such information is valuable in understanding the impacts of fishing activities, identifying sensitive habitats, and prioritizing conservation efforts.

The integration of technology in fisheries management and research has revolutionized the way these areas are approached. Remote sensing, acoustic telemetry, electronic tagging, data analytics and modeling, DNA barcoding, and underwater imaging technologies have greatly enhanced data collection, analysis, monitoring, and decision-making processes. However, it is important to acknowledge the challenges associated with technology integration, such as cost, data management, and technological capacity-building. Overcoming these challenges and continuous innovation in technology will contribute to more robust and sustainable fisheries management and research practices.

Advances in genetic tools and applications for fisheries and aquatic animal health

Genetic tools and applications have significantly contributed to the fields of fisheries and aquatic animal health. These tools serve as powerful methods for understanding genetic diversity, population structure, disease resistance, breeding programs, and conservation efforts. This note aims to explore the recent advances in genetic tools and their applications in fisheries and aquatic animal health, highlighting their benefits and potential challenges (Kattel and Wu, 2023)

1. Genomic sequencing

The advent of next-generation sequencing technologies has revolutionized the field of genetics. Genomic sequencing allows for the rapid and cost-effective determination of the complete DNA sequence of an organism's genome. This technology has been applied to fish species to gain insights into their genetic makeup, adaptive evolution, and understanding of disease resistance (Allendorf *et al.*, 2010). Genomic information aids in the identification of

functional genes related to specific traits and provides valuable resources for selective breeding and conservation purposes.

2. Single Nucleotide Polymorphism (SNP) markers

SNP markers are DNA variations that occur at the single nucleotide level and are widely distributed throughout the genome. Advances in genotyping technologies have made SNP markers a preferred choice for genetic studies in fisheries and aquatic animal health due to their abundance, easy detection, and cost-effectiveness (Palti, 2011). SNP markers are used in population genetics studies, parentage analysis, identification of disease-resistant individuals, and marker-assisted breeding programs.

3. Gene expression studies

Gene expression studies involve analyzing the activity levels of specific genes in an organism. Transcriptomic technologies, such as microarrays and RNA sequencing, allow for the examination of the entire transcriptome, providing insights into gene regulation and functional responses to various environmental factors and stressors (Ball *et al.*, 2015). Gene expression studies help identify biomarkers for disease diagnosis, understand the molecular mechanisms underlying adaptation, and develop strategies for improving fish health and productivity.

4. CRISPR/Cas9 gene editing

CRISPR/Cas9 gene editing technology has revolutionized the ability to precisely modify the genomes of various organisms. This tool allows for targeted gene knockouts, knock-ins, and mutations, offering unparalleled opportunities for functional genomics and breeding programs (Gratacap *et al.*, 2019). CRISPR/Cas9-based gene editing in fish species has the potential to develop disease-resistant strains, enhance growth performance, and improve other desirable traits.

5. Environmental DNA (eDNA) metabarcoding

eDNA metabarcoding is a non-invasive and efficient method that involves analyzing environmental DNA to detect and identify species present in aquatic habitats. This technique has become popular in fisheries and aquatic animal health for monitoring species distribution, biodiversity assessments, and early detection of invasive species (Kelly, 2016). eDNA metabarcoding offers a cost-effective and time-efficient approach for large-scale monitoring, aiding in the implementation of effective management strategies.

Advances in genetic tools and applications have revolutionized the study of fisheries and aquatic animal health. Genomic sequencing, SNP markers, gene expression studies, CRISPR/Cas9 gene editing, and eDNA metabarcoding have significantly contributed to understanding genetic diversity, disease resistance, breeding programs, and conservation efforts. While these tools provide immense potential, it is important to consider challenges such as data analysis, ethics, regulatory frameworks, and public acceptance. Continued development and

utilization of genetic tools will lead to better management and conservation of fisheries and aquatic animal health.

Emerging infectious diseases and disease surveillance in aquatic animals

Aquatic animals, both in the wild and in aquaculture settings, are vulnerable to various infectious diseases. In recent years, the emergence of new and re-emerging infectious diseases in aquatic animals has become a cause of concern for aquaculture industries, conservationists, and public health professionals. Disease surveillance plays a crucial role in monitoring and mitigating the impact of these infections. This note aims to provide an overview of emerging infectious diseases in aquatic animals and the importance of disease surveillance, emphasizing the need for effective monitoring and control measures.

Emerging infectious diseases in aquatic animals

Emerging infectious diseases refer to infections that are newly identified, or previously known diseases that have increased in incidence or geographic range. Aquatic animals are susceptible to a wide range of pathogens, including bacteria, viruses, fungi, and parasites, which can cause significant economic losses and threaten biodiversity. One such example is the global spread of infectious salmon anemia virus (ISAV), which has had a devastating impact on farmed Atlantic salmon (Miller *et al.*, 2017). Additionally, new virus strains have emerged in Asia, causing mass mortality events in shrimp farms, affecting the global aquaculture industry (Lightner, 2011, Goethel *et al.*, 2023).

Several factors contribute to the emergence of infectious diseases in aquatic animals. These include the increasing intensity of aquaculture practices, globalization and trade in live animals, habitat destruction, climate change, and the interaction between wild and farmed populations (Thompson *et al.*, 2004; Rohde *et al.*, 2015). These factors create pathways for pathogen transmission, facilitating the introduction and spread of infections.

Disease surveillance in aquatic animals

Disease surveillance is a fundamental component of any effective disease management strategy. It involves systematic and ongoing monitoring of the health status of aquatic animal populations, aiming to detect new and emerging diseases, understand their distribution and impact, and evaluate control measures. Surveillance allows for early detection and timely response, minimizing the spread of infectious agents and reducing the impact on affected populations.

Effective disease surveillance in aquatic animals involves the integration of various methodologies, including active monitoring, passive reporting, and diagnostic testing. Active monitoring includes regular sampling of aquatic animal populations, both in the wild and in aquaculture facilities, to monitor the presence of pathogens and track changes in disease prevalence and severity. Passive reporting relies on the submission of samples by farmers, fishermen, and other stakeholders to diagnostic laboratories, aiming to rapidly identify and

respond to disease outbreaks. Diagnostic testing plays a crucial role in confirming disease diagnoses, characterizing pathogens, and understanding their virulence and transmission dynamics.

Continued research and development of diagnostic tools are essential for disease surveillance in aquatic animals. This includes the development of rapid and accurate diagnostic tests, such as molecular techniques, which allow for early detection and identification of pathogens (Arias *et al.*, 2013). Furthermore, the use of advanced technologies, such as next-generation sequencing, can help identify new pathogens and monitor their evolution in real-time (Verner-Jeffreys *et al.*, 2016 and Das *et al.*, 2023).

The emergence of infectious diseases in aquatic animals is a significant threat to aquaculture industries, biodiversity, and public health. Disease surveillance plays a vital role in monitoring and managing these diseases. Through active monitoring, passive reporting, and diagnostic testing, disease surveillance provides valuable information that can guide effective control measures and facilitate early detection and response. Continued investment in research and development of diagnostic tools is necessary to enhance disease surveillance capabilities in aquatic animals, ultimately minimizing the impact of emerging infectious diseases.

Advances in vaccination and immunostimulants for improved aquatic animal health

Aquatic animals, including fish, crustaceans, and mollusks, play a crucial role in global food security and the economy. However, the aquaculture industry faces numerous challenges, such as infectious diseases, which significantly impact productivity, sustainability, and profitability. To mitigate these challenges, advances in vaccination and immunostimulants have emerged as promising strategies for improving aquatic animal health. This note aims to explore the recent developments in vaccination techniques and immunostimulants in the context of aquaculture.

1. Vaccination in aquaculture

Vaccination is a proven method for the prevention and control of infectious diseases in aquaculture. The development of effective vaccines has become a major focus of research and industry effort. Traditional vaccination methods often involve the administration of inactivated or attenuated pathogens to trigger an immune response in the host. However, recent advancements in vaccination techniques have provided more sophisticated approaches, including DNA vaccines, recombinant protein vaccines, subunit vaccines, and live vector vaccines.

1.1 DNA vaccines

DNA vaccines have gained considerable attention due to their potential to induce both cellular and humoral immune responses. These vaccines use plasmid DNA encoding specific antigenic proteins that are taken up by host cells, leading to antigen production and subsequent immune response. Several successful examples have been reported in fish, such as the use of

DNA vaccines against viral hemorrhagic septicemia virus (VHSV) in rainbow trout (Edwards *et al.*, 2017).

1.2 Recombinant protein vaccines

Recombinant protein vaccines rely on the production of specific antigens through genetic engineering techniques. By synthesizing recombinant proteins that mimic the pathogen's surface proteins, vaccines can stimulate an immune response without causing disease. For example, a recombinant vaccine against bacterial diseases in shrimp has shown promising results in terms of improved survival rates and disease resistance (Lightner *et al.*, 2018).

1.3 Subunit vaccines

Subunit vaccines contain purified fragments of pathogens, such as surface proteins or polysaccharides, that can induce an immune response. These vaccines are considered safer alternatives to whole-cell vaccines, as they eliminate the risk of pathogen replication and disease development. Subunit vaccines have shown efficacy in several aquatic species, including catfish and shrimp, against various bacterial and viral pathogens (Ammayappan *et al.*, 2018; Dolny *et al.*, 2019).

1.4 Live vector vaccines

Live vector vaccines employ genetically modified viruses or bacteria that act as carriers for specific antigens. These vectors replicate in the host, thereby delivering the antigen and stimulating an immune response. Examples of live vector vaccines in aquaculture include the use of attenuated strains of viral pathogens to induce protective immunity against diseases such as infectious pancreatic necrosis (IPN) in salmon (Garcia-Valtanen *et al.*, 2019).

2. Immunostimulants in aquaculture

Immunostimulants are substances that enhance the immune response of aquatic animals, improving their resistance to pathogens. These substances can be natural or synthetic and are often added to feed or water to ensure delivery to the animals. The application of immunostimulants is particularly useful when vaccines are unavailable or ineffective against certain pathogens.

2.1 Natural immunostimulants

Various natural compounds have been identified as immunostimulants in aquatic animals. For instance, β -glucans derived from yeast cell walls and various plant extracts have demonstrated significant immunostimulatory effects in fish (Dawood *et al.*, 2018). These substances enhance immune cell function, increase antibody production, and improve disease resistance.

2.2 Synthetic immunostimulants

Synthetic immunostimulants, such as levamisole and polyinosinic : polycytidylic acid, have also been investigated for their immunomodulatory effects in aquaculture. They can

modulate innate and adaptive immune responses, improving the overall immune status of aquatic animals and reducing the susceptibility to infections (Magnadottir, 2010).

Advances in vaccination techniques and the use of immunostimulants offer promising opportunities for improving aquatic animal health and disease management in the aquaculture industry. Vaccination strategies are evolving rapidly, encompassing DNA vaccines, recombinant protein vaccines, subunit vaccines, and live vector vaccines. Additionally, natural and synthetic immunostimulants play a critical role in supporting the immune system of aquatic animals, thereby enhancing their resistance to infectious agents. Further research and development in these fields will contribute to the sustainable growth of aquaculture and ensure food security in the future.

Environmental impacts and sustainable practices in fisheries and aquaculture

Fisheries and aquaculture are essential sectors that provide food, livelihoods, and economic opportunities worldwide. However, these industries can exert significant environmental pressures, including overfishing, habitat destruction, pollution, and the introduction of non-native species. The need to adopt sustainable practices has become paramount in ensuring the long-term viability of fisheries and aquaculture while minimizing negative environmental impacts. This note aims to explore the environmental impacts of these industries and highlight sustainable practices that can contribute to their conservation and sustainable development.

1. Environmental impacts in fisheries and aquaculture

1.1 Overfishing

Overfishing, the unsustainable harvesting of fish stocks beyond their reproductive capacity, remains a primary concern in global fisheries. It leads to depleted populations, loss of biodiversity, and ecosystem disruption. Implementing effective fisheries management strategies, such as catch quotas, size restrictions, and marine protected areas, is crucial to combat overfishing (Jennings *et al.*, 2017).

1.2 Habitat destruction

Certain fishing practices, such as bottom trawling and dredging, can cause significant damage to marine habitats, including coral reefs, seagrass beds, and sensitive benthic ecosystems. These destructive practices result in habitat degradation and the loss of essential nursery and feeding areas for marine species. Encouraging the use of sustainable fishing gear and promoting selective fishing techniques can help minimize habitat destruction (Kaiser *et al.*, 2017).

1.3 Pollution

Fisheries and aquaculture activities can contribute to pollution through the discharge of organic waste, excess nutrients, and chemicals. These pollutants can lead to eutrophication, harmful algal blooms, and water quality degradation. Promoting responsible practices, such as

proper waste management, wastewater treatment, and the use of eco-friendly aquaculture systems, is crucial to minimize pollution (Bostock *et al.*, 2018).

1.4 Introduction of non-native species

The escape or intentional release of non-native species from aquaculture facilities can have devastating ecological impacts. These species can become invasive, outcompeting native species, altering food webs, and causing significant ecological disruptions. Ensuring strict biosecurity measures and promoting responsible aquaculture practices can help prevent the introduction of non-native species (Naylor *et al.*, 2019).

2. Sustainable practices in fisheries and aquaculture

2.1 Ecosystem-based fisheries management

Implementing ecosystem-based fisheries management approaches considers the broader ecosystem dynamics rather than individual species. This approach aims to maintain the health and resilience of marine ecosystems by considering biological, ecological, and social aspects when making management decisions (Link *et al.*, 2015).

2.2 Aquaculture certification and best practices

Certification programs, such as the Aquaculture Stewardship Council (ASC) and Best Aquaculture Practices (BAP), contribute to sustainable aquaculture practices. These programs set standards for responsible farming practices, including environmental management, animal welfare, and social accountability. Compliance with these standards helps minimize environmental impacts and ensure the sustainability of aquaculture operations (Little *et al.*, 2018).

2.3 Closed-loop aquaculture systems

Closed-loop aquaculture systems, such as recirculating aquaculture systems (RAS), minimize the environmental footprint of fish farming. These systems use advanced filtration and water treatment technologies to continuously recycle and purify water, reducing the need for water exchange. RAS can significantly reduce water usage, waste discharge, and the risk of pollution (Eding *et al.*, 2019).

2.4 Sustainable seafood choices

Making informed choices as consumers can drive sustainable fisheries and aquaculture practices. Supporting seafood certification schemes, sourcing from well-managed fisheries, and promoting sustainable aquaculture products encourage responsible practices and help protect vulnerable fish stocks and ecosystems (Cashion *et al.*, 2017).

Addressing the environmental impacts of fisheries and aquaculture is vital for the conservation and sustainable development of these industries. Through the adoption of sustainable practices, such as ecosystem-based fisheries management, responsible aquaculture certification, closed-loop aquaculture systems, and sustainable seafood choices, we can minimize

negative environmental impacts, protect biodiversity, and ensure the long-term viability of fisheries and aquaculture for future generations.

Challenges and opportunities in marine conservation and restoration

Marine conservation and restoration are crucial for preserving the biodiversity and health of our oceans. However, the world's oceans face numerous challenges, including habitat degradation, overfishing, pollution, and climate change. This note aims to explore the key challenges and opportunities in marine conservation and restoration efforts, providing an overview of important initiatives and strategies undertaken to address these issues.

1. Habitat degradation

1.1 Coral reef conservation

Coral reefs are among the most biodiverse ecosystems on Earth and provide critical habitats for numerous marine species. However, they are vulnerable to a range of threats, including rising ocean temperatures, pollution, and destructive fishing practices. Efforts to conserve and restore coral reefs involve strategies such as coral gardening, species transplantation, and implementing marine protected areas (MPAs) to safeguard reef ecosystems (Donahue *et al.*, 2019).

1.2 Mangrove restoration

Mangroves serve as important nurseries for many marine species and provide protection against coastal erosion. However, they have been extensively cleared for coastal development and aquaculture. Restoration efforts have focused on replanting mangroves and creating protected areas to allow for their natural regeneration (Alongi, 2014).

2. Overfishing and sustainable fisheries

2.1 Implementing sustainable fishing practices

Overfishing disrupts fish populations and marine ecosystems. To ensure the long-term sustainability of fisheries, efforts have been made to establish fishing policies and practices that adhere to scientifically-determined catch limits, implement fishing gear modifications to minimize bycatch, and promote responsible fishing practices (Botsford *et al.*, 1997).

2.2 Marine protected areas

MPAs are designated areas that are set aside to protect marine resources and promote the recovery of species and habitats. By limiting or prohibiting fishing within these areas, MPAs allow fish populations to grow and habitats to replenish. The establishment and effective management of MPAs are essential for conserving marine biodiversity and supporting sustainable fisheries (Hilborn *et al.*, 2004).

3. Pollution and marine debris

3.1 Plastic pollution mitigation

Plastic pollution poses a significant threat to marine ecosystems, with floating plastic debris harming marine species through entanglement and ingestion. Initiatives focused on

reducing single-use plastics, promoting recycling programs, and implementing stricter waste management practices aim to mitigate this growing issue (McIlgorm *et al.*, 2020).

3.2 Oil spill response and restoration

Oil spills can cause devastating impacts on marine environments. Response efforts involve containing and cleaning up oil spills promptly, assessing the extent of damage, and implementing restoration projects to rehabilitate affected ecosystems. Research and technological advancements have improved oil spill response capabilities and restoration techniques (Wang *et al.*, 2016).

4. Climate change and adaptation

4.1 Marine protected areas as climate refuges

Marine protected areas can serve as refuges for species that may be able to adapt to climate change impacts by providing suitable habitats and reducing additional stressors. Designing resilient MPAs that account for changing ocean conditions is crucial for protecting vulnerable ecosystems (Burrows *et al.*, 2014).

4.2 Blue carbon ecosystems

Coastal ecosystems such as seagrass beds and salt marshes play a significant role in carbon sequestration. Protecting and restoring these "blue carbon" ecosystems can contribute to climate change mitigation efforts while providing additional benefits for marine biodiversity (Nellemann *et al.*, 2009).

Marine conservation and restoration efforts face considerable challenges due to habitat degradation, overfishing, pollution, and climate change. However, various initiatives and strategies have provided opportunities for addressing these challenges. The examples discussed in this note highlight the importance of coral reef and mangrove conservation, sustainable fisheries management, pollution mitigation, oil spill response, and the role of protected areas in climate change adaptation. It is critical that interdisciplinary collaboration, policy changes, and public awareness continue to support these efforts, fostering the long-term conservation and restoration of our marine ecosystems.

The impacts of climate change on fisheries and aquatic animal health

Climate change is a pressing global issue that poses significant threats to the world's fisheries and aquatic animal health. Rising ocean temperatures, changing sea levels, ocean acidification, and altered weather patterns are among the many impacts of climate change affecting aquatic ecosystems. This note explores the various ways in which climate change impacts fisheries and the health of aquatic animals, highlighting the challenges and potential strategies to mitigate these effects.

1. Changes in species distribution and abundance

1.1 Shifts in fish migration and distribution

Rising water temperatures can lead to changes in the geographical distribution and migration patterns of fish species. Warmer waters may cause fish populations to move to higher

latitudes or shift to deeper, cooler areas, disrupting ecosystems and posing challenges for fisheries management (Pecl *et al.*, 2017).

1.2 Altered productivity of marine ecosystems

Changes in ocean temperature and nutrient availability can significantly affect the productivity of marine ecosystems. Warmer waters can promote harmful algal blooms, which can lead to oxygen depletion and fish kills. In contrast, decreased nutrient availability due to changes in ocean circulation patterns can result in reduced productivity, impacting food availability and trophic interactions within the ecosystem (Beaugrand *et al.*, 2019).

2. Ocean acidification

Increasing atmospheric carbon dioxide (CO₂) levels not only result in global warming but also lead to ocean acidification. Ocean acidification affects the growth and survival of aquatic organisms with calcified structures, such as shellfish and coral reefs. Reduced shellfish growth and impaired coral reef structure pose significant challenges for fisheries and the overall health of marine ecosystems (Gattuso *et al.*, 2015).

3. Impact on aquatic animal health

3.1 Disease susceptibility and outbreaks

Climate change alters environmental conditions, potentially leading to increased susceptibility to diseases in aquatic animals. It can weaken their immune systems and trigger the emergence and spread of pathogens. For example, warming water temperatures have been linked to the spread of pathogens like *Vibrio* bacteria, affecting both wild and farmed populations of fish and shellfish (Harvell *et al.*, 2002).

3.2 Parasite dynamics

Changes in oceanographic conditions can influence the life cycles, abundance, and distribution of parasitic organisms, impacting the health and productivity of aquatic animals. Warmer waters may enhance the survival and reproduction rates of parasites, leading to increased infection rates in fish and other aquatic organisms (Lo *et al.*, 2016).

4. Mitigating the impacts

4.1 Adaptive fisheries management

Effective fisheries management should incorporate climate change considerations to accommodate shifts in species distribution and ensure sustainable fishing practices. This may involve dynamic fishing quotas and flexible management strategies that respond to changing environmental conditions and stock dynamics (Cheung *et al.*, 2016).

4.2 Promoting climate-resilient aquaculture practices

Aquaculture, as an alternative to wild capture fisheries, can play a role in ensuring a stable supply of fish while reducing pressure on wild populations. Implementing climate-resilient aquaculture practices, such as improved water management, genetically selecting heat-tolerant

species, and incorporating ecosystem-based approaches, can help mitigate the impacts of climate change on aquaculture systems (FAO, 2020).

Climate change poses significant challenges to the world's fisheries and the health of aquatic animals. The impacts include shifts in species distribution, altered productivity of marine ecosystems, ocean acidification, increased disease susceptibility, and changes in parasite dynamics. Mitigating these impacts requires adaptive fisheries management practices and the promotion of climate-resilient aquaculture. Collaboration among scientists, policymakers, and stakeholders is essential to develop strategies that minimize the impacts of climate change, protect aquatic ecosystems, and ensure the sustainability of fisheries and aquatic animal health.

Socioeconomic and policy considerations in fisheries and aquatic animal health research

Socioeconomic and policy considerations play a crucial role in fisheries and aquatic animal health research. Understanding the socioeconomic factors that affect the fishing industry and aquatic animal health is essential for the successful implementation of policies and strategies that ensure sustainable development and the well-being of coastal communities. This note aims to explore the significance of socioeconomic and policy considerations in fisheries and aquatic animal health research and highlight their importance in maintaining the health and sustainability of aquatic ecosystems.

Socioeconomic considerations:

1. Livelihoods and food security

The fishing industry is a significant source of livelihood for millions of people worldwide, particularly in coastal areas. It provides income, employment, and nutrition to many communities. Research should assess the socioeconomic impacts of aquatic animal diseases and the efficacy of health management strategies to safeguard these livelihoods and maintain food security.

2. Poverty alleviation and equity

In many developing countries, the fishing industry serves as a means for poverty alleviation and reducing income disparities. Policies and interventions in fisheries and aquatic animal health should ensure equitable access for small-scale fishers to resources, technologies, and markets. Research should focus on identifying interventions that support communities with limited resources while promoting inclusive growth.

3. Trade and market access

The globalization of fish trade has profound implications for both developed and developing nations. Ensuring the health of aquatic animals is vital to maintain market access and protect against potential trade disruptions. Research should explore the socioeconomic impacts of aquatic animal diseases on trade, including strategies to reduce barriers and enhance transparency and traceability in the global seafood market.

Policy considerations:

1. Regulatory frameworks

Effective management of fisheries and aquatic animal health requires clear and robust regulatory frameworks. Research should examine the effectiveness of existing policies and regulations in preventing the spread of diseases, preventing overfishing, and promoting sustainable practices. It should also focus on identifying policy gaps and suggesting evidence-based solutions to address them.

2. Stakeholder engagement

Inclusive stakeholder engagement is essential for the successful implementation of policies related to fisheries and aquatic animal health. Research should analyze existing mechanisms for stakeholder participation, assess the effectiveness of these mechanisms, and recommend approaches for enhancing stakeholder engagement and collaboration.

3. International cooperation

Aquatic animal diseases do not respect borders, necessitating international cooperation. Research should examine the role of regional and international organizations in coordinating efforts to improve aquatic animal health and fisheries management. It should propose mechanisms for strengthening cooperation, knowledge exchange, and capacity-building among nations.

Socioeconomic and policy considerations are integral to successful fisheries and aquatic animal health research. Understanding the social, economic, and policy contexts in which these industries operate is crucial for the creation of effective policies, regulations, and management strategies. By incorporating socioeconomic considerations and engaging stakeholders, research can help ensure the sustainability of aquatic ecosystems, safeguard the livelihoods of coastal communities, and protect global food security.

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PCR BASED METHODS IN FISH MICROBIOLOGY

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Introduction:

Developments in molecular biology have sped up the development of novel techniques for identifying fish diseases. Molecular diagnostic techniques are quicker, more accurate, and more sensitive than approaches using pathogen culture, serology, histology, and biological chemistry which are typically used to determine the sources of fish sickness. Molecular diagnostic techniques are useful for identifying particular pathogens that are challenging to cultivate in vitro or need a lengthy development period. They are also substantially faster at producing results than culture. It facilitates quicker bacteremia identification, especially for low levels of bacteria in specimens, and sooner informed decision-making.

The most important molecular techniques are PCR-based molecular diagnostic procedures, such as Random Amplified Polymorphic DNA (RAPD), Real-Time Polymerase Chain Reaction (RT-PCR), Multiplex Polymerase Chain Reaction (multiplex PCR), and others. In recent years, these have been used more and more to diagnose fish sickness. Disease can be diagnosed using molecular diagnostic techniques even in asymptomatic fish breakouts might be avoided. As a result, the need for antibiotic therapy can be decreased, and the emergence of germs resistant to antibiotics can be stopped. In this chapter, we make an effort to provide an overview of the potential of PCR-based molecular diagnostic techniques and their use in identifying fish pathogens.

PCR based methods in fish pathogen identification

Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction (PCR) is called as molecular photocopying. This is a quick and low-cost method used to copy brief DNA segments. Studies of isolated fragments of DNA are almost difficult without PCR amplification since considerable volumes of a sample's DNA are required for molecular and genetic analysis. Mullis *et al.* (1986), who invented PCR, received the 1993 Nobel Prize in Chemistry for his contributions to the field of DNA research.

How and why is PCR used?

The DNA generated by PCR can be used in a variety of laboratory techniques after it has been amplified. For instance, PCR was a key component of most mapping techniques used in the

Human Genome Project (HGP). A number of laboratory and clinical methods, such as DNA fingerprinting, the detection of germs or viruses (especially AIDS), and the identification of genetic abnormalities all benefit from the use of PCR.

PCR machine procedures

1. Denaturation

Using a thermal cycler, the solution inside the tube is heated to at least 94°C (201.2°F). Denaturation of double-stranded DNA is the process by which heat causes the hydrogen bonds in the original DNA sample to dissolve, separating the DNA into single strands (Borah, 2011; Sarah Maddocks & Jenkins, 2017).

2. Annealing

The sample mixture is subsequently cooled to a temperature of 50 to 60 °C (122 to 140 °F), which enables the DNA polymerase enzyme and DNA primers to attach to the distinct strands of DNA that were caused by the heat (this is known as annealing of the primers). The nucleotides (A, T, C, and G) from the newly added mixed solution will now pair with the variously separated strands of DNA produced by the heating procedure (Borah, 2011; Tymoczko *et al.*, 2011)

3. Extension

They combine to create a new complementary strand of DNA, often known as an extension of the DNA. From each of the single strands of the original sample molecule, a new duplicate double-stranded DNA molecule has been created (Caetano-Anollés, 2013; Drouin *et al.*, 2007). The temperature swings between 95°C and 50–60°C. The thermal cycler, which automatically repeats the heating and cooling cycles of the process, is then used to repeat the cycle approximately 20 to 40 times. Every time the cycler performs a heating/cooling cycle, the resulting DNA sequence is doubled. As a result, after 35 doubling cycles, a single short piece of DNA from one sample can be amplified to generate millions of copies (Baynes & Dominiczak, 2009)

Nested PCR

Two sets of unique primer pairs were used in two subsequent PCR amplifications in order to increase the sensitivity and specificity of the PCR reaction. Low-abundance genes may be amplified by the nested PCR since it uses less target DNA (Liop *et al.*, 2000). In a nutshell, the amplicons produced by the first amplification are subjected to a second amplification. Kamolvarin *et al.* (1993) provided the initial description of this method.

Due to numerous vessel openings required to complete the two amplification cycles, nested PCR has a significant risk of Eppendorf contamination. This might have led to a sharp increase in false positives. At extremely low bacterial infection densities—3–4 bacteria per gram of fish tissue—Nested PCR allowed the identification of *R. salmoninarum*, *V. vulnificus*, *A.*

hydrophila, *E. tarda*, *S. iniae*, and *P. damsela* (Farzadnia and Naeemipour, 2020). In Egypt, mixed infections of *Streptococcus* and *Myxobolus tilapia* coinfections in moribund Nile tilapia have been successfully diagnosed using nested PCR (Eissa *et al.*, 2021b).

Multiplex PCR

Using several primer pairs, multiplex PCR can concurrently target and amplify many distinct genes from various bacterial pathogens, producing specialized DNA products of various sizes. The first time this method was mentioned as a potential method to identify deletions in the dystrophin gene was in 1988 (Chamberlain *et al.*, 1988). This method is time- and money-efficient, with great accuracy. It also reduces pipette mistakes (Ador *et al.*, 2021). This technique has been used to simultaneously identify five different pathogenic bacteria (*Y. ruckeri*, *A. salmonicida subsp. salmonicida*, *A. hydrophila*, *R. salmoninarum*, and *F. columnare*), three different pathogenic bacteria (*A. hydrophila*, *F. columnare*, and *E. ictaluri*), four different pathogenic bacteria (*V. parahaemolyticus*, *V. cholerae*, *V. alginolyticus*, and *V. vulnificus*) (Adams and Thompson, 2011).

Three distinct pathogens, namely *A. salmonicida*, *F. psychrophilum*, and *Y. ruckeri*, were simultaneously identified using this technique. The simultaneous recognition of four different pathogens, including *F. psychrophilum*, *L. garvieae*, *Ps. putida*, and *Ps. aeruginosa*, was also accomplished using the mPCR. Additionally, *S. parauberis*, *S. iniae*, *L. garvieae*, and *S. difficilis* were all simultaneously identified using multiplex PCR (Mata *et al.*, 2004).

Identification of pathogens

Vibrio, *Aeromonas*, *Streptococcus*, and *Edwardsiella* were identified using multiplex PCR at extremely low bacterial concentrations of 100 CFU in fish tissues and 50 CFU in culture. To prevent reactions between target DNA and primers, a multiplex PCR could not use more than six primer pairs. The main drawback of this approach is that it may require multiple tries to standardize and that primer pair sets may become self-inhibitory. In Egypt, *A. hydrophila* and *Y. ruckeri* were found to co-infect farmed fish using multiplex PCR (El-Hady and Ahmed, 2014).

Real-time PCR

When SYBR Green dyes bind to double-stranded DNA, they release fluorescence through the excited fluorophore, allowing real-time PCR or quantitative PCR to count the amount of amplified products. RT-PCR, as opposed to regular PCR, identifies the targeted DNA amplification while the PCR progresses rather than at the end. Real-time PCR has a very high sensitivity and specificity and can detect extremely little amounts of DNA in various tissue samples. The RT-PCR process comprises a series of temperature changes and is repeated 25–50 times. The length of time and recommended temperature for each cycle of RT-PCR depend on a variety of variables, including the temperature at which the primers anneal, the quantity of

divalent ions present in the reaction, the concentration of deoxyribonucleotides (dNTPs), and the DNA synthesis enzyme (Austin, 2019).

This method eliminates the need for gel documentation while allowing for very accurate detection of low-copy DNA targets. The ability to quantify the DNA or RNA template present in the specimen is the main benefit (Tom *et al.*, 2004). For the development of the assay, the RT-PCR technology calls for more technical proficiency. Additionally, there are just a few standardized protocols and no RT-PCR kits for the many genes of the causative agents.

As a result, the assay is a costly and challenging test to scale. Even while this technique might show when pathogenic DNA material is present during an infection, it cannot tell whether the host is infected or not. Pathogenic bacteria in fish, like *A. salmonicida* and *S. parauberis*, are identified and measured using real-time PCR. *Francisella noatunensis* subsp. *orientalis* was detected with 99.2% accuracy and sensitivity in the farmed *O. niloticus*. According to Chapela *et al.* (2018), the multiplex qPCR was successfully used to detect concurrent infections with *A. salmonicida*, *V. anguillarum*, and *T. maritimum*.

The results showed the excellent specificity of this technique to identify coinfection scenarios or issues with natural contamination because they were consistently confirmed in all methodological replicates. Real-time PCR was used in Egypt to identify the co-infection of Nile tilapia with the tilapia lake virus and other *Aeromonas* species (Nicholson *et al.*, 2017).

Loop-mediated isothermal amplification

Nucleic acid amplification can be accomplished with the low-cost, straightforward, quick, and highly specific LAMP technique. To target particular genes, this instrument employs a Bst DNA polymerase and six distinct primer sequences. This polymerase enzyme lacks 5'-3' exonuclease activity but exhibits high 5'-3' DNA polymerase activity and strand displacement. The LAMP amplification reaction uses this enzyme. The amplified products, which produce > 500 mg/mL of PCR products, are stem-loop DNA structures with several inverted repeats of the target and cauliflower-like structures with multiple loops. (Nagamine *et al.*, 2001)

Conclusion:

As per the past studies, we conclude that the use of PCR in fishery science, have various horizons as they are useful in acute identification of pathogen. So, as we can diagnose new genetic sequences so as the mild change in fish pathogen may lead in variable modes of transmission.

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TERMITE: FRIEND AND FOE FOR MANKIND

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Introduction:

Termites are social insects with an extraordinary range of polymorphic forms belongs to order Isoptera and live social lives. It is now clearly established that they are a very specialised form of cockroach, with far more complex social systems than other cockroaches and with a far wider range of diets. Termites live in colonies, with reproductive stage (kings and queens), soldiers and drone. Morphological and anatomical adaptations of termite are caste-specific, with structures evolving independently in reproductives, the winged individual female in the colony with 5 to 7.5 cm in length, creamy white abdomen marked with transverse dark brown stripes. The abdomen is dilated and distended because of enormous growth of ovaries, gut and fat bodies and this condition is known as physogastry. It lays about 1000-3000 eggs in a day and lives up to 10-15 years. King intermittently fertilizes the queen. It helps the queen in the construction of nuptial chamber and also in rearing first brood. Workers are developed from the unfertilized eggs and play an important role in preparing the nest, tunnel, collection of food and care of the eggs as well as young ones. They occupy more than 90% in a termite colony and soldiers defend the colony against intruders and they occupy only 10% of the colony. In mandibulate soldiers head is large well sclerotized with well developed mandibles, while in case of nasute soldiers head is drawn into a nozzle shaped projection at the tip of which opens frontal gland. The modifications seen in termite societies are similar to those found in the somatic parts of multicellular organisms, leading to the idea that a termite colony is best thought of as a single organism (superorganism). The structures that termites build, the mounds and nests, might also be defined as part of this organism. Termites live either underground in soil, in wood or construct lofty attractive earthen mounds or carton nests by making colony. Mounds and nests contribute greatly to the well-being of termite colonies by providing shelter, fortifications and climate control. They feed on cellulose, and the social structure of their groups is highly similar to that of ants and bees. These insects, have evolved independently. Despite being quite different from ants, termites also enjoy the name of white ants, entirely due to their appearance. Overall, termites have amongst the most complex social, anatomical and structural adaptations of any animal.

Distribution and diversity

Termites are found on all continents except Antarctica, due to their soft cuticles, termites do not inhabit cool or cold habitats. The diversity of termite species is low in North America and Europe (10 species known in Europe and 50 in North America), but is high in South America, where over 400 species are known of the 2,972 termite species currently classified, 1,000 species are found in Africa, where mounds are extremely abundant in certain regions. Approximately 1.1 million active termite mounds can be found in the northern Kruger National Park alone (Meyer, 1999). In Asia, there are 435 species of termite among them 300 species recorded in India. Out of 300 species, 35 have been reported damaging agricultural crops and buildings (Rajagopal, 2002). In addition to naturally occurring termites, many species have been inadvertently transported by humans from their native habitats to new parts of the world. Termites, particularly *Cryptotermes* and *Coptotermes*, have been accidentally transported with wooden articles such as shipping crates, boat timbers, lumber and furniture. Dry-wood termites (*Cryptotermes* species) live in small colonies in wood and tolerate long periods of dryness, they can survive for many years in seasoned wood and furniture and can easily be transported over long distances. Members of the family Rhinotermitidae (*Coptotermes*) require access to moisture and cannot survive prolonged dry periods (Sanderson, 1996). There are three ecological groups of termites: dampwood, drywood and subterranean. Dampwood termites are found only in coniferous forests and drywood termites are found in hardwood forests subterranean termites live in widely diverse areas. One species in the drywood group is the West Indian drywood termite (*Cryptotermes brevis*), which is an invasive species in Australia (Heather, 1971).

Behaviour

Communication

Most termites are blind, so communication primarily occurs through chemical, mechanical and pheromonal cues (Costa-Leonardo, 2010). These methods of communication are used in a variety of activities, including foraging, locating reproductives, construction of nests, recognition of nestmates, nuptial flight, fighting enemies, and defending the nests (Costa-Leonardo, 2010). The most common way of communicating is through antennation. A number of pheromones are known, including contact pheromones (which are transmitted when workers are engaged in trophallaxis or grooming) and alarm, trail and sex pheromones. The alarm pheromone and other defensive chemicals are secreted from the frontal gland. Trail pheromones are secreted from the sternal gland and sex pheromones derive from two glandular sources like the sternal and tergal glands. When termites go out in search of food, they forage in columns along the ground through vegetation. A trail can be identified by the faecal deposits or runways that are covered by objects. Workers release pheromones on these trails, which are detected by other

nestmates through olfactory receptors (Krishna, 2015). Termites can also communicate through mechanical cues, vibrations, and physical contact. These signals are frequently used for alarm communication or for evaluating a food source (Evans, 2007). Termites can distinguish nestmates and non-nestmates through chemical communication and gut symbionts. Chemicals consisting of hydrocarbons released from the cuticle allow the recognition of alien termite species (Richard, 2013). Each colony has its own distinct odour. This odour is a result of genetic and environmental factors such as the termites' diet and the composition of the bacteria within the termites' intestines (Dronnet, 2006).

Defence

Termites rely on alarm communication to defend a colony (Reinhard, 2001). Alarm pheromones release when the nest has been breached or being attacked by enemies or potential pathogens. Termites always avoid nestmates infected with *Metarhizium anisopliae* spores through vibrational signals released by infected nestmates (Dronnet, 2006). Intense jerking and secretion of fluids from the frontal gland and defecating faeces containing alarm pheromones (Reinhard, 2001). In some species, some soldiers block tunnels to prevent their enemies from entering the nest, and they may deliberately rupture themselves as an act of defence (Wilson, 1977). In cases where the intrusion is coming from a breach that is larger than the soldier's head, soldiers form a phalanx-like formation around the breach and bite at intruders (Belbin, 2013). To termites, any breach of their tunnels or nests is a cause for alarm. When termites detect a potential breach, the soldiers usually band their heads, apparently to attract other soldiers for defence and to recruit additional workers to repair any breach. Additionally, an alarmed termite bumps into other termites which causes them to be alarmed and to leave pheromone trails to the disturbed area, which is also a way to recruit extra workers. The pantropical subfamily Nasutitermitinae has a specialised caste of soldiers, known as nasutes, that have the ability to exude noxious liquids through a horn-like frontal projection that they use for defence (Wilson, 2014). Nasutes have lost their mandibles through the course of evolution and must be fed by workers. A wide variety of monoterpene hydrocarbon solvents have been identified in the liquids that nasutes secrete (Miura, 2000). Similarly, Formosan subterranean termites have been known to secrete naphthalene to protect their nests. Soldiers of the species *Globitermes sulphureus* commit suicide by autothysis – rupturing a large gland just beneath the surface of their cuticles. The thick, yellow fluid in the gland becomes very sticky on contact with the air, entangling ants or other insects that are trying to invade the nest (Chen, 1998). Another termite, *Neocapriterme taracua*, also engages in suicidal defence. Workers physically unable to use their mandibles while in a fight form a pouch full of chemicals, then deliberately rupture themselves, releasing toxic chemicals that paralyse and kill their enemies. The soldiers of the neotropical termite family Serritermitidae have a defence strategy which involves front gland

autothysis, with the body rupturing between the head and abdomen. When soldiers guarding nest entrances are attacked by intruders, they engage in autothysis, creating a block that denies entry to any attacker (Sobotnik, 2012). Workers use several different strategies to deal with their dead, including burying, cannibalism and avoiding a corpse altogether. To avoid pathogens, termites occasionally engage in necrophoresis, in which a nestmate carries away a corpse from the colony to dispose it elsewhere. Which strategy is used depends on the nature of the corpse a worker is dealing with *i.e.* the age of the carcass (Sun, 2013).

Termite: Multifold roll towards man

Friend: An extremely beneficial

These small creatures are a part of the natural ecosystem and contribute significantly to most of the ecosystems on the earth. The significance of termites for ecosystem functioning is widely acknowledged and receives considerable attention from the scientific community, with much effort to disentangling their specific contributions to ecosystem functioning (Davies *et al.*, 2014). Termites are important in both dry and humid tropical forests, where they are consumers of the plant necromass, helping in the processes of nutrient cycling and soil formation (Lee and Wood, 1971). A great role in the cycles of biogenic elements in tropical forest ecosystems belongs to termites that consume up to 50 per cent of the leaf litter (Brauman, 2000). They are often referred to as ecosystem engineers because they shape the environment through their action. Bonachela *et al.* (2015) reported that in many arid ecosystems, termite nests impart substrate heterogeneity by altering soil properties, thereby enhancing plant growth. Further more, they noticed that mound-field landscapes are more robust to aridity, suggesting that termites may help stabilize ecosystems under global change. Termite mounds shape many environmental properties, as their soils differ from surrounding “matrix” soils in physical and chemical composition, which enhance vegetation growth (Sileshi *et al.*, 2010), creating “islands of fertility” (Sileshi *et al.*, 2010 and Davies *et al.*, 2014).

Termites can be beneficial to agriculture, such as by boosting crop yields and enriching the soil. Termites and ants can re-colonise untilled land that contains crop stubble, which colonies use for nourishment when they establish their nests. The presence of nests in fields enables larger amounts of rain water to soak into the ground and increases the amount of nitrogen in the soil, both essential for the growth of crops (Evans, 2011). The increased soil fertility and moisture found near termite mounds can have pronounced effects on vegetation communities and their productivity (Sileshi *et al.*, 2010). Previous studies have found that woody vegetation growing on termite mounds increased density (Moe *et al.* 2009), tree height (Levick *et al.*, 2010), species richness (Traore *et al.*, 2008), functional diversity (Joseph *et al.*, 2014), and reproductive output (Brody *et al.*, 2010). Termites have long been studied because of their uncommon diet and complex hindgut microbiota. Researchers have discovered that enzymes

found in a termite digestive system could aid in biofuel production from woody biomass. The lignocellulolytic system in wood feeding termites has some unique system advantages and can potentially serve as a model system to improve our current biomass bioconversion technology for fuels and chemicals (Ben Guerrero *et al.*, 2015). The use of termite mound samples is an appropriate media in the search for concealed mineralization in complex regolith environments (Arhin *et al.*, 2015). Affam and Arhin (2006) recognized termite mounds as a good geochemical sample media for gold exploration, and its validation had also been confirmed by Arhin and Nude (2010) in northern Ghana.

Some termite species are used as food by humans or are fed to livestock (Figueiredo, 2015). These insects are particularly important in impoverished countries where malnutrition is common, as the protein from termites can help to improve the human diet. Termites are consumed in many regions globally, but this practice has only become popular in developed nations in recent years (Figueirêdo, 2015). Termites are consumed by people in many different cultures around the world. In many parts of Africa, the alates are an important factor in the diets of native populations (Nyakupfuka, 2013). Termite alates are high in nutrition with adequate levels of fat and protein. They are regarded as pleasant in taste, having a nut-like flavour after they are cooked (Nyakupfuka, 2013). Alates are collected when the rainy season begins. During a nuptial flight, they are typically seen around lights to which they are attracted, and so nets are set up on lamps and captured alates are later collected. The wings are removed through a technique that is similar to winnowing. The best result comes when they are lightly roasted on a hot plate or fried until crisp. Oil is not required as their bodies usually contain sufficient amounts of oil. Termites are typically eaten when livestock is lean and tribal crops have not yet developed or produced any food, or if food stocks from a previous growing season are limited (Nyakupfuka, 2013). In addition to Africa, termites are consumed in local or tribal areas in Asia and North and South America. In Australia, Indigenous Australians are aware that termites are edible but do not consume them even in times of scarcity; there are few explanations as to why. Termite mounds are the main sources of soil consumption (geophagy) in many countries including Kenya, Tanzania, Zambia, Zimbabwe and South Africa. Researchers have suggested that termites are suitable candidates for human consumption and space agriculture, as they are high in protein and can be used to convert inedible waste to consumable products for humans (Katayama, 2008).

Foe: An extremely injurious

They pose serious threat to a wide range of agricultural crops, structures, especially wooden materials and prove themselves a major insect-pest to humankind. Termite cause severe damage to supporting structural timber, flooring, paneling, window and door frame, furniture,

books, fabrics, leather, carpets and lumber in storage etc. It damages the crops right from sowing to till harvest. Termites are principally cellulose feeder.

Owing to their wood-eating habits, many termite species can do significant damage to unprotected buildings and other wooden structures (Su, 2000). Termites play an important role as decomposers of wood and vegetative material, and the conflict with humans occurs where structures and landscapes containing structural wood components, cellulose derived structural materials and ornamental vegetation provide termites with a reliable source of food and moisture (Thorne, 1999). Their habit of remaining concealed often results in their presence being undetected until the timbers are severely damaged, with only a thin exterior layer of wood remaining, which protects them from the environment. Of the 3,106 species known, only 183 species cause damage; 83 species cause significant damage to wooden structures. In North America, 18 subterranean species are pests, in Australia, 16 species have an economic impact in the Indian subcontinent 26 species are considered as pests and in tropical Africa, 24. In Central America and the West Indies, there are 17 pest species. Among the termite genera, *Coptotermes* has the highest number of pest species of any genus, with 28 species known to cause damage (Su, 2000). Less than 10% of drywood termites are pests, but they infect wooden structures and furniture in tropical, subtropical and other regions. Dampwood termites only attack lumber material exposed to rainfall or soil Drywood termites thrive in warm climates, and human activities can enable them to invade homes since they can be transported through contaminated goods, containers and ships. Colonies of termites have been seen thriving in warm buildings located in cold regions (Grace, 1991). Some termites are considered invasive species. *Cryptotermes brevis*, the most widely introduced invasive termite species in the world, has been introduced to all the islands in the West Indies and to Australia. In addition to causing damage to buildings, termites can also damage food crops. Termites may attack trees whose resistance to damage is low but generally ignore fast-growing plants. Most attacks occur at harvest time; crops and trees are attacked during the dry season (Sands, 1973). In Australia, at a cost of more than A\$1.5 billion per year, termites cause more damage to houses than fire, floods and storms combined. The damage caused by termites costs the southwestern United States approximately \$1.5 billion each year in wood structure damage, but the true cost of damage worldwide cannot be determined (Flores, 2010). Drywood termites are responsible for a large proportion of the damage caused by termites (Su, 1990).

Termites can be major agricultural pests, particularly in East Africa and North Asia, where crop losses estimated from 3-100 per cent in Africa (Mitchell, 2002). In South America, cultivated plants such as eucalyptus, upland rice and sugarcane can be severely damaged by termite infestations, with attacks on leaves, roots and woody tissue. Termites can also attack other plants, including cassava, coffee, cotton, fruit trees, maize, peanuts, soybeans and

vegetables. Mounds can disrupt farming activities, making it difficult for farmers to operate farming machinery; however, despite farmers' dislike of the mounds, it is often the case that no net loss of production occurs.

A classical case of termite damage was reported from a Northern India town where termites damaged currency notes worth Rs. 10 M (US \$ 222,000) kept in a steel chest inside the State Bank of India branch (Sacks, 2011). Annual losses caused by termites in the USA and Japan are 1000 and 800 M US\$. In India the losses have been estimated around 35.12 M US\$. Globally, the estimated loss due to termite damage is about 50 billion US\$ annually (Subekti *et al.*, 2015).

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CELLULAR REGENERATION FRONTIERS: EXPLORING THE HORIZONS OF TISSUE REPAIR AND THERAPEUTICS

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Introduction:

Stem cell research and regenerative medicine represent revolutionary fields that hold the promise of transforming healthcare by harnessing the remarkable potential of stem cells to repair and regenerate damaged tissues and organs (Thomson *et al.*, 1998). Stem cells, with their unique ability to self-renew and differentiate into various cell types, offer unprecedented opportunities for developing innovative therapies for a wide range of medical conditions. This chapter delves into the intricacies of stem cell research, the principles governing stem cell biology, their applications in regenerative medicine, the ethical considerations that accompany this scientific pursuit, and the exciting future prospects that lie ahead.

Stem cell basics

At the heart of stem cell research lies the foundational concept of pluripotency, the remarkable ability of certain cells to give rise to a multitude of specialized cell types. Stem cells can be broadly classified into embryonic stem cells (ESCs) and adult or somatic stem cells. ESCs, derived from early-stage embryos, are pluripotent and hold the potential to develop into any cell type in the body. Adult stem cells, on the other hand, are specialized cells found in various tissues, and while they have a more limited differentiation potential, they play a crucial role in tissue maintenance and repair.

Types of stem cells

1. Embryonic Stem Cells (ESCs): These pluripotent cells are obtained from embryos during the blastocyst stage of development. The remarkable plasticity of ESCs makes them valuable tools for studying development and for potential therapeutic applications. However, ethical considerations surround their use due to the destruction of embryos.

2. Induced Pluripotent Stem Cells (iPSCs): iPSCs are a groundbreaking advancement achieved through cellular reprogramming, where adult cells are transformed into a pluripotent state (Takahashi *et al.*, 2007). This technology bypasses the ethical concerns associated with ESCs and allows for the generation of patient-specific stem cells, offering the potential for personalized regenerative therapies.

3. Adult or Somatic Stem Cells: These specialized stem cells exist in various tissues, such as bone marrow, skin, and brain. They play a pivotal role in maintaining tissue integrity and participating in repair processes, though their differentiation potential is more limited compared to pluripotent stem cells.

Applications of stem cell research in regenerative medicine

Stem cell research has ushered in a new era of regenerative medicine, offering innovative solutions to address a wide array of medical conditions that were once considered untreatable. The unique ability of stem cells to self-renew and differentiate into various cell types makes them invaluable tools for repairing and regenerating damaged tissues and organs. In this section, we delve into the diverse applications of stem cells in regenerative medicine, showcasing recent advancements and promising therapies.

1. Neurological disorders

Stem cell-based therapies hold significant potential for treating neurological disorders that were previously deemed irreversible. For instance, Parkinson's disease, characterized by the loss of dopamine-producing neurons, has been targeted using stem cell-derived dopamine neurons. In a landmark study, researchers transplanted these neurons into Parkinson's patients, leading to improvements in motor function and quality of life (Barker *et al.*, 2013). Similarly, spinal cord injuries, which often result in permanent paralysis, are being tackled through stem cell interventions. Experimental treatments involving neural stem cells are showing promise in promoting nerve regeneration and functional recovery in animal models.

2. Cardiac regeneration

Stem cells are playing a pivotal role in regenerating damaged heart tissue following heart attacks. Recent studies have explored the use of stem cell-derived cardiac muscle cells to replace scar tissue, improve heart function, and reduce the risk of heart failure. These therapies aim to restore blood flow, enhance tissue healing, and ultimately prevent the progression of heart disease (Menasché *et al.*, 2015).

3. Diabetes treatment

Type 1 diabetes is characterized by the loss of insulin-producing beta cells in the pancreas. Stem cell-based approaches aim to replenish these lost cells. Scientists have succeeded in differentiating stem cells into functional beta-like cells capable of producing insulin. Transplantation of these cells into diabetic animal models has shown promising results in regulating blood glucose levels, offering hope for a potential cure for diabetes (Millman *et al.*, 2016).

4. Tissue engineering and organ transplantation

One of the most transformative applications of stem cell research lies in tissue engineering and the creation of functional organs for transplantation. Scientists are utilizing stem

cells to generate tissues such as skin, cartilage, and even entire organs like kidneys and livers. These bioengineered tissues hold the potential to address the critical shortage of donor organs and alleviate the challenges associated with organ rejection.

5. Musculoskeletal injuries and disorders

Stem cell therapies are being explored for musculoskeletal injuries and disorders, including bone fractures, cartilage defects, and degenerative joint diseases. Mesenchymal stem cells (MSCs) extracted from bone marrow or adipose tissue have shown promise in promoting bone and cartilage regeneration, reducing pain, and improving joint function.

6. Ophthalmic applications

Degenerative eye conditions such as age-related macular degeneration and retinitis pigmentosa have limited treatment options. Stem cell-based approaches involving the differentiation of retinal cells offer potential strategies to restore vision. Scientists are working on transplanting stem cell-derived retinal cells into patients' eyes to replace damaged cells and potentially halt or reverse vision loss.

7. Skin repair and wound healing

Stem cells are being harnessed for skin repair and wound healing, particularly in cases of chronic wounds, burns, and tissue damage. Stem cell-based therapies aim to accelerate healing, reduce scarring, and promote the regeneration of functional skin tissue.

8. Blood disorders

Hematopoietic stem cells from bone marrow or cord blood have been used for decades in bone marrow transplantation to treat blood-related disorders such as leukemia, lymphoma, and sickle cell anemia. Recent advances in stem cell therapies are enhancing the efficiency and safety of these treatments.

Challenges and ethical considerations

While the potential of stem cell research is immense, it is not without its challenges and ethical dilemmas. The use of ESCs raises concerns about embryo destruction and the moral implications surrounding their sourcing. Additionally, there is a risk of teratoma formation, an unintended consequence where stem cells give rise to tumors, highlighting the importance of thorough safety assessments (Song *et al.*, 2019).

Emerging technologies and future directions

Stem cell research is rapidly evolving, driven by innovative technologies like gene editing (e.g., CRISPR/Cas9) that allow precise manipulation of stem cell genomes. Researchers are working to refine differentiation protocols, enhance safety measures, and develop effective delivery methods for therapeutic applications. The integration of stem cell therapies with other cutting-edge approaches such as biomaterials, 3D printing, and tissue engineering holds great promise for creating functional and transplantable tissues and organs.

Conclusion:

Stem cell research and regenerative medicine stand at the forefront of scientific and medical advancement, offering a potential paradigm shift in how we approach healthcare and disease treatment. While navigating ethical considerations is essential, the potential to alleviate suffering, restore function, and improve quality of life is undeniable. As the field continues to progress, collaborative efforts between researchers, clinicians, ethicists, and policymakers will be instrumental in unlocking the full potential of stem cells and ushering in a new era of regenerative therapies. With ongoing discoveries, breakthrough technologies, and unwavering dedication, the profound impact of stem cell research is poised to reshape the medical landscape and offer renewed hope to individuals seeking effective treatments for previously untreatable conditions.

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