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FROM CELLS TO ECOSYSTEMS: EXPLORING LIFE SCIENCE RESEARCH VOLUME I

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From Cells to Ecosystems: Exploring Life Science Research Volume I

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PREFACE

The vast tapestry of life, from the smallest cells to the most complex ecosystems, is a subject of endless fascination and critical importance. It is a domain where every discovery, no matter how small, can have profound implications for our understanding of the natural world and our place within it. "From Cells to Ecosystems: Exploring Life Science Research" is a celebration of this exploration, capturing the essence of scientific inquiry across the diverse and interconnected fields of life science.

This book brings together a collection of research and insights that span the full spectrum of life sciences. Each chapter delves into a different aspect of the biological world, offering readers a comprehensive overview of the latest advancements and enduring questions that drive this field forward. From molecular biology and genetics to ecology and environmental science, the breadth of topics covered reflects the intricate and multifaceted nature of life itself.

The creation of "From Cells to Ecosystems" has been a journey marked by collaboration, discovery, and a shared commitment to advancing our understanding of life. We have had the honor of working with leading scientists, researchers, and educators whose contributions are at the forefront of their respective fields. Their work not only expands our knowledge but also inspires new questions and avenues for future research.

Our aim with this book is to provide a resource that is both informative and inspiring. We seek to highlight the connections between different areas of life science research, emphasizing the importance of a holistic understanding of biological systems. By showcasing the diversity of life science studies, we hope to foster a deeper appreciation for the complexity and beauty of the natural world.

"From Cells to Ecosystems" is dedicated to the scientists and researchers who dedicate their lives to exploring the mysteries of life. Your passion, perseverance, and curiosity are the driving forces behind the advancements we celebrate in these pages. We also extend our gratitude to the educators who inspire the next generation of life scientists, ensuring that the pursuit of knowledge continues to thrive.

Editors

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ROLE OF POLYAMINES IN ROS GENERATION IN ASYMBIOTIC REGENERATED PLANTS OF ORCHIDS

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Abstarct:

Stress such as metal stress, osmotic shock, culture medium dehydration, mechanical stress, hypoxia etc. are common during plant tissue culture. Overproduction of reactive oxygen species (ROS) is a marker of stress. Although high level ROS is deleterious for cell but low level ROS (H_2O_2) can function in signaling and crucial for plant developmental processes. Several studies were reported the role of ROS mainly superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) during in-vitro plant regeneration. It was well established that low level H_2O_2 can function as signaling molecule from plant development (*in vivo* and *in vitro*) to stress response because of its long half-life and membrane permeability. Activity of different antioxidant enzymes regulates ROS level in cell. Three important polyamines in plant such as Putrescine (Put), spermine (spm) and spermidine (spd) are playing important role in plant development (in vivo and in vitro) to stress response. Polyamines catabolism can produce ROS, on the other hand polyamines are also able to detoxify ROS. In this review, we focused on role of polyamines and ROS in plant regeneration during *in-vitro* culture with special emphasis on orchid tissue culture.

Keywords: Reactive Oxygen Species, Antioxidant Enzymes, Polyamines, *In-Vitro* Culture, Orchids, Plant Regeneration

Abbrevaiations:

ROS: Reactive oxygen species, Put: Putrescine, Spd: Spermidine, Spr: Spermine,
PLB: Protocorm-like body, SOD: Superoxide dismutase, NOX: NADPH oxidase,
POX: Peroxidase, Prx: Class III peroxidase, PO: Polyphenol oxidase, AO: Ascorbate oxidase,
CAT: Catalase, APX: Ascorbate peroxidase, MDHR: Monodehydroascorbate reductase

Introduction:

Orchids belong to the family orchidaceae, distributed across the globe except Antarctica (Wraith *et al.,* 2020). Many orchid species are under threat to extinction (Wang *et al.,* 2009, Fay *et al.,* 2018 and Wraith *et al.,* 2020). Orchids are harvested, grown and

traded for variety of purposes such as ornamental plants, medicine and food (Hinsley *et al.,* 2018). Recent studies indicate its strong potential to fight against Alzhimer's disease, parkinson's disease and other neurodegenerative diseases (Zeng *et al.,* 2018). In-vitro plant tissue culture techniques are very important tools for disease-free plant production, rapid multiplication of rare plant genotypes, plant genome transformation and production of commercially valuable plant metabolites (Espinosa-Leal *et al.,* 2018). Several research efforts have been invested in the goal of development of efficient multiplication system by modifying culture media. Although in-vitro culture of orchids takes lots of time (Wang *et al.,* 2009, Saiprasad *et al.,* 2004). Due to lack of storage reserves (endosperm) in orchid seed, food must be supplied by endomycorrhizal fungus to orchid seed during the period of germination to achlorophyllous stage of protocorm development at natural condition (Valadares *et al.,* 2014). Some orchids are achlorophyllous throughout the life cycle therefore they are mycoheterotrophic in whole life (Valadares *et al.,* 2014). Mycoheterotrophic achlorophyllous protocorm stage may convert to green protocorms of mixotrophic or fully photoautotrophic nature (Valadares *et al.,* 2014). Valadares *et al.,* (2014) reported the differential accumulation of proteins in mycohetrotrophic and mixotrophic or photoautotrophic stage of protocorm development in orchid, *Oncidium sphacelatum*. Polyamines are important plant hormone that play role in macromolecular biosynthesis, plant growth, development and stress response (Saiprasad *et al.,* 2004, Wang *et al.,* 2009, and Chen *et al.,* 2019). Putrescine (put), spermidine (spd) and spermine (spm) are three important polyamines in plant. Polyamines catabolism leads to H_2O_2 production (Chen *et al.,* 2019).

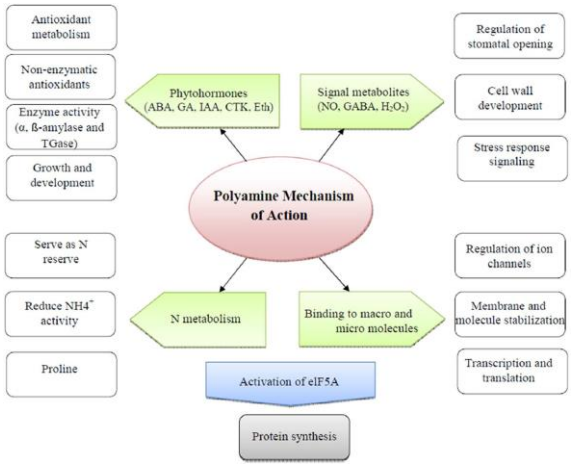

Figure 1: General role of Polyamines in plant growth and development (Mustafavi *et al.,* 2018)

Higher level of reactive oxygen species (ROS) accumulation lead to oxidative stress in plants, although low concentration of H_2O_2 act as a signaling molecule due to its long half life and high permeability to membrane (Gill *et al.,* 2010). Elaborate antioxidant system (enzymatic and non-enzymatic) can scavenge ROS, and a balance is maintained between productions and scavenging of ROS (Gill *et al.,* 2010). ROS production, an unavoidable process of aerobic metabolism, play role in plant growth, development and stress response (Gill *et al.,* 2010, Majumdar *et al.,* 2016, 2018, 2019). Polyamines have dual roles in regulation of ROS level – as a ROS producer and ROS scavenger (Saha *et al.,* 2015, Pooja *et al.,* 2019). In this review, we discussed the roles of different polyamines and ROS during *in-vitro* plant regeneration.

Polyamines and *in-vitro* plant regeneration:

Use of various elicitors, altering the media components, the strength of media, pH, precursor feeding etc. have all contributed tremendously in the in-vitro techniques used for culturing rare, endemic and medicinal plants for the commercial purposes. Owing to the demand for the plant products and drugs, the search for the other superior novel methods to increase its quantity and quality has not been stopped. Thus, one such method is the use of chemical compounds with many amino groups which serves as an additional source of nitrogen in the media and these organic compounds are called polyamines. Polyamines are known to play a wide role in plant physiological processes helping them in differentiation, inducing totipotency, increasing cell division and also in molecular signaling (Bhaskar *et al.,* 2021). In living organism, polyamines are present in free form, covalently conjugated and noncovalently conjugated form (Chen *et al.,* 2019). Polyamines have one or more amino (NH2) group as cation, which is responsible for its biological activity (Chen *et al.,* 2019). Three important polyamines in plant such as Putrescine (Put), spermine (spm) and spermidine (spd) are playing important role in plant development to stress response (Pal *et al.,* 2021). It was suggested that polyamine level is related to regenerating potential of cultured protoplast (Papadakis *et al.,* 2002). In orchid *Dendrobium* 'sonia', Put treatments (0.4 mM) lead to more PLB (Protocorm-like bodies) production, but spd (0.2 mM) as well as all concentration of spm treatments were significantly reduces PLBs (Saiprasad *et al.,* 2004). Scholten (1998) reported the similar results; positive effect of put and negative effect of spm on growth and development in micropropagation of acacia, tomato, cacti and lilac. Precursors of put can promote the plantlet formation from auxillary buds and PLBs in taro (Sabapathy and Nair, 1992). In orchid, *Dendrobium huoshanense*, a study speculated

that the conversion of put to spd instead of higher level endogenous put is necessary for conversion of PLBs to shoot (Wang *et al.,* 2009). Several study have been reported the promotive effect of particular polyamine in in-vitro tissue culture in carrot (Feirer *et al.,* 1984), *Medicago sativa* L. (Meijer and Simmonds, 1988), *Brassica campestris* (Chi *et al.,* 1994), rice (Bajaj and Rajam, 1995, 1996), mango (Litz and Schaffer, 1987), *Coffea canephora* (Kumar *et al.,* 2008), Chinese radish (Pua *et al.,* 1996), cucumber (Zhu and Chen, 2005), *Araucaria angustifolia* (Silveira *et al.,* 2006), sugarbeet (Hagege *et al.,* 1994), barely (Cho and Kasha, 1989). A correlation between polyamines mediated enhanced conversions of PLBs to shoots with higher cytokinins to indole-3-acetic acid (IAA) ratio have been reported in orchid, *Dendrobium huoshanense* (Wang *et al.,* 2009). Wang *et al.,* (2009) also suggested that polyamine treatment stimulate higher activity of IAA oxidase and lower activity as well as downregulated expression of cytokinin oxidase which led to higher cytokinins to indole-3-acetic acid (IAA) ratio during polyamine treatment. Polyamines catabolism can produce ROS, on the other hand polyamines are also able to detoxify ROS, and thus polyamines may be an important regulator of ROS metabolism during in-vitro development of orchids, which warns for further investigation.

Oxidative metabolism and *in-vitro* plant regeneration:

Stress such as osmotic shock, culture medium dehydration, metal stress, hypoxia, mechanical stress etc. are common in in-vitro tissue culture which can lead to overproduction of ROS, and this stress cue can initiate signaling for regeneration during in-vitro culture (Libik-Konieczny *et al.,* 2015, 2017). However many study indicates that oxidative stress is associated with plant recalcitrance (Siminis *et al.,* 1993, Siminis *et al.,* 1994, Papadakis *et al.,* 2002, Tang *et al.,* 2004a, Batkova *et al.,* 2008, Paul *et al.,* 2014) but sufficient H_2O_2 production crucial for regeneration during in-vitro plant regeneration (Marco *et al.,* 1996, Libik-Konieczny *et al.,* 2017). During in-vitro culture, fully differentiated and non-dividing cells of explant dedifferentiate which re-enter the cell cycle and proliferate, and plant regeneration takes place either by somatic embryogenesis or organogenesis. It has been proposed that ROS have role in specific plant cell cycle checkpoints and cell wall loosening required for cell elongation (Papadakis *et al.,* 2002). Activities of antioxidant enzymes and ROS production during in-vitro regenaration were studied by several workers in many plant species. In *Gladiolus* plant, scavenging of ROS by antioxidants induces shoot organogenesis (Paul *et al.,* 2014). Frank *et al.,* (1995) reported that higher activity of SOD and lower activity of other antioxidant enzymes lead to higher

accumulation of ROS (H_2O_2), which is responsible for vitrification in in-vitro culture of *Prunus avium*. Endogenous H_2O_2 accumulation has been reported to promote somatic embryogenesis in *Lycium barbarum* (Kairong *et al.,* 1999). Although severe oxidative stress is damaging but somatic embryogenesis prefers more stress environment than shoot organogenesis (Gupta *et al.,* 2004). In *Gladiolus hybridus*, increase of SOD activity along with lower activity of CAT and POX lead to H_2O_2 accumulation during somatic embryogenesis where as lower SOD activity along with higher CAT as well as POX activity lead to scavenging of ROS which promote shoot organogenesis (Gupta *et al.,* 2004). Tian *et al.,* (2003) suggested the role of H_2O_2 as a messenger for shoot organogenesis of strawberry callus. Due to long half-life and membrane permeability, H_2O_2 works as novel signaling molecule mediates plant developmental process to stress response (Li *et al.,* 2007, 2009a, 2009b; Gill *et al.,* 2010; Kar 2011; Das 2017). H_2O_2 accumulation mediates morphogenesis of callus during in-vitro culture of *Mesembryanthemum crystallinum*, and the activity of SOD, CAT was also studied (Libik *et al.,* 2005). In plant stress response, polyamines can mediate ROS homeostasis via Scavenging of ROS and activating antioxidant enzymes (Dhansu *et al.,* 2019). In in-vitro culture of *Pinus virginiana*, exogenous polyamine application can reduce browning of callus as well as improving regeneration by increasing activities of antioxidant enzyme: APX, GR and SOD (Tang *et al.,* 2004b). Free polyamines also can function in scavenging of $O_2^{\cdot-}$ and H_2O_2, while its conjugated form scavenge other ROS (Dhansu *et al.,* 2019). Valadares *et al.,* (2014) speculated that in orchid protocorm the cell specific defense response modulates fungal growth and peloton formation in symbiotic cells, while inhibits hyphal growth in meristimatic and photosynthetic tissue. Cu, Zn SOD, ascorbate peroxidase (APX) and monodehydroascorbate reductase (MDHR) were upregulated in green protocorms compared to mycoheterotrophic protocorm of orchid, *Oncidium sphacelatum*, suggesting their roles in scavenging of ROS during photosynthesis (Valadares *et al.,* 2014). Enhanced activity of antioxidant enzymes such as polyphenol oxidase (PO), ascorbate oxidase (AO), catalase (CAT) and peroxidase (POX) in symbiotic or mycoheterotrophic protocorms of orchids, *Dactylorhiza purpurella* and *Cymbidium* hybrid were also studied (Blakeman *et al.,* 1976). Zeng *et al.,* (2018) reported the greater accumulation of four POX in mycorrhizal protocorm of orchidaceae member, *Gastrodia elata*. Higher activity of POX and CAT were reported in PLB as compared to ptotocorm at stage IV of development in orchid, *Dendrobium hookerianum* (Paul *et al.,* 2014). It was suggested that CAT and POX play important role in growth and differentiation during shoot

induction (Paul *et al.,* 2014). Specific marker proteins at different stages of development of protocorm and PLB were indicated by Paul *et al.,* (2014). In the same orchid plant (*Dendrobium hookerianum)* patteren of polyphenol oxidase activity in different stages of development in PLB and protocorm were reported, and the activity remains more or less same from stage IV of development in both system (Paul *et al.,* 2014). Membrane bound NADPH oxidase (NOX) is an important apoplastic ROS producing enzyme in plants which produces $O_2^{\cdot-}$ in apoplast. Several studies have been confirmed the important role of NOX in plant developmental processes to stress response (Kar *et al.,* 2011, Singh *et al.,* 2014; Das and Kar, 2017; Majumdar *et al.,* 2018). Polyamines neutralizes lipid peroxidation through downregulation of NADP(H) oxidase/NADP(H) peroxidase activity (Pooja *et al.,* 2019). Spd may inhibit NADPH oxidase in cucumber during chilling stress to protect membrane structure (Dhansu *et al.,* 2019). Levels of put, spm and spd were increases in cucumber plant during chilling stress (Pooja *et al.,* 2019). Libik-Konieczny *et al.,* (2015) reported that NOX is responsible for H_2O_2 accumulation in vascular cylinder cells, where as peroxidase mediates H_2O_2 accumulation in cortex cells during in-vitro hypocotyl culture of ice plant (Mesembryanthemum crystallinum). This same study indicates the accumulation of $O_2^{\cdot-}$ and H_2O_2 in distinct zones that may play different roles during rhizogenesis from hypocotyl in in-vitro culture. Mammalian-like NADPH oxidase and NAD(P)H oxidase-peroxidase both are involved in ROS production in tobacco protoplast culture but in grape protoplast culture NAD(P)H oxidase-peroxidase was suggested for ROS production (Papadakis *et al.,* 1999, 2002). The NOX activity in different stages of protocorm development of orchid never been studied, emerged as an important area of future research.

The apoplastic ROS cascade or network has extensively been reported to be involved in cellular signal perception and transduction mechanisms. Apoplastic ROS, a minorfraction of total cellular ROS, accumulates because of low redox buffer state of the cell wall that creates a condition favourable for signaling (Podgorska *et al.,* 2017, Libik-Konieczny *et al.,* 2015). Apoplastic ROS emerged as an important player in morphogenic response and regeneration during in-vitro culture (Marco *et al.,* 1996, Libik-Konieczny *et al.,* 2015). Another important enzyme in apoplastic ROS metabolism is class III peroxidase (Prx), which is also a source of $O_2^{\cdot-}$ or H_2O_2 via oxidative cycle or produces $OH^{\cdot}$ from H_2O_2 and $O_2^{\cdot-}$ in hydroxylic cycle in the apoplast (Podgorska *et al.,* 2017). Insufficient data regarding roles of apoplastic oxidative metabolism in in-vitro plant regeneration warns for further study. Hydroxyl radical ($^{\cdot}OH$; most reactive ROS form) non-enzymatically cleaves

cell wall polysaccharides and causes wall loosening resulting in cell growth thus effectively enhancing the plastic extensibility of the cell wall (Das *et al.,* 2017, Majumdar *et al.,* 2019). Recent study reported the role of ˙OH in axis growth of germinating seed (Singh *et al.,* 2015). Study the role of ˙OH accumulation at different developmental stages in in-vitro plant regeneration is required. Roles of polyamines in mediation of oxidative regulation may be important for understanding the regulation of morphogenesis during in-vitro culture.

Future prospective:

Accumulation of $O_2^{\cdot-}$ and H_2O_2 at different developmental stages during in-vitro plant regeneration have been studied. Roles of some antioxidant enzymes were also elucidated. But lack of information regarding the exact role of different ROS ($O_2^{\cdot-}$, H_2O_2 and .OH), apoplastic ROS signaling and role of polyamines on regulation of ROS metabolism at different stages of in-vitro plant regeneration of orchids warns for further investigation.

References:

1. Bajaj S, Rajam MV (1996) Polyamine Accumulation and Near Loss of Morphogenesis in Long-Term Callus Cultures of Rice (Restoration of Plant Regeneration by Manipulation of Cellular Polyamine Levels). Plant Physiol. 112(3):1343-1348 doi: 10.1104/pp.112.3.1343
2. Bajaj S, Rajam MV, (1995) Efficient plant regeneration from long term callus cultures of rice by spermidine. Plant Cell Rep 14 (11):717–720
3. Batkova P, Pospisilova J and Synkova H (2008) Production of reactive oxygen species and development of antioxidative systems during in vitro growth and ex vitro transfer. Biologia Plantarum 52 (3): 413-422, 2008
4. Bhaskar, Rakesh & Wudali, Sudheer & Nagella, Praveen. (2021). Role of polyamines in plant tissue culture: An overview. Plant Cell, Tissue and Organ Culture (PCTOC). 145. 1-20. 10.1007/s11240-021-02029-y.
5. Blakeman JP, Mokahel MA AND Hadley G (1976) Effect of mycorrhizal infection on respiration and activity of some oxidase enzymes of orchid protocorms. New Phytol 77:697-704
6. Chen D, Shao Q, Yin L, Younis A and Zheng B (2019) Polyamine Function in Plants: Metabolism, Regulation on Development, and Roles in Abiotic Stress Responses. Front. Plant Sci. 9:1945. doi: 10.3389/fpls.2018.01945

7. Chi GL, Lin WS, Lee JEE, Pua EC (1994) Role of polyamines on de novo shoot morphogenesis from cotyledons of Brassica campestris ssp. pekinensis (Lour.) Olsson in vitro. Plant Cell Rep. 13, 323–329.
8. Cho UH, Kasha KJ, (1989) Ethylene production and embryogenesis from anther cultures of barely (Hordeum vulgare). Plant Cell Rep. 8:415–417
9. Das S, Kar RK (2017) Reactive oxygen species-mediated promotion of root growth under mild water stress during early seedling stage of Vigna radiata (L.) Wilczek. J Plant Growth Regul 36: 338-347
10. Espinosa-Leal CA, Puente-Garza CA & García-Lara S (2018) In vitro plant tissue culture: means for production of biological active compounds. Planta **248:**1–18 doi:10.1007/s00425-018-2910-1
11. Fay MF (2018) Orchid conservation: How can we meet the challenges in the twenty-first century? Botanical Studies 59: 16
12. Feirer RP, Mignon G, Litvay JD (1984) Arginine decarboxylase and polyamines required for embryogenesis in wild carrot. Science 223:1433–5
13. Frank T, Kevers C, Gaspar T (1995) Protective enzymatic systems against activated oxygen species compared in normal and hyperhydric shoots of Prunus avium L. raised in vitro. Plant Growth Regul 16:253–256
14. Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants, Plant Physiology and Biochemistry 48:909-930, https://doi.org/10.1016/j.plaphy.2010.08.016
15. Gupta SD, Datta S (2004). Antioxidant enzyme activities during in vitro morphogenesis of gladiolus and the effect of application of antioxidants on plant regeneration. Biol Plant. 47:179–183
16. Hagege D, Kevers C, Genus J, Gaspar T (1994) Ethylene production and polyamine content of fully habituated sugar beet calli. J. Plant Physiol. 143:722–725
17. Hinsley A, Boer HJD, Fay MF, Gale SW, Gardiner LM, Gunasekara RS, Kumar P, Masters S, Metusala D, Roberts DL, Veldman S, Wong S and Phelps J (2018) A review of the trade in orchids and its implications for conservation. Botanical Journal of the Linnean Society 186, 435–455
18. Kairong C, Gengsheng X, Xinmin L, Gengmei X, Yafu W (1999) Effect of hydrogen peroxide on somatic embryogenesis of Lycium barbarum L. Plant Sci 146:9–16

19. Kar RK (2011) Plant responses to water stress, role of reactive oxygen species. Plant Signal Behav 6: 1741-1745
20. Kumar V, Giridhar P, Chandrashekar A, Ravishankar GA (2008) Polyamines influence morphogenesis and caffeine biosynthesis in in vitro cultures of Coffea canephora P. ex Fr. Acta Physiol Plant. 30:217–23
21. Li S, Xue L, Xu S, Feng H, An L (2007) Hydrogen peroxide involvement in formation and development of adventitious roots in cucumber. Plant Growth Regul 52:173–180
22. Li S, Xue L, Xu S, Feng H, An L (2009a) Hydrogen peroxide acts as a signal molecule in the adventitious root formation of mung bean seedlings. Environ Exp Bot 65:63–71
23. Li S, Xue L, Xu S, Feng H, An L (2009b) IBA-induced changes in antioxidant enzymes during adventitious rooting in mung bean seedlings: the role of H2O2. Environ Exp Bot 66:442–445
24. Libik M, Konieczny R, Pater B, S´lesak I and Miszalski Z (2005) Differences in the activities of some antioxidant enzymes and in H2O2 content during rhizogenesis and somatic embryogenesis in callus cultures of the ice plant. Plant Cell Rep 23:834–841 doi: 10.1007/s00299-004-0886-8
25. Libik-Konieczny M, Kozieradzka-Kiszkurno M, Desel C, Michalec-Warzecha Z, Miszalski Z & Konieczny R (2015) The localization of NADPH oxidase and reactive oxygen species in in vitro-cultured Mesembryanthemum crystallinum L. hypocotyls discloses their differing roles in rhizogenesis. Protoplasma 252:477–487 doi: 10.1007/s00709-014-0692-2
26. Libik-Konieczny M, Kozieradzka-Kiszkurno M, Michalec-Warzecha Z, Miszalski Z, Bizan J, Konieczny R (2017) Influence of anti- and prooxidants on rhizogenesis from hypocotyls of Mesembryanthemum crystallinum L. cultured in vitro. Acta Physiol Plant. 39:166
27. Litz RE, Schaffer B (1987) Polyamines in adventitious and somatic embryogenesis in mango (Mangifera indica L.). J Plant Physiol. 128:251–8
28. Majumdar A, Kar RK (2016) Integrated role of ROS and Ca2+ in blue light-induced chloroplast avoidance movement in leaves of Hydrilla verticillata (L.f) Royle. Protoplasma 253: 1529-1539. doi: 10.1007/s00709-015- 0911-5
29. Majumdar A, Kar RK (2018) Congruence between PM H+ -ATPase and NADPH oxidase during root growth: a necessary probability. Protoplasma 255: 1129-1137. doi: 10.1007/s00709-018-1217-1

30. Majumdar A, Kar RK (2019) Orchestration of Cu-Zn SOD and class III peroxidase with upstream interplay between NADPH oxidase and PM H+ -ATPase mediates root growth in Vigna radiata (L.) Wilczek. J Plant Physiol 232: 248- 256. doi: 10.1016/j.jplph.2018.11.001
31. Marco AD and Roubelakis-Angelakis KA (1996) The Complexity of Enzymic Control of Hydrogen Peroxide Concentration May Affect the Regeneration Potential of PIant ProtopIasts. Plant Physiol. 11 O: 137-145
32. Meijer EGM, Simmonds J, (1988) Polyamine levels in relation to growth and somatic embryogenesis in tissue cultures of Medicago sativa L., J. Exp. Bot. 39:787–794.
33. Mustafavi SH, Naghdi Badi H, Sekara A et al (2018) Polyamines and their possible mechanisms involved in plant physiological processes and elicitation of secondary metabolites. Acta Physiol Plant 40:102
34. Pál M, Szalai G, Kinga OG, Janda T (2021) Unfinished story of polyamines: Role of conjugation, transport and light-related regulation in the polyamine metabolism in plants. Plant Science 308:110923, doi:10.1016/j.plantsci.2021.110923.
35. Papadakis AI, Roubelakis-Angelakis KA (2002) Oxidative stress could be responsible for the recalcitrance of plant protoplasts Plant Physiol Biochem. 40:549–559
36. Papadakis AK, Roubelakis-Angelakis KA (1999) The Generation of Active Oxygen Species Differs in Tobacco and Grapevine Mesophyll Protoplasts. Plant Physiol. Biochem. 40:549–559
37. Paul S, Kumaria S, Tandon P (2014) Comparative study on the changes of proteins and oxidative enzymes occurring in protocorms and protocorm-like bodies systems of development in the orchid Dendrobium hookerianum. Acta Physiol Plant. doi: 10.1007/s11738-014-1588-7
38. Podgorska A, Burian M, Szal B (2017) Extra-cellular but extra-ordinarily important for cell: Apoplastic reactive oxygen species metabolism. Frontiers Plant Sci 8: 1353
39. Pooja D, Goyal V, Devi S and Munjal R (2019) Role of Polyamines in Protecting Plants from Oxidative Stress. Reactive Oxygen, Nitrogen and Sulfur Species in Plants: Production, Metabolism, Signaling and Defense Mechanisms, Volume 1, First Edition. John Wiley & Sons Ltd.
40. Pua, EC, Sim GE, Chi GL, Kong LF (1996) Synergistic effect of ethylene inhibitors and putrescine on shoot regeneration from hypocotyl explants of Chinese radish (Raphanus sativus L. var. Longipinnatus Bailey) in vitro. Plant Cell Rep. 15:685–690

41. Sabapathy, S., Nair, H., 1992. In vitro propagation of taro, with spermine, arginine and ornithine. I. Plantlet regeneration from primary shoot apices and axillary buds. Plant Cell Rep. 11 (5–6), 290–294
42. Saha J, Brauer EK, Sengupta A, Popescu SC, Gupta K and Gupta B (2015) Polyamines as redox homeostasis regulators during salt stress in plants. Front. Environ. Sci. 3:21. doi: 10.3389/fenvs.2015.00021
43. Saiprasad GVS, Raghuveer P, Khetarpal S, Chandra R (2004) Effect of various polyamines on production of protocorm-like bodies in orchid—Dendrobium 'Sonia'. Scientia Horticulturae, 100:161–168
44. Scholten, HJ (1998) Effect of polyamines on the growth and development of some horticultural crops in micropropagation. Sci. Hortic. 77, 83–88
45. Silveira V, Santa-Catarina C, Tun NN, Scherer GFE, Handro W, Guerra MP, Floh EIS (2006) Polyamine effects on the endogenous polyamine contents, nitric oxide release, growth and differentiation of embryogenic suspension cultures of Araucaria angustifolia (Bert.) O. Ktze. Plant Sci. 171:91–8
46. Siminis CI, Kanellis AK, Roubelakis-Angelakis KA (1993) Differences in protein synthesis and peroxidase isoenzymes between recalcitrant and regenerating protoplasts. Physiol Plant. 87:263–270
47. Siminis CJ, Kanellis AK, Roubelakis-Angelakis KA (1994) Catalase is differentially expressed in dividing and nondividing protoplasts. Plant Physiol. 105:137
48. Singh KL, Chaudhuri A, Kar RK (2014) Superoxide and its metabolism during germination and axis growth of Vigna radiata (L.) Wilczek seeds. Plant Signaling & Behavior doi:14.4161/psb.29278
49. Singh KL, Chaudhuri A, Kar RK (2015) Role of peroxidase activity and Ca2+ in axis growth during seed germination. Planta 242: 997-1007
50. Tang W, Harris LC, Outhavong V, Newton RJ (2004a) Antioxidants enhance in vitro plant regeneration by inhibiting the accumulation of peroxidase in Virginia pine (Pinus virginiana Mill.) Plant Cell Rep. 22:871–877 DOI 10.1007/s00299-004-0781-3
51. Tang W, Newton RJ and Outhavong V (2004b) Exogenously added polyamines recover browning tissues into normal callus cultures and improve plant regeneration in pine. Physiologia Plantarum 122: 386–395. 2004, doi: 10.1111/j.1399-3054.2004.00406.x

52. Tian M, Gu Q, Zhu M (2003) The involvement of hydrogen peroxide and antioxidant enzymes in the process of shoot organogenesis of strawberry callus. Plant Sci 165:701–707
53. Valadares RBS & Perotto S & Santos EC & Lambais MR (2014) Proteome changes in Oncidium sphacelatum (Orchidaceae) at different trophic stages of symbiotic germination. Mycorrhiza 24:349–360 doi: 10.1007/s00572-013-0547-2
54. Wang Y, Luo J, Wu H, Jin H (2009) Conversion of protocorm-like bodies of Dendrobium huoshanense to shoots: The role of polyamines in relation to the ratio of total cytokinins and indole-3-acetic acidindole-3-acetic acid, Journal of Plant Physiology 166:2013-2022, doi:10.1016/j.jplph.2009.06.008.
55. Wraith J, Norman P, Pickering C (2020) Orchid conservation and research: An analysis of gaps and priorities for globally Red Listed species. Ambio doi: 10.1007/s13280-019-01
56. Zeng X, Li Y, Ling H *(2018)* Revealing proteins associated with symbiotic germination of *Gastrodia elata* by proteomic analysis. Bot Stud. **59:**8 doi:10.1186/s40529-018-0224-z
57. Zhu C, Chen Z (2005) Role of polyamines in adventitious shoot morphogenesis from cotyledons of cucumber in vitro. Plant Cell Tissue Organ Cult. 81:45–53

METAGENOMICS: UNRAVELLING THE MICROBIAL WORLD IN AGRICULTURE

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Abstract:

Metagenomics, the study of genetic material recovered directly from environmental samples, has emerged as a powerful tool in understanding the complex microbial communities that play important roles in agriculture. This chapter provides an overview of metagenomics in agricultural contexts, covering its introduction, historical background, techniques, applications in soil health and crop production, case studies in agricultural systems, challenges and limitations, and future directions. The introduction sets the stage by highlighting the importance of microbial communities in agricultural ecosystems and the need to explore their diversity and functions through metagenomic approaches. The historical background traces the evolution of metagenomics, from its inception to its current status as a key tool in agricultural research. Metagenomic techniques are discussed in detail, including DNA extraction, sequencing technologies, and bioinformatics analysis, showcasing their utility in unraveling the genetic potential of soil and plant-associated microbiomes. Applications in soil health and crop production demonstrate how metagenomics aids in understanding nutrient cycling, disease suppression, and plant-microbe interactions crucial for sustainable agriculture. Case studies from agricultural systems worldwide illustrate the practical implications of metagenomic insights, showcasing success stories in improving crop productivity, soil fertility, and resilience to environmental stresses. Challenges and limitations such as data interpretation complexities, technological constraints, and ethical considerations are also addressed, highlighting areas for further research and refinement. Looking ahead, future directions in metagenomics include integration with other omics technologies, prospects for precision

agriculture through real-time monitoring and targeted interventions, and overcoming challenges through advances in data analysis and public engagement. This chapter provides a comprehensive overview of metagenomics' contributions to unraveling the microbial world in agriculture, paving the way for innovative and sustainable agricultural practices.

Introduction:

Definition of metagenomics:

Metagenomics is the study of genetic material recovered directly from environmental samples, enabling the comprehensive analysis of microbial communities without the need for culturing individual species. This approach uses high-throughput sequencing technologies to identify and characterize the collective genome of all microorganisms present in a sample, often referred to as the microbiome. Metagenomics provides insights into the diversity, structure, and functional potential of microbial communities in various environments, including soil, water, and the plant rhizosphere (Handelsman *et al.,* 1998).

Importance of studying microbial communities in agriculture:

The study of microbial communities in agriculture is of paramount importance due to the critical roles these microorganisms play in soil health, plant growth, and overall ecosystem functioning. Soil microbiomes, for instance, are integral to nutrient cycling, organic matter decomposition, and the suppression of soil-borne pathogens (Van der heijden *et al.,* 2008). Understanding the composition and functionality of these microbial communities can lead to the development of sustainable agricultural practices that enhance crop productivity and soil fertility.

Microbial communities influence plant health through various mechanisms, including the fixation of atmospheric nitrogen, solubilization of phosphate, production of growth-promoting hormones, and protection against pathogens (Mendes *et al.,* 2011). By harnessing beneficial microbes, it is possible to reduce the reliance on chemical fertilizers and pesticides, thus promoting more environmentally friendly and sustainable farming practices (Singh *et al.,* 2020).

Metagenomic approaches have revolutionized our ability to study these complex microbial ecosystems. Traditional microbiological methods, which rely on culturing microbes in the laboratory, often miss the vast majority of microbial diversity due to the inability to culture many organisms (Riesenfeld *et al.,* 2004). Metagenomics overcomes

these limitations by allowing the examination of entire microbial communities in their natural environments, providing a more holistic understanding of their roles and interactions.

In recent years, advances in metagenomic techniques have enabled detailed studies of agricultural soils, revealing how microbial communities respond to different farming practices, crop types, and environmental conditions (Hartmann *et al.,* 2015). This knowledge is beneficial for developing strategies to improve soil health and enhance the resilience of agricultural systems to climate change and other stresses.

By unravelling the complexities of microbial communities through metagenomics, scientists can identify key microbial species and functions that support plant health and productivity. This information can be used to design microbial inoculants, biofertilizers, and biopesticides that optimize the performance of crops in various agricultural settings (Schlaeppi & Bulgarelli, 2015). Ultimately, the integration of metagenomics into agricultural research and practice holds great promise for advancing sustainable agriculture and ensuring food security in the face of global challenges.

Metagenomics plays an important role in identifying plant traits that enhance resilience to environmental stresses like drought, salinity, and extreme temperatures, contributing to the development of hardier crop varieties. Recent advancements in multi-omics, high-throughput culturing, computational biology, and synthetic biology have greatly enhanced our comprehension of the structure and function of native microbial communities. By employing metagenomics methods, we can effectively understand microbiome activity and function, which can then be correlated with plant physiology, genetics, metabolism, and other host processes. This integrated approach generates comprehensive and actionable insights for optimizing plant microbiome systems. (Benitez alfonso *et al.,* 2023).

Historical background:

Evolution of metagenomic techniques:

The field of metagenomics, which involves the study of genetic material recovered directly from environmental samples, has its roots in the late 20th century. Initially, microbial studies were limited to culturable organisms, leaving a significant portion of microbial diversity unexplored. The advent of DNA sequencing technologies in the 1970s, particularly the development of the Sanger sequencing method, marked the beginning of the genomic era and laid the groundwork for metagenomic studies (Sanger *et al.,* 1977).

The term "metagenomics" was first coined by Jo Handelsman and her colleagues in 1998, highlighting the need to study microbial communities as a whole, rather than focusing on individual species (Handelsman *et al.,* 1998). This approach was revolutionary, as it allowed scientists to analyse the vast majority of microorganisms that are not easily cultured in the lab.

With the development of high-throughput sequencing technologies in the early 2000s, such as 454 pyrosequencing and later Illumina sequencing, the field of metagenomics experienced significant advancements. These technologies enabled the sequencing of complex microbial communities at an unprecedented scale, providing deeper insights into microbial diversity and function (Margulies *et al.,* 2005; Bentley *et al.,* 2008).

Key discoveries and developments:

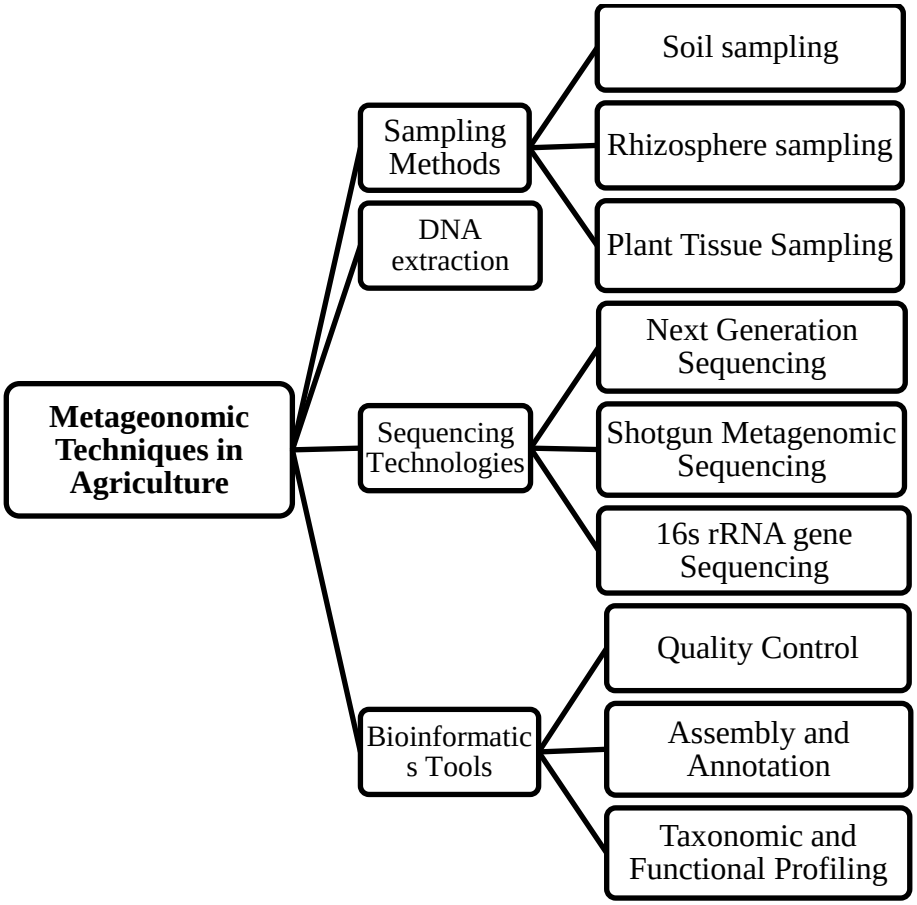
One of the seminal studies in metagenomics was conducted by Venter *et al.,* (2004), who used shotgun sequencing to analyse the microbial communities in the Sargasso Sea. This study revealed a previously unrecognized diversity of marine microbes and demonstrated the power of metagenomics to uncover novel genes and metabolic pathways (Venter *et al.,* 2004).

Another pivotal moment in metagenomics was the Human Microbiome Project (HMP), launched in 2007. The HMP aimed to characterize the microbial communities associated with the human body and their impact on health and disease. The project's findings underscored the importance of microbial communities in human health and set the stage for similar studies in other ecosystems, including agricultural soils (Turnbaugh *et al.,* 2007).

In agriculture, metagenomic techniques have been applied to study soil microbiomes, revealing their critical roles in nutrient cycling, plant health, and disease suppression. For example, a study by Mendes *et al.,* (2011) demonstrated how specific soil microbes can protect plants from pathogenic fungi, emphasizing the potential of metagenomics in developing sustainable agricultural practices (Mendes *et al.,* 2011).

The integration of metagenomics with other omics technologies, such as transcriptomics, proteomics, and metabolomics, has further expanded our understanding of microbial ecosystems. This holistic approach allows for a more comprehensive analysis of microbial functions and interactions within their environments, paving the way for innovations in agriculture and beyond (Knight *et al.,* 2018).

Metagenomic techniques:



Metagenomics is a powerful approach for studying microbial communities in their natural environments. It involves the comprehensive analysis of genetic material recovered directly from environmental samples, providing insights into the diversity, composition, and functional potential of microbial communities.

This section details the key metagenomic techniques used in agricultural research, including sampling methods, DNA extraction, sequencing technologies, and bioinformatics tools.

Sampling methods:

Effective metagenomic studies begin with robust sampling methods to ensure that the collected samples accurately represent the microbial communities in their natural habitats. Common sampling methods in agricultural metagenomics include:

1. Soil sampling: Soil samples are collected using sterilized tools to prevent contamination. Samples can be taken from various depths and locations within a field to capture spatial variability. Composite sampling, where multiple sub-samples are mixed, is often used to obtain a representative sample (Ranjan *et al.,* 2016).

2. Rhizosphere sampling: The rhizosphere, the soil region near plant roots, harbors a unique microbial community. Rhizosphere samples are collected by gently shaking off the soil adhering to roots and then using a brush or scalpel to collect the remaining soil closely associated with the roots (Edwards *et al.,* 2015).

3. Plant tissue sampling: To study endophytic microorganisms, plant tissue samples such as leaves, stems, and roots are surface-sterilized and then processed to extract microbial DNA from within the tissues (Compant *et al.,* 2010).

DNA extraction, sequencing technologies, and bioinformatics tools:

Once samples are collected, the next steps involve extracting DNA, sequencing it, and analysing the data using bioinformatics tools.

1. DNA extraction:

Efficient DNA extraction is critical for obtaining high-quality metagenomic data. Commercial DNA extraction kits (e.g., MoBio PowerSoil DNA Isolation Kit) are commonly used due to their ability to handle various sample types and minimize contaminants that can inhibit downstream processes (Lombard *et al.,* 2011).

Protocols typically involve lysing cells to release DNA, removing contaminants (e.g., humic acids in soil), and purifying the DNA for sequencing.

2. Sequencing technologies:

I. Next-Generation Sequencing (NGS): NGS technologies, such as Illumina sequencing, enable high-throughput sequencing of metagenomic samples, providing comprehensive data on microbial diversity and function (Caporaso *et al.,* 2012).

II. Shotgun Metagenomic Sequencing: This approach sequences all the DNA present in a sample, offering a broad view of the genetic material and allowing for the identification of functional genes and pathways (Simon & Daniel, 2011).

III. 16S rRNA Gene Sequencing: Targeted sequencing of the 16S ribosomal RNA gene is widely used to identify and classify bacteria and archaea. This method provides taxonomic insights but limited functional information (Tremblay *et al.,* 2015).

Bioinformatics Tools:

I. Quality Control: Tools like FastQC and Trimmomatic are used to assess and improve the quality of raw sequencing data by trimming low-quality bases and removing adapters (Bolger *et al.,* 2014).

II. Assembly and Annotation: Metagenomic data are assembled into longer contiguous sequences (contigs) using assemblers like MEGAHIT or SPAdes. These contigs are

then annotated using databases such as NCBI's RefSeq or IMG/M to identify genes and predict their functions (Li *et al.,* 2015; Nurk *et al.,* 2017).

III. Taxonomic and Functional Profiling: Tools like QIIME and MetaPhlAn2 provide taxonomic profiles by comparing sequence data against reference databases. Functional profiling tools like HUMAnN2 and KEGG help in identifying metabolic pathways and gene functions within the microbial communities (Segata *et al.,* 2012; Franzosa *et al.,* 2018).

Applications in Soil health and crop production

1. Soil microbiome analysis:

Metagenomics, the study of genetic material recovered directly from environmental samples, has revolutionized our understanding of the soil microbiome. The soil microbiome, composed of diverse microbial communities, plays a crucial role in maintaining soil health and enhancing crop production. Metagenomic approaches allow for a comprehensive analysis of these microbial communities, providing insights into their composition, diversity, and functional potential.

One of the primary applications of soil microbiome analysis through metagenomics is identifying microbial taxa and understanding their ecological roles. High-throughput sequencing technologies, such as Illumina and Oxford Nanopore, enable the detailed characterization of microbial diversity and the detection of previously unculturable microorganisms (Shokralla *et al.,* 2012). By analysing soil samples from different agricultural settings, researchers can assess how farming practices, crop types, and environmental conditions influence microbial communities. This information is critical for developing strategies to enhance beneficial microbial populations and suppress harmful ones, ultimately leading to improved soil health and crop productivity.

2. Impact on nutrient cycling, disease suppression, and plant growth:

The soil microbiome significantly impacts nutrient cycling, disease suppression, and plant growth. Metagenomic studies have shed light on the complex interactions between soil microorganisms and their environment, revealing how these interactions affect agricultural outcomes.

I. Nutrient cycling: Soil microorganisms play essential roles in nutrient cycling by decomposing organic matter, fixing atmospheric nitrogen, and solubilizing phosphorus. Metagenomic analyses have identified specific genes and metabolic pathways involved in these processes, providing insights into how microbial

communities contribute to soil fertility. For instance, nitrogen-fixing bacteria, such as those from the genera Rhizobium and Azospirillum, possess genes for nitrogenase enzymes that convert atmospheric nitrogen into ammonia, a form usable by plants (Zhong *et al.,* 2009). Similarly, phosphate-solubilizing bacteria release organic acids that solubilize bound phosphates, making them available for plant uptake (Rodriguez *et al.,* 2006).

II. Disease suppression: The soil microbiome also plays a vital role in suppressing soil-borne plant pathogens. Metagenomic studies have identified microbial taxa and genes associated with disease suppression, such as those involved in the production of antibiotics, siderophores, and lytic enzymes. For example, the presence of Pseudomonas species that produce antifungal compounds has been correlated with reduced incidence of soil-borne diseases like Fusarium wilt (Haas & Défago, 2005). By enhancing populations of such beneficial microbes, it is possible to naturally suppress pathogens and reduce the reliance on chemical pesticides.

III. Plant growth: Metagenomics has provided insights into how soil microorganisms promote plant growth through various mechanisms, including hormone production, nutrient mobilization, and stress tolerance. Certain soil bacteria, such as Bacillus and Pseudomonas species, produce phytohormones like indole-3-acetic acid (IAA), which can stimulate root elongation and enhance nutrient uptake (Spaepen *et al.,* 2007). Additionally, microorganisms can induce systemic resistance in plants, making them more resilient to environmental stresses and pathogen attacks.

Case studies in agricultural systems

1. Example 1: Soil microbiome analysis for enhanced nutrient cycling

Study: A study by Smith *et al.,* (2018) conducted metagenomic analysis of soil microbiomes in maize fields.

Findings: The study revealed a diverse microbial community involved in nutrient cycling processes, including nitrogen fixation and phosphorus solubilization.

Impact: Understanding the soil microbiome's role in nutrient availability led to targeted management practices, resulting in improved nutrient uptake by maize plants and increased crop yields.

2. Example 2: Microbial diversity and disease suppression in vineyards

Study: Jones *et al.,* (2020) investigated the microbial diversity in vineyard soils using metagenomic techniques.

Findings: The study identified beneficial microbes with antagonistic properties against fungal pathogens known to affect grapevines.

Impact: Implementing practices to promote the proliferation of these beneficial microbes led to reduced disease incidence, healthier vines, and ultimately, improved grape yields.

3. Example 3: Microbiome analysis for enhanced soil health in organic farming

Study: Brown *et al.,* (2019) conducted metagenomic analysis of soil microbiomes in organic farming systems.

Findings: The study highlighted the presence of microbial species associated with soil aggregation, organic matter decomposition, and disease suppression.

Impact: Implementing organic farming practices that promote microbial diversity and activity resulted in improved soil structure, enhanced nutrient availability, and overall better soil health, leading to increased crop yields over time.

These case studies demonstrate the effectiveness of metagenomic studies in agricultural systems. By understanding and harnessing the power of microbial communities, farmers and researchers can optimize soil health, nutrient cycling, disease suppression, and ultimately achieve improvements in crop yields and sustainability.

Challenges and limitations:

Metagenomics, while a powerful tool in understanding the microbial world in agriculture, comes with its own set of challenges and limitations. This section discusses the technical and analytical challenges faced in metagenomic studies and the efforts required to address biases and improve accuracy.

Technical and analytical challenges

1. Complexity of Microbial Communities: Agricultural environments host diverse and complex microbial communities, making it challenging to accurately characterize and analyse all microbial species present.
2. Sample collection and preservation: Proper sample collection and preservation techniques are crucial for maintaining microbial diversity and integrity during metagenomic analysis. Challenges arise in ensuring uniformity and consistency across samples, especially in large-scale studies.
3. DNA extraction and sequencing: Extracting high-quality DNA from environmental samples, particularly those with complex matrices like soil or plant tissues, can be technically demanding. Sequencing errors and biases can occur during the amplification and sequencing processes, affecting data accuracy.

4. Bioinformatics analysis: Processing and analyzing metagenomic data require advanced bioinformatics tools and computational resources. Challenges include data storage, processing large datasets, and accurate taxonomic classification of microbial sequences.

Addressing biases and improving accuracy

1. Standardization of protocols: Standardizing sample collection, DNA extraction, library preparation, and sequencing protocols is essential to minimize technical biases and ensure reproducibility across studies.
2. Quality control measures: Implementing stringent quality control measures at each step of the metagenomic workflow helps identify and mitigate errors, ensuring data accuracy and reliability.
3. Normalization and statistical methods: Utilizing normalization techniques and robust statistical methods can help address biases introduced during sequencing and data processing, allowing for accurate comparisons between samples.
4. Reference databases and annotation: Access to comprehensive reference databases and accurate annotation tools is crucial for taxonomic and functional profiling of metagenomic data, improving the reliability of results.

Future directions

1. Integration with other omics technologies

Metagenomics is poised to integrate with other omics technologies such as meta transcriptomics, metaproteomic, and metabolomics to achieve a comprehensive understanding of microbial communities in agricultural systems (Nayak *et al.,* 2021).

By combining metagenomic data with gene expression (meta transcriptomics), protein abundance (metaproteomic), and metabolite profiles (metabolomics), researchers can unravel complex interactions within microbial communities and their impact on crop health and productivity (Li *et al.,* 2020).

2. Prospects for precision agriculture

Metagenomic insights can inform precision agriculture strategies by providing real-time monitoring of soil and plant microbiomes, leading to targeted interventions for nutrient management, disease control, and environmental sustainability (Mendes *et al.,* 2022).

Integration of metagenomic data with remote sensing, IoT (Internet of Things), and machine learning technologies can enable farmers to make data-driven decisions for

optimizing resource use and maximizing crop yield while minimizing inputs (Chen *et al.,* 2019).

Conclusion:

Metagenomics has emerged as a transformative tool in understanding and harnessing the microbial diversity within agricultural systems. This chapter has delved into various aspects of metagenomics, from its historical roots to its current applications and future directions. The introduction set the stage by highlighting the significance of microbial communities in soil health and crop production. It emphasized the need for advanced techniques like metagenomics to unravel the complexities of these microbial ecosystems. The historical background provided insights into the evolution of metagenomic techniques, from early studies using Sanger sequencing to the modern era of high-throughput sequencing technologies. This journey showcased the rapid advancements that have enabled researchers to explore microbial communities at unprecedented depths.

Metagenomic techniques were discussed in detail, including sample collection, DNA extraction, sequencing methodologies, and bioinformatics analysis. These techniques have revolutionized our ability to characterize microbial diversity, identify functional genes, and understand microbial interactions within agricultural environments. The applications in soil health and crop production demonstrated how metagenomics has been applied to assess soil microbiomes, identify beneficial microorganisms, and optimize soil management practices. From enhancing nutrient cycling to promoting plant growth promotion and disease suppression, metagenomics has offered valuable insights for sustainable agriculture.

Case studies in agricultural systems provided concrete examples of how metagenomics has been used to address specific challenges, such as soil degradation, pest management, and crop productivity. These studies highlighted the practical implications of metagenomic research in real-world agricultural settings. Despite its promise, challenges and limitation in metagenomics were acknowledged. Issues such as data analysis complexities, standardization of protocols, and interpretation of results remain areas of active research and development. Looking ahead, the future directions of metagenomics were outlined. Integration with other omics technologies, prospects for precision agriculture, and advancements in bioinformatics tools were discussed as key areas for further exploration. The potential for metagenomics to drive sustainable agricultural practices and mitigate environmental challenges was underscored.

In conclusion, metagenomics represents a powerful tool for unraveling the microbial world in agriculture. Its continued advancement and integration with other cutting-edge technologies hold immense promise for enhancing soil health, crop productivity, and environmental sustainability in the years to come. By leveraging the insights gained from metagenomic studies, we can pave the way for a more resilient and food-secure agricultural future.

References:

1. Benitez-Alfonso, Y., Soanes, B. K., Zimba, S., Sinanaj, B., German, L., Sharma, V., & Foyer, C. H. (2023). Enhancing climate change resilience in agricultural crops. *Current biology*, *33*(23), R1246-R1261.
2. Bentley, D. R., Balasubramanian, S., Swerdlow, H. P., Smith, G. P., Milton, J., Brown, C. G., & Smith, A. J. (2008). Accurate whole human genome sequencing using reversible terminator chemistry. Nature, 456(7218), 53-59.
3. Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics, 30(15), 2114-2120.
4. Brown, A. *et al.,* (2019). "Metagenomic analysis of soil microbiomes in organic farming systems." Journal of Sustainable Agriculture, 35(3), 123-135.
5. Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S. M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J. A., Smith, G., & Knight, R. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. The ISME Journal, 6(8), 1621-1624.
6. Chen, J., Li, B., Zheng, H., Lin, X., & Chen, J. (2019). Application of Internet of Things Technology in Precision Agriculture. In 2019 IEEE International Conference on Systems, Man and Cybernetics (SMC) (pp. 3375-3380). IEEE.
7. Compant, S., Clement, C., & Sessitsch, A. (2010). Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role in plant growth and ecosystem sustainability. International Journal of Molecular Sciences, 11(3), 761-787.
8. Edwards, J., Johnson, C., Santos-Medellín, C., Lurie, E., Podishetty, N. K., Bhatnagar, S., Eisen, J. A., & Sundaresan, V. (2015). Structure, variation, and assembly of the root-associated microbiomes of rice. Proceedings of the National Academy of Sciences, 112(8), E911-E920.

9. Escobar-Zepeda, A., de León, A. V. P., & Sanchez-Flores, A. (2015). The road to metagenomics: From microbiology to DNA sequencing technologies and bioinformatics. Frontiers in Genetics, 6, 348.
10. Fierer, N. (2017). Embracing the unknown: Disentangling the complexities of the soil microbiome. Nature Reviews Microbiology, 15(10), 579-590.
11. Franzosa, E. A., McIver, L. J., Rahnavard, G., Thompson, L. R., Schirmer, M., Weingart, G., Lipson, K. S., Knight, R., Caporaso, J. G., Segata, N., & Huttenhower, C. (2018). Species-level functional profiling of metagenomes and metatranscriptomes. Nature Methods, 15(11), 962-968.
12. Haas, D., & Défago, G. (2005). Biological control of soil-borne pathogens by fluorescent pseudomonads. Nature Reviews Microbiology, 3(4), 307-319.
13. Handelsman, J., Rondon, M. R., Brady, S. F., Clardy, J., & Goodman, R. M. (1998). Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. Chemistry & Biology, 5(10), R245-R249.
14. Hartmann, M., Frey, B., Mayer, J., Mäder, P., & Widmer, F. (2015). Distinct soil microbial diversity under long-term organic and conventional farming. ISME Journal, 9(5), 1177-1194.
15. Jansson, J. K., & Hofmockel, K. S. (2018). Soil microbiomes and climate change. Nature Reviews Microbiology, 16(1), 35-50.
16. Jones, B. *et al.,* (2020). "Microbial diversity and disease suppression in vineyards: A metagenomic approach." Journal of Agricultural Science, 45(2), 210-225.
17. Knight, R., Vrbanac, A., Taylor, B. C., Aksenov, A., Callewaert, C., Debelius, J., ... & Dorrestein, P. C. (2018). Best practices for analysing microbiomes. Nature Reviews Microbiology, 16(7), 410-422.
18. Kuczynski, J., Stombaugh, J., Walters, W. A., González, A., Caporaso, J. G., & Knight, R. (2011). Using QIIME to analyze 16S rRNA gene sequences from microbial communities. Current Protocols in Bioinformatics, 36(1), 10-17.
19. Li, D., Liu, C. M., Luo, R., Sadakane, K., & Lam, T. W. (2015). MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics, 31(10), 1674-1676.
20. Li, X., Cui, X., & Chen, J. (2020). Metatranscriptomics: Opening the Black Box of Microbial Community Dynamics in Agricultural Systems. Frontiers in Microbiology, 11, 791.

21. Lombard, N., Prestat, E., van Elsas, J. D., & Simonet, P. (2011). Soil-specific limitations for access and analysis of soil microbial communities by metagenomics. FEMS Microbiology Ecology, 78(1), 31-49.
22. Margulies, M., Egholm, M., Altman, W. E., Attiya, S., Bader, J. S., Bemben, L. A., & Rothberg, J. M. (2005). Genome sequencing in microfabricated high-density picolitre reactors. Nature, 437(7057), 376-380.
23. Mendes, R., Garbeva, P., & Raaijmakers, J. M. (2011). The rhizosphere microbiome: Significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiology Reviews, 35(5), 957-979.
24. Mendes, R., Kruijt, M., de Bruijn, I., Dekkers, E., van der Voort, M., & Schneider, J. (2022). Metagenomics for Precision Agriculture: Harnessing Microbial Diversity for Sustainable Crop Production. Trends in Biotechnology, 40(3), 246-259.
25. Nayak, A. P., Green, S. J., Vimal, S., Kumar, P. S., & Polz, M. F. (2021). From Metaomics to Deep Learning: Recent Advances in Microbial Community Analysis. Annual Review of Microbiology, 75, 431-450.
26. Nurk, S., Meleshko, D., Korobeynikov, A., & Pevzner, P. A. (2017). metaSPAdes: a new versatile metagenomic assembler. Genome Research, 27(5), 824-834.
27. Ranjan, K., Richa, K., & Singh, V. (2016). Soil sampling and methods of analysis. In D. L. Sparks (Ed.), Encyclopedia of Soils in the Environment (pp. 460-468). Elsevier.
28. Riesenfeld, C. S., Schloss, P. D., & Handelsman, J. (2004). Metagenomics: Genomic analysis of microbial communities. Annual Review of Genetics, 38, 525-552.
29. Rodriguez, H., Fraga, R., Gonzalez, T., & Bashan, Y. (2006). Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Plant and Soil, 287(1-2), 15-21.
30. Sanger, F., Nicklen, S., & Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. Proceedings of the National Academy of Sciences, 74(12), 5463-5467.
31. Schlaeppi, K., & Bulgarelli, D. (2015). The plant microbiome at work. Molecular Plant-Microbe Interactions, 28(3), 212-217.
32. Segata, N., Waldron, L., Ballarini, A., Narasimhan, V., Jousson, O., & Huttenhower, C. (2012). Metagenomic microbial community profiling using unique clade-specific marker genes. Nature Methods, 9(8), 811-814.

33. Shokralla, S., Spall, J. L., Gibson, J. F., & Hajibabaei, M. (2012). Next-generation sequencing technologies for environmental DNA research. Molecular Ecology, 21(8), 1794-1805.
34. Simon, C., & Daniel, R. (2011). Metagenomic analyses: past and future trends. Applied and Environmental Microbiology, 77(4), 1153-1161.
35. Singh, B. K., Trivedi, P., Egidi, E., Macdonald, C. A., & Delgado-Baquerizo, M. (2020). Crop microbiome and sustainable agriculture. Nature Reviews Microbiology, 18(11), 601-602.
36. Smith, C. *et al.,* (2018). "Soil microbiome analysis for enhanced nutrient cycling in maize fields." Soil Biology and Biochemistry, 22(1), 45-58.
37. Spaepen, S., Vanderleyden, J., & Remans, R. (2007). Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiology Reviews, 31(4), 425-448.
38. Tremblay, J., Singh, K., Fern, A., Kirton, E. S., He, S., Woyke, T., Lee, J., Chen, F., Dangl, J. L., Tringe, S. G. (2015). Primer and platform effects on 16S rRNA tag sequencing. Frontiers in Microbiology, 6, 771.
39. Turnbaugh, P. J., Ley, R. E., Hamady, M., Fraser-Liggett, C. M., Knight, R., & Gordon, J. I. (2007). The human microbiome project. Nature, 449(7164), 804-810.
40. van der Heijden, M. G., Bardgett, R. D., & van Straalen, N. M. (2008). The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters, 11(3), 296-310.
41. Venter, J. C., Remington, K., Heidelberg, J. F., Halpern, A. L., Rusch, D., Eisen, J. A., ... & Smith, H. O. (2004). Environmental genome shotgun sequencing of the Sargasso Sea. Science, 304(5667), 66-74.
42. Zhong, W. H., Gu, T., Wang, W. T., Zhang, B., Lin, X. G., Huang, Q. R., & Shen, W. S. (2009). The effects of mineral fertilizer and organic manure on soil microbial community and diversity. Plant and Soil, 326(1-2), 511-522.

CLIMATE CHANGE AND ITS CUMULATIVE EFFECT ON FISH REPRODUCTION

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Abstract:

Climate change poses a significant threat to global ecosystems, including fisheries and aquaculture, with profound implications for biodiversity, food security, and socioeconomic development. This review explores the impact of climate change on fish reproduction, focusing on the complex interactions between environmental factors, hormonal regulation, and reproductive processes. Rising temperatures, altered precipitation patterns, and increased CO_2 concentrations disrupt fish reproductive cycles, leading to changes in breeding behaviors, sex ratios, and reproductive success rates. Elevated temperatures induce thermal-induced masculinization, altering sex differentiation and skewing sex ratios within populations. The endocrine system, crucial for regulating reproductive functions, is susceptible to temperature fluctuations and chemical pollutants exacerbated by climate change, further exacerbating reproductive disruptions. Additionally, stressors such as habitat loss and hypoxia compound the challenges faced by fish populations. Mitigating the impacts of climate change on fish reproduction requires interdisciplinary approaches, including sustainable fisheries management, habitat conservation, and adaptation strategies. Collaboration among governments, scientific institutions, and local communities is essential to implement effective mitigation and adaptation measures. Education and awareness initiatives are vital to empower stakeholders to address the multifaceted challenges posed by climate change. While climate change presents significant threats to fisheries and aquaculture, it also offers opportunities for innovative solutions and transformative action. By prioritizing sustainability, innovation, and cooperation, society can safeguard the resilience of marine and freshwater ecosystems and ensure a sustainable future for fisheries and aquaculture amidst a changing climate.

Keywords: Climate Change, Fish Reproduction, Adverse Effect, Fisheries, Temperature.

Introduction:

Climate change is an incredibly significant environmental concern that is affecting the entire world. It is a global phenomenon that demands our attention and action. The fourth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC) in 2007 has brought the threats of climate change to the forefront of our attention. It has become clear that climate change poses a significant risk to both human society and natural ecosystems. As a result, addressing this issue has become a top priority. This text highlights the often-overlooked significance of fisheries and aquaculture. It emphasizes the undeniable impact of climate change on these sectors and the communities they support, particularly those located along coastlines and riversides. According to Dutta *et al.,* (2020), it is a major issue that we must address in the present time. The major consequences of climate change are quite significant. They encompass abrupt shifts in temperature, unpredictable patterns of rainfall, and the occurrence of extreme climate events. The impact of climate change on food production systems is becoming increasingly severe. Unusual seasonality and disease outbreaks are exacerbating the existing issues, leading to a decline in productivity under harsh environmental conditions (Siddique *et al.,* 2022). The evidence supporting the notion that our climate is changing at a faster pace than in previous periods is becoming increasingly compelling. The reliance on fossil fuels as an energy source has seen a significant increase since the Industrial Revolution. The reliance on fossil fuels for power generation is a significant concern, with approximately 80% of the world's energy coming from these non-renewable sources (Bolin *et al.,* 1986; ACIA, 2004).

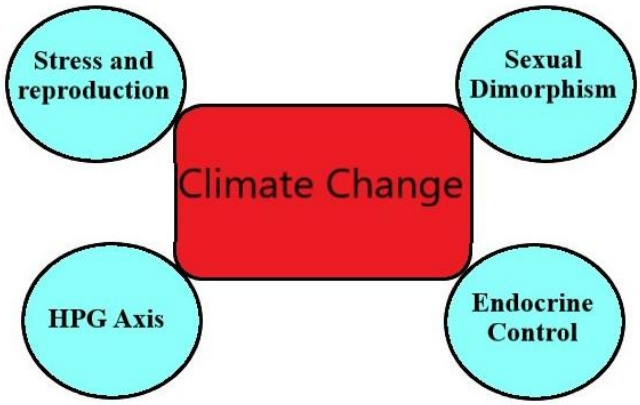

Figure 1: Effect of climate change in fish reproduction

Aquaculture has emerged as the most rapidly expanding food production sector globally, making a significant contribution of about 50% to the animal protein consumed by

humans (Dawood, 2021). The Food and Agriculture Organization (FAO) predicts a significant increase in aquaculture production by 2030, with estimates suggesting a growth of 53% (FAO, 2020). But the primary ramifications of climate change encompass sudden fluctuations in temperature, unpredictable patterns of precipitation, and occurrences of severe climatic phenomena. Climate change is exacerbating the preexisting challenges associated with atypical seasonal patterns and disease outbreaks, resulting in the degradation of food production systems through reduced productivity in adverse environmental conditions (Ray *et al.,* 2019). The unfavorable effects of climate change are expected to have significant implications for fisheries and aquaculture, particularly in relation to the ecosystems, biodiversity, breeding patterns, productivity, and socioeconomic development (Klinger *et al.,* 2017).

The authors Pörtner and Farrell (2008) highlight that reproduction in fish is a physiological process that is limited to a specific temperature range. As a result, even slight fluctuations in water temperature can have a significant impact on this crucial process, as noted by Zucchetta *et al.,* (2012). The study by Migaud *et al.,* (2010) explores the reproductive seasonality of various fish species in temperate climates. It highlights the importance of thermal conditions and day length in determining the timing of reproduction for most fish. The sensitivity of spawning phenology to temperature has been demonstrated in numerous fish species, as highlighted by McQueen and Marshall (2017). The study conducted by Kjesbu *et al.,* (2010) demonstrates that warmer temperatures have a significant impact on the reproductive cycle of fish in laboratory settings. It was observed that higher temperatures accelerate ovarian development and lead to an earlier onset of spawning. In a study conducted by Audzijonyte *et al.,* (2016), it was found that alterations in temperature can have a significant impact on the maturation process, regardless of body growth. This can lead to smaller adult body sizes as a result of the earlier allocation of energy towards reproduction. The study conducted by Tobin and Wright (2011) demonstrates that temperature has a direct impact on promoting earlier reproductive investment by influencing the rate of gonad development. The objective of this review is to provide a comprehensive overview of the existing literature on climate change and its possible impacts on the reproductive system of fish (Fig.1).

Methods:

This review on the cumulative impact of climate change on fish reproduction utilized a comprehensive technique to collect and analyze relevant data from past

publications to present research. A comprehensive search was undertaken in academic databases like PubMed, Google Scholar, and ScienceDirect using keywords such as "climate change", "fish reproduction", and "endocrine control". The search included articles from multiple decades to ensure a thorough grasp of the topic's development. Studies were selected based on certain criteria to focus on the relationship between climate change and fish reproductive physiology. The criteria included relevance to the study subject, methodological rigor, and publication in peer-reviewed publications. The data extraction approach required careful examination of each chosen article to gather important findings, methods used, and significant conclusions related to the influence of climate change on fish reproductive processes. Citation tracking was used to find important works and follow the evolution of ideas across time. This iterative procedure enabled the incorporation of both fundamental research and recent developments in the field. By combining information from many sources such as empirical research, reviews, and meta-analyses, a thorough understanding of the overall impact of climate change on fish reproduction was achieved. Critical evaluation approaches were used to evaluate the quality and trustworthiness of the literature contained, assuring the integrity of the review conclusions. This study offers a comprehensive analysis of the current information, exploring the complex relationship between climate change and the hormonal regulation of fish reproductive.

Sexual dimorphism:

Climate change poses a significant risk to the world's biodiversity since it has the potential to influence the physiology of species as well as the distribution of populations Poloczanska *et al.,* (2013). Temperature can trigger the development of sexual dimorphism in certain fish species, such as the Mosquitofish. Chance D. observed that Mosquitofish have sexual dimorphism, where females can be double the size of males. According to the temperature-size rule, smaller body sizes are anticipated in higher temperatures. Therefore, it is predicted that higher temperatures result in a higher proportion of males. These findings suggest that temperature could be a factor influencing the skewed sex ratios in animals with sexual size dimorphism, like mosquitofish. Findings indicate that rising temperatures could alter sex ratios in natural environments, leading to significant ecological and evolutionary impacts. Temperature during development can greatly impact the heart structure and swimming abilities of zebrafish, as shown by Dimitriadi *et al.,* (2018).

Endocrine control:

Climate change presents substantial obstacles to the endocrine regulation of fish reproduction. Rising temperatures are changing aquatic ecosystems and affecting hormone balance. Temperature fluctuations can impact the timing and outcome of reproductive cycles, resulting in mismatches in spawning behavior and decreased reproductive success rates. Increased CO_2 concentrations in water can affect fish endocrine systems, potentially impacting hormone synthesis and receptor sensitivity. This can lead to changes in behaviors associated to partner selection and courtship rituals, ultimately influencing population dynamics and genetic diversity. Pollutants and pollutants worsened by climate change add more complexity to the situation. Disrupting endocrine function can have widespread effects on ecosystems, impacting fish and their predators, emphasizing the necessity for thorough mitigation efforts. Although it is believed that the influence of water temperature on spawning rhythm is secondary to that of photoperiod for the majority of temperate species (Bromage *et al.,* 1993), there may be a significant interaction between the two (Devauchelle *et al.,* 1988). Water temperature can also exert a large influence on spawning rhythm. In numerous research on photoperiod, the impacts of temperature have frequently been overlooked (Bye, 1984). Furthermore, in situations when photoperiod regimes are superimposed on regular temperature cycles, the synchronizing effects of the latter may be obscured (Scott *et al.,* 1984).

Temperature and the HPG axis:

The hypothalamic-pituitary (HP) axis serves as the connection between the central nervous system, specifically the hypothalamus, and the endocrine system, specifically the pituitary gland (Fig.2) (Löhr and Hammerschmidt, 2011). The hypothalamus plays a crucial role in maintaining the internal balance of vertebrate organisms, known as homeostasis. It achieves this by directly influencing the pituitary gland through synaptic neurotransmission and the release of neuroendocrine releasing peptides (Biran *et al.,* 2015). The pituitary gland, regarded as a central gland in the endocrine system, is comprised of two distinct lobes: the anterior pituitary or adenohypophysis, which is composed of glandular cells responsible for secreting pituitary hormones, and the posterior pituitary or neurohypophysis, which contains nerve bundles originating from the hypothalamus and other regions of the brain (Zohar *et al.,* 2010).

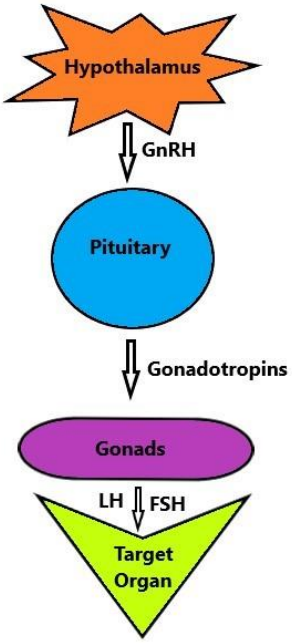

Figure 2: HPG axis of fish

The reproductive process of fish exhibits a distinct rhythmic nature, which is controlled by cyclical environmental conditions, specifically photoperiod and temperature. Fish in their native environments at moderate latitudes experience the regular variations in light and darkness throughout the day and year. This includes longer days in summer, shorter days in winter, and intermediate durations in spring and autumn. They also experience slow variations in water temperature during the day (higher during the day, lower during the night and early morning), as well as seasonal variations (hot waters in summer, colder waters in winter, and intermediate temperatures in spring and fall). The fluctuations in daylight length and water temperature are cyclical and consistent over years, serving as dependable cues for fish to coordinate their physiological rhythms. As a response to these recurring fluctuations, certain species engage in reproduction at specific seasons of the year and precise moments of the day (Falcón *et al.,* 2010). The pineal organ in fish is a crucial neural structure responsible for perceiving photoperiod and temperature information. It also converts this information into nerve and neuroendocrine signals, specifically neurotransmitters and melatonin. These signals enable the synchronization of various rhythmic processes in the individual, particularly those related to reproduction, with the surrounding environment. Numerous melatonin production patterns in freshwater and marine fish species have been observed thus far. (Vera *et al.,* 2007).

The direct neural connection to the pituitary gland in teleost fish, along with the discovery of a distinct neurosecretory terminal near a specific type of pituitary cell, has

allowed scientists to identify and understand the brain factors that play a role in regulating the release of pituitary hormones. In close proximity to gonadotrophic (LH and FSH) cells, the identification of terminals that secrete gonadotrophin-releasing hormone (GnRH), dopamine, neuropeptide Y (NPY), and gamma-amino butyric acid (GABA) has resulted in the production of valuable neuroanatomical evidence that can be used to understand the role that these neurohormones and neurotransmitters play in the secretion of gonadotrophins and the effects that they have on fish reproduction. (Kah *et al.,* 1992).

The primary direct and indirect effects arising from global climate changes that are expected to impact fish reproductive behavior include temperature, acidity, hypoxia, and alterations in pluviosity patterns. Ice melting in arctic and alpine locations can significantly alter the salinity and composition of water. Due to the warming of ocean waters, several fish species will undergo migration towards higher latitudes, resulting in alterations in photoperiod and light quality. Various stimuli will be detected by distinct sensory pathways and trigger diverse neuroendocrine mediators. Thermoreceptors in teleost fish are believed to be situated in the lateral line, a tissue that is in constant communication with the central nervous system and is responsible for sensing mechanical stimuli (Sullivan, 1954). However, it is generally agreed upon that these thermoreceptors are dispersed throughout the body and likely connected to the spinal nerves (Fontaine, 1976).

Mechanothermal nociceptors, which are sensory receptors that respond to extreme heat above 20ºC, have been discovered in the trigeminal nerve of the head of rainbow trout. These nociceptors are not sensitive to cold temperatures. The characteristics of these nociceptors are strongly associated with the behavior of rainbow trout in both natural and high-temperature stream pools. This information is supported by studies conducted by Ashley *et al.,* in 2007 and Sneddon in 2003. TRPV1 is an ion channel that selectively allows cations to pass through. It is triggered by high temperature and capsaicin, and is found in several vertebrates. In zebrafish, TRPV1 seems to function as a molecular sensor for environmental heat, as suggested by Gau *et al.,* (2013). It is also possible that TRPV1 has a similar role in other fish species, as proposed by Gracheva and Bagriantsev (2015).

Temperature changes can greatly disturb the intricate hormonal equilibrium essential for fish reproduction. High temperatures can speed up hormone production, causing early maturation and spawning in certain species. Prolonged exposure to high temperatures can inhibit reproductive hormones, leading to disruptions in sexual development and spawning. Hormonal imbalances can affect different elements of

reproduction, such as gamete production, fertilization success, and offspring survivability. Temperature fluctuations can cause imbalances in hormone levels, resulting in distorted sex ratios across fish populations, which can impact genetic diversity and population dynamics in the long run. Changes in reproductive hormone levels can impact reproductive behaviors like wooing displays and mate choice, thereby changing breeding cycles and decreasing reproductive success. Moreover, fluctuations in temperature might worsen hormonal imbalances, potentially intensifying the impact of pollution or habitat loss on fish reproductive health. Comprehending the complex relationship between temperature and reproductive hormones is crucial for anticipating and lessening the effects of climate change on fish populations. Efforts to conserve aquatic biodiversity must take into account intricate physiological linkages to guarantee the enduring sustainability of freshwater and marine ecosystems. In goldfish, the pre-ovulatory steroid pheromone stimulates the production of LH and milt, regardless of whether the fish is isolated or in a group. On the other hand, the postovulatory prostaglandin pheromone only produces these effects when the fish is in a group. This suggests that the pre-ovulatory steroid pheromone acts through a straightforward neuroendocrine reflex, while the postovulatory prostaglandin pheromone has an indirect effect (Sorensen *et al.,* 1989; Stacey, 2014; Zheng and Stacey, 1997). The LH hormone stimulates a range of steroids and prostaglandins in the downstream pituitary-gonad axis. These include pheromones and pheromone metabolites, as demonstrated in studies on goldfish and Mozambique tilapia (Huertas *et al.,* 2014; Stacey, 2014).

Stress and reproduction:

Ectothermic animals, like fish, are highly affected by changes in water temperature. This environmental stimulus has a significant impact on numerous biochemical responses and physiological processes, including reproduction. An increase in water temperature can significantly impact the health and behavior of fish species. Higher temperatures can boost metabolic rates, causing increased energy expenditure and potentially harming overall health. This can compromise immunological responses, rendering fish more vulnerable to illnesses and infections. Moreover, changes in temperatures can disturb typical behavioral patterns, like feeding, mating, and migration, affecting individual fitness and population dynamics. Fluctuations in water temperature can impact the dispersion of oxygen and other dissolved gases in aquatic settings, which can alter respiratory processes and potentially lead to hypoxia or hypercapnia. Fish may exhibit changed swimming patterns or

reduced activity levels in response to physiological stressors in order to cope with environmental stress. Rising temperatures can disturb the timing of developmental phases, like hatching and larval growth, causing mismatches with food supply and higher fatality rates. Temperature fluctuations can induce genetic reactions in fish populations, possibly resulting in adaptations or changes in species composition over time. The intricate relationship between increasing water temperatures and the well-being and actions of fish highlights the critical necessity for thorough conservation measures to reduce the effects of climate change on aquatic environments. Collectively, these modifications can have adverse impacts on the processes of sex determination and differentiation, initial maturation, gametogenesis, migration timing, spawning patterns, and reproductive output (Jonsson and Jonsson, 2009; Wedekind and Küng, 2010; Blanco-Vives *et al.,* 2011; Dorts *et al.,* 2012; Villamizar *et al.,* 2012).

High temperatures can harm fish gonads, which are essential for their reproductive functions necessary for population survival. Heat stress can hinder the development of reproductive organs and the synthesis of reproductive cells, resulting in decreased fertility and success in reproduction. Elevated temperatures frequently interfere with hormonal signaling pathways crucial for reproductive functions, including sex determination and maturation. Damage caused by heat to gonadal tissues can lead to abnormalities such as degeneration and necrosis, which can affect future reproductive ability. Continual exposure to high temperatures might worsen these effects, intensifying the reduction in population of afflicted fish species. Conservation plans need to consider the susceptibility of fish gonads to temperature stress in the face of climate change. The primary negative impact of climate change seems to be the rise in water temperature, which has been found to particularly harm the gonads, according to Miranda *et al.,* (2013). Elevated temperature in fish can have permanent impacts at critical stages of early development. These effects include impaired larval growth, increased occurrence of deformities, and disruption of sex determination and differentiation. This can lead to functional masculinization. (Strüssmann and Patiño, 1999; Piferrer *et al.,* 2005).

Elevated temperatures can trigger physiological changes in fish, including the induction of male characteristics. This phenomenon, known as "thermal-induced masculinization," can occur when temperatures exceed normal ranges during critical periods of development. High temperatures may disrupt hormonal balance, leading to the expression of male traits in genetically female fish. Such alterations can impact

reproductive dynamics and potentially skew sex ratios within fish populations. Understanding these effects is crucial for managing aquatic ecosystems in the face of climate change. Gonadotrophin-inhibitory hormone (gnih) and its receptor (gnihr) expression was reduced during the critical thermosensitive period in developing sea bass, according to a recent study (Paullada-Salmerón *et al.,* 2017). Elevated temperature conditions are known to induce male characteristics in fish species (Piferrer *et al.,* 2005).

Conclusion:

Ultimately, the effects of climate change on fisheries and aquaculture have evident consequences for ecosystems, biodiversity, and socioeconomic progress. The research highlights the necessity for taking proactive steps to reduce these impacts and guarantee the sustainability of these crucial species. Rising temperatures and erratic precipitation patterns significantly impact the reproductive processes of fish, causing disruptions in breeding cycles, changes in sex ratios, and reduced reproductive success rates. The alterations not only endanger fish populations but also pose a threat to food security and the livelihoods of communities reliant on fisheries and aquaculture. Society must take decisive action to address these concerns. It is crucial to promptly take action to decrease greenhouse gas emissions and restrict more global warming. Transitioning to renewable energy sources, adopting carbon pricing mechanisms, and improving energy efficiency are essential solutions in this context. Conservation and restoration of aquatic habitats including mangroves, coral reefs, and wetlands can mitigate the effects of climate change on fisheries and aquaculture. Adaptive management strategies are crucial for enhancing resilience in response to climatic variability. This involves applying ecosystem-based strategies to fisheries management, advocating for sustainable aquaculture methods, and improving monitoring and surveillance systems to detect shifts in fish populations and reproductive patterns. Collaborative efforts among governments, scientific institutions, civil society organizations, and local communities are essential to promote innovation and the sharing of knowledge in climate adaption methods. Education and awareness initiatives are essential to provide stakeholders with the necessary information and resources to effectively tackle the implications of climate change. Individuals may help protect fisheries and aquaculture in a changing climate by advocating sustainable consumption, backing local food systems, and enhancing community resilience. Climate change provides a significant threat to fisheries and aquaculture, but it also offers a chance for revolutionary action. Society can reduce the effects of climate change on fish reproduction and ensure a

strong future for marine and freshwater ecosystems by focusing on sustainability, innovation, and cooperation.

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References:

1. Agriculture Organization of the United Nations. Fisheries Department. (2018). *The state of world fisheries and aquaculture*. Food and Agriculture Organization of the United Nations.
2. Ashley, P. J., Sneddon, L. U., & McCrohan, C. R. (2007). Nociception in fish: stimulus–response properties of receptors on the head of trout Oncorhynchus mykiss. *Brain research*, *1166*, 47-54.
3. Assessment, A. C. I. (2004). *Impacts of a warming Arctic-Arctic climate impact assessment* (p. 144).
4. Audzijonyte, A., Fulton, E., Haddon, M., Helidoniotis, F., Hobday, A. J., Kuparinen, A., ... & Waples, R. S. (2016). Trends and management implications of human-influenced life-history changes in marine ectotherms. *Fish and Fisheries*, *17*(4), 1005-1028.
5. Biran, J., Tahor, M., Wircer, E., & Levkowitz, G. (2015). Role of developmental factors in hypothalamic function. *Frontiers in neuroanatomy*, *9*, 47.
6. Blanco-Vives, B., Vera, L. M., Ramos, J., Bayarri, M. J., Mananos, E., & Sánchez-Vázquez, F. J. (2011). Exposure of larvae to daily thermocycles affects gonad development, sex ratio, and sexual steroids in Solea senegalensis, kaup. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, *315*(3), 162-169.
7. Bolin, B., Do¨o¨s, B. R., Jager, J., Warrick, R.A. (1986). The greenhouse effect, climatic change, and ecosystems. John Wiley and Sons, Chichester, UK
8. Bromage, N., Mazorra, C., Bruce, M., Brown, N., & Shields, R. (2000). Halibut culture. *Encyclopedia of aquaculture*, 425-432.
9. Bye, V. J. (1989). The role of environmental factors in the timing of reproductive cycles. *Fish reproduction: strategies and tactics*.

10. Chance, D. L. Understanding the effects of temperature on sex ratio in a sexually dimorphic fish species. *URL: https://norriscenter. ucsc. edu/student-projects/chance-mosquito-fish. pdf*.
11. Dawood, M. A. (2021). Nutritional immunity of fish intestines: Important insights for sustainable aquaculture. *Reviews in Aquaculture*, *13*(1), 642-663.
12. Devauchelle, N., Alexandre, J. C., Le Corre, N., & Letty, Y. (1988). Spawning of turbot (*Scophthalmus maximus*) in captivity. *Aquaculture*, *69*(1-2), 159-184.
13. Dimitriadi, A., Beis, D., Arvanitidis, C., Adriaens, D., & Koumoundouros, G. (2018). Developmental temperature has persistent, sexually dimorphic effects on zebrafish cardiac anatomy. *Scientific Reports*, *8*(1), 8125.
14. Dorts, J., Grenouillet, G., Douxfils, J., Mandiki, S. N., Milla, S., Silvestre, F., & Kestemont, P. (2012). Evidence that elevated water temperature affects the reproductive physiology of the European bullhead Cottus gobio. *Fish Physiology and Biochemistry*, *38*, 389-399.
15. Dutta, J., Sen, T., Mitra, A., Zaman, S., & Mitra, A. (2020). Brief commentary on the impact of global climate change on fisheries and aquaculture with special reference to India. *Bangladesh J. Zool*, *48*(2), 457-463.
16. Falcon, J., Migaud, H., Munoz-Cueto, J. A., & Carrillo, M. (2010). Current knowledge on the melatonin system in teleost fish. *General and comparative endocrinology*, *165*(3), 469-482.
17. Fontaine, M. (1976). Physiological mechanisms in the migration of marine and amphihaline fish. In *Advances in marine biology* (Vol. 13, pp. 241-355). Academic Press.
18. Gau, P., Poon, J., Ufret-Vincenty, C., Snelson, C. D., Gordon, S. E., Raible, D. W., & Dhaka, A. (2013). The zebrafish ortholog of TRPV1 is required for heat-induced locomotion. *Journal of Neuroscience*, *33*(12), 5249-5260.
19. Gracheva, E. O., & Bagriantsev, S. N. (2015). Evolutionary adaptation to thermos-sensation. *Current opinion in neurobiology*, *34*, 67-73.
20. Huertas, M., Almeida, O. G., Canário, A. V., & Hubbard, P. C. (2014). Tilapia male urinary pheromone stimulates female reproductive axis. *General and Comparative Endocrinology*, *196*, 106-111.

21. IPCC, T. (2007). Climate change 2007: synthesis report. *Contribution of working groups I, II and III to the fourth assessment report of the intergovernmental panel on climate change*, 104.
22. Jonsson, B., & Jonsson, N. (2009). A review of the likely effects of climate change on anadromous Atlantic salmon Salmo salar and brown trout Salmo trutta, with particular reference to water temperature and flow. *Journal of fish biology*, *75*(10), 2381-2447.
23. Kah, O., Trudeau, V. L., Sloley, B. D., Chang, J. P., Dubourg, P., Yu, K. L., & Peter, R. E. (1992). Influence of GABA on gonadotrophin release in the goldfish. *Neuroendocrinology*, *55*(4), 396-404.
24. Kjesbu, O. S., Righton, D., Krüger-Johnsen, M., Thorsen, A., Michalsen, K., Fonn, M., & Witthames, P. R. (2010). Thermal dynamics of ovarian maturation in Atlantic cod (Gadus morhua). *Canadian Journal of Fisheries and Aquatic Sciences*, *67*(4), 605-625.
25. Klinger, D. H., Levin, S. A., & Watson, J. R. (2017). The growth of finfish in global open-ocean aquaculture under climate change. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1864), 20170834.
26. Klinger, D. H., Levin, S. A., & Watson, J. R. (2017). The growth of finfish in global open-ocean aquaculture under climate change. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1864), 20170834.
27. Knobil, E., & Neill, J. D. (Eds.). (1998). *Encyclopedia of reproduction* (Vol. 1). San Diego: Academic Press.
28. Löhr, H., & Hammerschmidt, M. (2011). Zebrafish in endocrine systems: recent advances and implications for human disease. *Annual review of physiology*, *73*, 183-211.
29. McQueen, K., & Marshall, C. T. (2017). Shifts in spawning phenology of cod linked to rising sea temperatures. *ICES Journal of Marine Science*, *74*(6), 1561-1573.
30. Migaud, H., Davie, A., & Taylor, J. F. (2010). Current knowledge on the photoneuroendocrine regulation of reproduction in temperate fish species. *Journal of fish biology*, *76*(1), 27-68.
31. Miranda, L. A., Chalde, T., Elisio, M., & Strüssmann, C. A. (2013). Effects of global warming on fish reproductive endocrine axis, with special emphasis in pejerrey *Odontesthes bonariensis*. *General and Comparative Endocrinology*, *192*, 45-54.

32. Paullada-Salmerón, J. A., Loentgen, G. H., Cowan, M., Aliaga-Guerrero, M., del Carmen Rendón-Unceta, M., & Muñoz-Cueto, J. A. (2017). Developmental changes and day-night expression of the gonadotropin-inhibitory hormone system in the European sea bass: effects of rearing temperature. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *206*, 54-62.
33. Piferrer, F., Blázquez, M., Navarro, L., & González, A. (2005). Genetic, endocrine, and environmental components of sex determination and differentiation in the European sea bass (*Dicentrarchus labrax* L.). *General and comparative endocrinology*, *142*(1-2), 102-110.
34. Poloczanska, E. S., Brown, C. J., Sydeman, W. J., Kiessling, W., Schoeman, D. S., Moore, P. J., ... & Richardson, A. J. (2013). Global imprint of climate change on marine life. *Nature Climate Change*, *3*(10), 919-925.
35. Pörtner, H. O., & Farrell, A. P. (2008). Physiology and climate change. *Science*, *322*(5902), 690-692.
36. Scott, A. P., Baynes, S. M., Skarphedinsson, O., & Bye, V. J. (1984). Control of spawning time in rainbow trout, *Salmo gairdneri*, using constant long daylengths. *Aquaculture*, *43*(1-3), 225-233.
37. Siddique, M. A. B., Ahammad, A. S., Mahalder, B., Alam, M. M., Hasan, N. A., Bashar, A., ... & Haque, M. M. (2022). Perceptions of the impact of climate change on performance of fish hatcheries in Bangladesh: An empirical study. *Fishes*, *7*(5), 270.
38. Sorensen, P. W., Stacey, N. E., & Chamberlain, K. J. (1989). Differing behavioral and endocrinological effects of two female sex pheromones on male goldfish. *Hormones and Behavior*, *23*(3), 317-332.
39. Stacey, N. (2014). Hormonally derived pheromones in teleost fishes. *Fish pheromones and related cues*, 33-88.
40. Tobin, D., & Wright, P. J. (2011). Temperature effects on female maturation in a temperate marine fish. *Journal of Experimental Marine Biology and Ecology*, *403*(1-2), 9-13.
41. Vera, L. M., De Oliveira, C., López-Olmeda, J. F., Ramos, J., Mananos, E., Madrid, J. A., & Sánchez-Vázquez, F. J. (2007). Seasonal and daily plasma melatonin rhythms and reproduction in Senegal sole kept under natural photoperiod and natural or controlled water temperature. *Journal of pineal research*, *43*(1), 50-55.

42. Villamizar, N., Ribas, L., Piferrer, F., Vera, L. M., & Sánchez-Vázquez, F. J. (2012). Impact of daily thermocycles on hatching rhythms, larval performance and sex differentiation of zebrafish. *PLoS One*, *7*(12), e52153.
43. Wedekind, C., & Kueng, C. (2010). Shift of spawning season and effects of climate warming on developmental stages of a grayling (Salmonidae). *Conservation Biology*, *24*(5), 1418-1423.
44. Zheng, W., & Stacey, N. E. (1997). A steroidal pheromone and spawning stimuli act via different neuroendocrine mechanisms to increase gonadotropin and milt volume in male goldfish *Carassius auratus*. *General and Comparative Endocrinology*, *105*(2), 228-238.
45. Zohar, Y., Muñoz-Cueto, J. A., Elizur, A., & Kah, O. (2010). Neuroendocrinology of reproduction in teleost fish. *General and comparative endocrinology*, *165*(3), 438-455.
46. Zohar, Y., Muñoz-Cueto, J. A., Elizur, A., & Kah, O. (2010). Neuroendocrinology of reproduction in teleost fish. *General and comparative endocrinology*, *165*(3), 438-455.
47. Zucchetta, M., Cipolato, G., Pranovi, F., Antonetti, P., Torricelli, P., Franzoi, P., & Malavasi, S. (2012). The relationships between temperature changes and reproductive investment in a Mediterranean goby: Insights for the assessment of climate change effects. *Estuarine, Coastal and Shelf Science*, *101*, 15-23.

ROLE OF 16S RIBOSOMAL RNA GENE SEQUENCING STUDY IN ANIMAL SCIENCE

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Abstract:

Recent years have seen an increase in the use of 16S rRNA gene sequencing for microbiome investigations in animal science, as sequencing costs have decreased and bioinformatic tools have grown in strength and ease of use. Microbiome research can be scary because of the convergence of various sciences such as molecular biology, microbiology, microbial ecology, computer science, and bioinformatics, in addition to the standard considerations when performing an animal science study. This review aims to address typical challenges and concerns that come up when planning an animal microbiome study from design through analysis, as well as to provide a starting point for animal scientists who are not as experienced with 16S rRNA gene sequencing and analyses. An outline of 16S rRNA gene sequencing's benefits and drawbacks is provided in this review; Wet lab considerations include field handling, microbial cell lysis, low biomass samples, library preparation, and sequencing controls. Computational considerations include identifying contamination, accounting for uneven sequencing depth, constructing diversity metrics, assigning taxonomy, testing for differential abundance, and, lastly, data availability. Experimental design considerations include study design, sample size, sample pooling, sample locations, and sequencing controls. We emphasize some particular factors by species and sample type in addition to basic considerations.

Keywords: 16S rRNA, Bacteriome, Bioinformatics

Introduction:

Complex colonies of microbes inhabit almost all habitats on Earth. The ability to fully characterize these microbial communities is limited by culture-based studies, which restrict scientific discovery to the microbes that can be grown in laboratory conditions. Traditionally, these environments have been studied by obtaining and classifying pure microbiological laboratory cultures. The "great plate count anomaly" (1) refers to the difference between the number of bacteria counted under a microscope and the viable

colonies on an agar plate; estimates suggest that only about 1% of bacteria can be cultivated using conventional methods. The creation of methods that do not require culture, such as next-generation sequencing (NGS) in conjunction with bioinformatic analysis, has made it possible to thoroughly characterize intricate microbial communities, or microbiomes.

The discovery that 181 additional microbial genomes related to previously undiscovered species after 396 human gut microbiome samples were analyzed is one illustration of the potency of NGS when combined with bioinformatic advances. The concurrent development of diverse "-omics" technologies has facilitated the resolution of significant inquiries regarding microbiomes, such as the types of microorganisms and genes present, their capabilities, and the roles they play (3). Among these methods, 16S ribosomal RNA gene sequencing is the least expensive both monetarily and computationally (commonly reduced to 16S rRNA or just 16S).

This method focuses on particular genes that enable the taxonomic classification and assessment of diversity of the bacterial and archaeal microbiome (it excludes fungal, viral, protozoal, and eukaryotic species from a sample). More animal scientists have been able to include 16S rRNA gene sequencing into their research projects over the past ten years thanks to a combination of declining DNA sequencing prices and more accessibility to bioinformatic tools. The number of research publications using the term "microbiome" in the Journal of Animal Science increased from four in 2010 to 184 in 2020—72 full-length articles and 112 abstracts—in just one year. As more scientists implement 16S rRNA gene sequencing in their studies, they ought to be knowledgeable about terms relevant to the microbiome.

16S rRNA gene sequencing: what is it?

Because the ribosomal RNA gene is present in every organism, Carl Woese was the first to propose using it as a marker for evolutionary relatedness (4). Only a small portion of microbial DNA is targeted by the 16S rRNA gene sequencing technique, which offers important insights into the diversity and identity of microbial communities. Bacterial ribosomes consist of a big [50S] and a small [30S] subunit. The 16S rRNA gene codes for the RNA component of the 30S subunit of a prokaryotic ribosome. since of its structure and involvement in cellular function, this gene, which is present in all bacteria and archaea, has been referred to as a "molecular clock" since it facilitates the determination of phylogeny and the separation of species (5).

The 16S rRNA gene is made up of nine hypervariable (6) and eight highly conserved sections, with a length of around 1,550 base pairs (bp). In a 16S rRNA gene sequencing investigation, one or more hypervariable regions are amplified and sequenced using broad-range primers that bind to conserved areas. The taxonomic composition and diversity of the sample are then reconstructed using the data in these locations, which is accomplished by comparing the sequences to databases of known organisms. Depending on the use, 16S rRNA hypervariable fragments as tiny as 100 bp can occasionally yield valid phylogenetic classifications, making widely used and reasonably priced short-read sequencing platforms—like Illumina—suitable for microbiome study.

Although sequencing the whole 16S rRNA gene can yield more information, doing so will cost more money and time, which may outweigh the benefits of this method of high-throughput microbiome sequencing. Similar to this, strain typing bacterial species has been found to benefit from sequencing across rRNA genes to include intergenic regions, although this method necessitates much larger time and financial commitments. In conclusion, 16S rRNA gene sequencing for current microbiome study is relatively inexpensive (often less than US $50 per sample) and can offer a survey of the bacterial and archaeal communities within a sample without the need for culture.

Applicability and constraints of 16S rRNA gene sequencing

Prior to choosing a specific microbiome analysis method, think about your research questions and if 16S rRNA gene sequencing will be able to provide answers. For instance, the metabolic capability or activity of a microbial community cannot be described by 16S rRNA gene sequencing. This is due to the fact that the 16S rRNA gene is a housekeeping gene that is present in all prokaryotes. Variations in its sequence simply signify phylogenetic divergence, which has no bearing on factors like antibiotic resistance, virulence, metabolic capability, etc. Furthermore, there might not be enough sequence variability in the 16S rRNA region to differentiate between different species or strains (such as a commensal strain of Escherichia coli and a pathogenic strain).

The arbitrary "total" that the sequencer imposes, which means that the whole absolute microbial load, or abundance, is not reflected, is another drawback of sequencing-based methods. Total bacterial load cannot be determined solely by 16S rRNA gene sequencing; instead, quantitative techniques such quantitative polymerase chain reaction (qPCR) are needed (7). Since 16S rRNA gene sequencing yields information on the types and relative quantity of microorganisms present, the data it produces can be compared to a

census or "taking attendance." This makes it possible to analyze the similarities and differences between numerous samples and to utilize diversity estimates to characterize the microbial ecology of a single sample.

One known drawback of this strategy is that different organisms have varying amounts of the 16S rRNA gene in their genomes, which causes relative abundance to be skewed toward those with more copies. Despite this known limitation, there are no reliable tools to address this problem, and it is standard practice to ignore the 16S rRNA gene copy number by bacterium (8). Notably, using specific primers rather than broad range primers would be preferable if you are searching for a particular bacterium because modest amounts of the target bacterium could be overlooked by 16S rRNA gene sequencing on its own, leading to a false negative.

The experimental unit, statistical power, and sample size

Numerous dependent variables, such as all the bacterial taxa present in a sample, are routinely analyzed simultaneously in microbiome studies as opposed to just one, like rate of gain and carcass size. However, many of the same experimental design considerations, like effect and sample size, experimental unit, and study design, must be assessed. While several publications describe techniques for power analysis in microbiome studies, these instruments are restricted to certain studies or have particular design requirements (e.g., case–control studies). Unfortunately, power analysis is still uncommon in microbiome investigations because of the complexity of these studies, which includes rare species, unclear effect sizes, sequencing depth, and bioinformatic tools. If studies that are comparable to the one you want to perform have been done before (9) however, this is still rare for the majority of animal sciences topics. However, if you are able to create a pilot sample set, you may be able to determine the sample and effect sizes more precisely. Sample size and power may become more estimable when more microbiome data are collected and made available to the public (10). Before the study begins, the experimental and sampling unit (one animal and one fecal grab) should also be decided upon, with special attention to the pooling of samples (see below). Lastly, even though a single sample could provide tens of thousands of sequencing reads, established practices for experimental design and biological replication still need to be adhered to.

Even with the fact that many microbiome studies have reduced sample sizes because of financial constraints, an investigator still needs to be mindful of sample size and

degree of freedom limitations and avoid subjecting microbiome data to unduly complex study designs.

Additionally, when pooling, you lose the ability to determine what microbes may be interacting in a single community. For example, if you are interested in which organisms may be associated with the presence of pathogens in a fecal microbiome, pooling across feedlot pen would limit your ability to address that research question if there is a difference between pens. Additionally, pooling may result in rare taxa not being detected as well as possible alterations in alpha and beta diversity. Therefore, like all suggestions made in this review, your choices in designing the sampling scheme should be carefully weighed against your research questions.

Combining samples

The pooling of samples is one particular factor that is closely related to sample size. Samples can be combined according to the raw material (e.g., equal amounts of meat, excrement, or rumen fluid) or according to the extraction method (e.g., equal weights or volumes). In addition to economic savings and the ability to use more representative experimental units of a microbiome (20 fecal grabs from a pen as opposed to one), pooling raises the possibility that variations between individual animals or groups may go unnoticed. A number of factors related to animal homology need to be taken into account when pooling samples, including husbandry, background, geography, and production method. If applicable, vertical integration of the production system should be taken into consideration; for instance, cattle at a feedlot are probably not from chickens in several houses at the same place might come from the same breeder, but all the stocker sources would be different. Indeed, the majority of the observed microbiome changes in feedlot research intended to assess a feed additive (11), were explained by the cattle source. The diet, veterinary care, and geographic history of animals can all be used to inform pooling technique; however, these host-level parameters are frequently unknown. Although pooling across these characteristics should be taken into consideration in an experimental design, such as a block, pooling should ideally be carried out across the most homologous group of animals that can be found, such as the same pen, barn, treatment group, or pasture. As with any animal research study, the optimal case would be to administer the treatment to the most similar group. will lessen variance that is not partitioned. Type II errors may occur if the pool's internal variance is greater than its external variation. When pooling, it is necessary to weigh the trade-off between bigger experimental units that may

be more representative of the overall population and smaller experimental units that are more homologous in type.

The ideal site for sampling

There are several factors to take into account while choosing the best places for samples, and these factors should be concentrated on meeting the goal or hypothesis of the research endeavor. The biological relevance of a sample site has to be taken into account. For instance, can variations in the microbiota of the chicken gastrointestinal tract (GIT) be inferred from cloacal swabs, fecal collections, or poultry litter? For instance, uncommon taxa in the ceca are not well represented in cloacal swabs, despite the correlation between cloacal swabs and cecal composition in broilers. Furthermore, in certain commercial production situations, quantifying individual animal phenotypes can be costly and often impracticable. Two instances of selecting a sampling location from two distinct species are as follows: 1) selecting the section of selecting a sample from a bovine GIT to use as a representative example of the chicken GIT in a broiler investigation.

Fecal and/or ruminal sampling is frequently done in the case of the bovine GIT, where numerous research have concentrated on the reticulorumen because of its key function in nutrition and metabolism. However, whereas fecal or colorectal samples are frequently used as a stand-in for rumen microbial activity, this could be problematic depending on the study's goals. If investigating elements such nutrient degradability (12), the effect of a nutritional supplement or diet on fecal pathogen shedding, then using fecal samples may be necessary. The digestion process, the absorption of fatty and amino acids, the post-ruminal breakdown of starch and cellulose, and the development of the mucosal immune system, all of which emphasize the variations between the sections of the lower gastrointestinal tract in cattle. This problem is not species-specific, and numerous investigations have used various methodologies to comprehend the microbiome of the chicken gastrointestinal tract. Previously, researchers used other methods, including fecal samples, to study the microbiota instead of the GIT because of the cost of euthanizing fowl to get digesta samples within the GIT. Due to this endeavor, inaccurate treatment effects on the microbiota were assumed (13). As an illustration, The cecal microbiota is not fully represented by the microorganisms found in feces, though. found that the bacteria in the feces and ceca of 163 birds in three trials' worth of fecal microbiota were comparable. albeit not in the same proportional amounts. Consequently, sampling from the feces or other readily accessible matrices may not be adequate for reflecting the site of interest

when attempting to find proxies for ease of sampling. For these microbiome research, selecting the best sample location is a crucial—and frequently challenging—step in project and experimental design. The location of the sample for amplicon-based rRNA research must eventually be determined by taking into account the study's hypothesis, objective, and/or knowledge of the physiology of the target tissue and/or host species.

The significance of precise metadata

All of the different data points that are collected to describe the biological specimens are referred to as "metadata." The study of 16S rRNA sequences from host-associated or environmental samples might benefit greatly from these data, which offer an essential context. For bioinformatic and downstream analysis, appropriately organizing and producing metadata from experiment data is essential. Preserving comprehensive metadata has several advantages. Crucially, as much information as possible—such as sample locations, dates, and times—as well as the identity of the sample source, whether the sample is single or pooled, treatments, and other observations made during the study—should be gathered in order to produce adequate and accurate metadata. After information is gathered, it should be suitably preserved in a standardized manner to enable allowing other parties or partners to be able to quickly understand the data (14) outlined some guidelines for formatting and gathering metadata; these guidelines should be adhered to, particularly if one plans to submit data to a publicly accessible database, which is sometimes necessary prior to publishing. Whatever bioinformatics tool is used for statistical analysis, taxonomic assignment, diversity, and other downstream analyses, the collection of precise metadata enhances such analyses. Accurate metadata is crucial for studies since bioinformatic pipelines need consistent names and formats in order to input data correctly. Furthermore, statistical analyses are frequently incorporated into these sequence-processing pipelines, which makes structuring metadata essential for performing multiple comparisons between variables. Accurate metadata can enhance reproducibility of collected data as well as data observations (15).

If your animal science research involves working with industry partners (farms, pastures, processing facilities, or retail outlets) that do not want to be recognized, you should take extra care when gathering metadata. Certain databases might need the precise location of the sample collection site, for instance. Before the study begins, it is crucial to think through appropriate communication, nondisclosures, and terminology that can be used to characterize the sample location. This could include generic regional data from a

manuscript (like a commercial feedlot in the Midwest) and a geographic location (like the latitude and longitude of the university lab where it was processed) in your publicly accessible metadata. For bioinformatic analysis of the data, precise and well-organized metadata is essential. When gathering data, care should be made so that different sources can comprehend the metadata for analysis. All things considered, precise metadata make it easier to locate data, use it in analyses, and raise the possibility that it will be employed in more research down the road.

Managing and storing in the field

For 16S rRNA gene sequencing, optimal sample preservation methods are essential for accurate results since numerous factors might change the integrity of the sample during collection (16). The best approach is to immediately freeze a sample after field collection (at least at −20 °C), although this is sometimes not feasible for field investigations. The amount of time between collection and freezing has been shown to considerably affect the outcome of 16S rRNA gene sequencing. For instance, one study on feces looked at six distinct window of time—from one to thirty-six hours—between the time of sample collection and freezing. The study discovered that the freezing window had a substantial impact on the microbiome composition results. Furthermore, a comparison study of feces' storage revealed that samples were immediately frozen. at -80 °C compared to those kept in 95% ethanol at ambient temperature before freezing showed stability for some bacterial populations, while Actinobacteria and other bacterial populations showed considerable changes. However, three distinct techniques—95% ethanol, Flinders Technology Associates cards, and the OMNIgene Gut kit—all adequately preserve samples at room temperature in an extensive investigation involving over 1,200 samples. Long-term storage at -80 °C has also been evaluated; while alterations are inevitable because of the duration of storage, these changes are comparable to inter- and intra-subject variance and are thus still appropriate for microbiome research (16). Maintaining a clean work area is another crucial element that is frequently disregarded in 16S rRNA gene sequencing investigations. In agricultural contexts, environmental contamination poses a serious risk, hence it's important to take precautions to sanitize work locations. Prior to sampling, barn surfaces should be cleaned with ethanol or a 10% bleach solution. Cleaning should be done in between samples to prevent cross-contamination. tests of dirt, for instance, left on a tabletop can contaminate subsequent tests. Even though it takes time, setting up a sterile or clean workspace before sampling is crucial to lowering the possibility of microbiological contamination. The use of

sterile sample swabs or collection containers is one component of a clean sampling environment. Not For 16S rRNA gene sequencing operations, any brand or type is appropriate, but it's important to get sterile, DNA-free swabs, bins, and tubes. Ensuring the workspace is sterilized and adhering to uniform freezing protocols on time will yield precise and superior 16S rRNA gene sequencing outcomes.

Cell lysis

The outer boundary, cell wall, and/or membrane must be efficiently disrupted or lysed in order to collect representative grade genomic DNA for the purpose of amplifying the 16S rRNA gene and sequencing the targeted region. The process of breaking down or destroying the cell wall and/or membrane in order to liberate intercellular materials like DNA, RNA, proteins, or organelles is known as cell lysis. Currently, there are several techniques, such as mechanical, physical, chemical, and enzymatic lysis, to damage the cell wall and/or membrane.

Depth of sequencing (Library preparation)

The V4 hypervariable region has gained popularity since it is used in the Earth Microbiome Project and has been proved to offer appropriate resolution and generate optimal community clustering with short-length reads. Sequencing tools can generate single- or paired-end reads that enable sequencing of the required amplicons, regardless of the variable region. Researchers are encouraged to sequence a mixture of nearby hypervariable regions because recent developments in the sequencing chemistry used to produce short reads have allowed for read lengths up to 300 base pairs (and roughly 500 bp if using paired-end reads). The benefit of doing this is that longer amplicons include more phylogenetic information, which helps to clarify any existing taxonomic uncertainties. while classifying a taxon using sequences. Although it can be somewhat random, the selection of the hypervariable region or regions to sequence should ideally be based on factors unique to the field or inquiry. For instance, the V4-V5 hypervariable region is preferred in arctic microbial communities because it offers better taxonomic coverage and resolution of archaeal groups, which make up a small but essential part of the arctic marine environment in the female genital tract; the V3-V4 hypervariable region is preferred because it can identify bacterial species linked to human skin diseases and vaginal health; and the V1-V3 hypervariable region is preferred because the microbial communities recovered there closely resemble those reported from deep shotgun metagenomic assays (17).

Recognizing contaminants

The most straightforward method for detecting and removing contamination in silico is to survey high-biomass samples using narrow taxonomic-range primers (such as genus- or species-specific probes). Single reads or contigs can be taxonomically assigned by alignment against a taxonomy database like Silva, Greengenes, or RDP after completing preliminary quality control procedures (such as size selection, quality filtering, adaptor cutting, and chimera testing). Sequences that have taxonomic designations outside of the primer set's predicted taxonomic range could be marked as nontarget and removed from further processing. Taxonomy-based contaminant identification procedures are therefore frequently seen in widely used amplicon analysis pipelines, such as QIIME2. Identifying contaminants in low-biomass samples becomes more complicated when broad taxonomic primers are used for the survey. The combined impacts in this case are 1) reduced biomass, leading to Confounding factors for in silico contaminant identification and removal include: 1) a lower number of target genes; 2) the presence of contaminant chromosomal DNA, which is common in PCR regents and nucleic acid extraction; and 3) the possibility of true overlap in the expected taxonomy of target and nontarget sequences (20).

Summary:

When appropriate procedures are adhered to, 16S rRNA gene sequencing can offer ecological insights that conventional microbiological techniques alone are unable to provide. All stages of a study, including planning, wet lab, bioinformatics, and statistical analysis, involve concerns specific to the microbiome. However, these concerns can be addressed and problems can be minimized with careful planning. Reviews of the literature closely related to your study or other scientists doing similar work are appropriate sources of solutions. When questions do arise regarding any specific portion of an experiment, the first thing you should do is ask how it relates to your specific hypothesis, as there is no one-size-fits-all answer. When examining a novel ecological niche with minimal to no prior knowledge, a brief pilot research with a limited number of samples will provide numerous answers. queries about sampling location and depth of sequencing.

References:

1. Staley, J. T., and Konopka A.. 1985. Measurement of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. *Annu. Rev. Microbiol*. 39:321–346.

2. Nielsen, H. B., Almeida M., Juncker A. S., Rasmussen S., Li J., Sunagawa S., Plichta D. R., Gautier L., Pedersen A. G., Le Chatelier E., . et al.; MetaHIT Consortium; MetaHIT Consortium. 2014. Identification and assembly of genomes and genetic elements in complex metagenomic samples without using reference genomes. *Nat. Biotechnol*. 32:822–828.
3. Addis, M. F., Tanca A., Uzzau S., Oikonomou G., Bicalho R. C., and Moroni P.. 2016. The bovine milk microbiota: insights and perspectives from -omics studies. Mol. Biosyst. 12:2359–2372. doi: 10.1039/c6mb00217j
4. Olsen, G. J., and Woese C. R.. 1993. Ribosomal RNA: a key to phylogeny. *FASEB J*. 7:113–123.
5. Duchêne, S., Holt K. E., Weill F. X., Le Hello S., Hawkey J., Edwards D. J., Fourment M., and Holmes E. C.. 2016. Genome-scale rates of evolutionary change in bacteria. *Microb. Genom*.
6. Clarridge, J. E., 3rd. 2004. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clin. Microbiol. Rev*. 17:840–862, table of contents.
7. Gloor, G. B., Macklaim J. M., Pawlowsky-Glahn V., and Egozcue J. J.. 2017. Microbiome datasets are compositional: and this is not optional. *Front. Microbiol*. 8:2224.
8. Louca, S., Doebeli M., and Parfrey L. W.. 2018. Correcting for 16S rRNA gene copy numbers in microbiome surveys remains an unsolved problem. *Microbiome* 6:41.
9. Debelius, J., Song S. J., Vazquez-Baeza Y., Xu Z. Z., Gonzalez A., and Knight R.. 2016. Tiny microbes, enormous impacts: what matters in gut microbiome studies? *Genome Biol*. 17:217
10. McDonald, D., Birmingham A., and Knight R.. 2015. Context and the human microbiome. *Microbiome* 3:52.
11. Huebner, K. L., Martin J. N., Weissend C. J., Holzer K. L., Parker J. K., Lakin S. M., Doster E., Weinroth M. D., Abdo Z., Woerner D. R., . *et al.,* 2019. Effects of a *Saccharomyces cerevisiae* fermentation product on liver abscesses, fecal microbiome, and resistome in feedlot cattle raised without antibiotics. *Sci. Rep*. 9:2559.
12. Kaltenegger, A., Humer E., Pacífico C., and Zebeli Q.. 2021. Feeding dairy cows bakery by-products enhanced nutrient digestibility, but affected fecal microbial composition and pH in a dose-dependent manner. *J. Dairy Sci*. 104:7781–7793

13. Schreuder, J., Velkers F. C., Bouwstra R. J., Beerens N., Stegeman J. A., de Boer W. F., van Hooft P., Elbers A. R. W., Bossers A., and Jurburg S. D.. 2020b. An observational field study of the cloacal microbiota in adult laying hens with and without access to an outdoor range. *Anim. Microbiome* 2:28.
14. Yilmaz, P., Kottmann R., Field D., Knight R., Cole J. R., Amaral-Zettler L., Gilbert J. A., Karsch-Mizrachi I., Johnston A., Cochrane G., . *et al.,* 2011. Minimum information about a marker gene sequence (MIMARKS) and minimum information about any (x) sequence (MIxS) specifications. *Nat. Biotechnol*. 29:415–420.
15. Tap, J., Cools-Portier S., Pavan S., Druesne A., Öhman L., Törnblom H., Simren M., and Derrien M.. 2019. Effects of the long-term storage of human fecal microbiota samples collected in RNAlater. *Sci. Rep*. 9:601.
16. Claassen-Weitz, S., Gardner-Lubbe S., Mwaikono K. S., du Toit E., Zar H. J., and Nicol M. P.. 2020. Optimizing 16S rRNA gene profile analysis from low biomass nasopharyngeal and induced sputum specimens. *BMC Microbiol*. 20:113.
17. Meisel, J. S., Hannigan G. D., Tyldsley A. S., SanMiguel A. J., Hodkinson B. P., Zheng Q., and Grice E. A.. 2016. Skin microbiome surveys are strongly influenced by experimental design. *J. Invest. Dermatol*. 136:947–956.
18. Bokulich, N. A., Kaehler B. D., Rideout J. R., Dillon M., Bolyen E., Knight R., Huttley G. A., and Gregory Caporaso J.. 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome* 6:90
19. Love, M. I., Huber W., and Anders S.. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*. 15:550.
20. Rothrock, M. J., Jr, Locatelli A., Glenn T. C., Thomas J. C., Caudill A. C., Kiepper B. H., and Hiett K. L.. 2016. Assessing the microbiomes of scalder and chiller tank waters throughout a typical commercial poultry processing day. *Poult. Sci*. 95:2372–2382.

ISOLATED D-PINITOL FROM AERIAL PARTS OF SOYBEAN PLANTS PLAYS A PROTECTIVE ROLE ON DOXORUBICIN INDUCED CARDIOTOXICITY IN RAT MODEL: HISTOPATHOLOGICAL STUDIES OF HEART

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Abstract:

Introduction: D-Pinitol, a polyol is a potent antioxidant agent and anti-inflammatory agent. Hence, this study was carried out to evaluate whether D-Pinitol administration prior to induction of cardiotoxicity using Doxorubicin would protect cardiac histopathological changes in an experimental rat model.

Methods: Ten groups of sixty Swiss Albino mice were taken for administration: Control, Doxorubicin (5 mg/kg), D-Pinitol (400 mg/kg, 300 mg/kg, 200 mg/kg & 100 mg/kg), and D-Pinitol (400 mg/kg, 300 mg/kg, 200 mg/kg & 100 mg/kg) + Doxorubicin (5 mg/kg). DOX-induced cardiac toxicity was characterized by the histopathological observations.

Results: Myocyte necrosis was clearly seen in the group that received just doxorubicin therapy. Administering D-Pinitol before receiving Doxorubicin minimized myocyte necrosis and degeneration of myocytes.

Conclusions: Administration of D-Pinitol to Doxorubicin-challenged rats ameliorated alterations in histological cardiac damage compared with the Doxorubicin alone treated group. As a result, D-Pinitol supplements may help lessen the cardiac damage caused by doxorubicin.

Keywords: Cardiotoxicity; Doxorubicin; D-Pinitol; Histopathology.

Introduction:

Globally, the two leading causes of mortality and morbidity are heart diseases and cancer. Chemotherapy is an important treatment modality for many different types of malignancies, including breast cancer. (Gürses I., 2014) Numerous chemotherapy medicines are used to treat a number of neoplastic illnesses. The extent and frequency of

side effects at therapeutic levels is one trait that distinguishes anticancer medications from other therapies. (Remesh A., 2012) An outstanding class of chemotherapy drugs used to treat a variety of hematological and solid cancers are anthracyclines. The most potent and widely used anthracycline is doxorubicin (DOX). (Subburaman S., 2014) DOX causes an abundance of free radicals, oxidative stress, along with substantial inflammatory reactions in a number of tissues. (Cortés-Funes H, 2007) The accumulation of lipid peroxides in free-radical-induced oxidative stress brings hydrophilic moieties into the membrane's hydrophobic phase, altering membrane permeability and function in cells. (Suntres ZE., 2011) This causes myocardial structural integrity to deteriorate and cardiac function to decline, culminating in cardiotoxicity. (Quiles, JL., 2002; Woodley-Cook, J., 2006; Saalu, L.C., 2009) DOX also causes inflammation in the vascular and heart, which raises levels of inflammatory cytokines. (Liu, Y., 2006; Mercuro, G., 2007) Therefore, one potential treatment strategy to lessen the cardiotoxicity of DOX is to combine it with cardioprotective drugs. D-Pinitol (D-P), a polyol, is a soluble carbohydrate found in all sections of *Glycine max* L. Merr. (Soybean plants). (Mercuro, G., 2007; Jayasooriya, RGPT., 2015). Because of its diverse pharmacological effects, D-P has sparked a lot of attention as a natural medication. D-P possesses a range of therapeutically beneficial qualities, including cardioprotective (Sripathi, S.K., 2013).) due to its anti-inflammatory, (López-Domènech, S., 2018).) and antioxidant (Rengarajan, T., 2014). capabilities. As a result, the role of D-P in reducing undesirable effects following DOX therapy was studied in this study. antioxidant properties.

Materials and Methods:

Ethics statement:

The Institutional Animal Ethics Committee (IAEC) of Adhiparasakthi College of Pharmacy (Reg. No. 409/PO/Re/S/01/CPCSEA) approved the experimental protocol. The approval number was APCP/IAEC/2019-2020/1.

Materials required:

Hematoxylin and eosin stain (Himedia, India), Giemsa (Himedia, India), Formaldehyde (Labogens, India), Paraffin Wax (Labogens, India), Diluent for smear preparation: 5% bovine serum albumin (BSA) in Phosphate buffered saline (Himedia, India) and Microscope (Olympus Optical Co., Germany).

Experimental animal:

Both male and female Swiss Albino mice of weight 25–30 g were kept in a 12-hour light/dark cycle. Animals were acclimatized by maintaining them in a clean environment according to CPCSEA guidelines. (Yadav, A.R. 2020)

Methodology:

Treatment protocol:

Ten groups of animals were utilized in this study (six mice in each group - Table 1). D-P (all doses viz, 100, 200, 300 & 400 mg/kg) was given for fifteen days to mice and DOX (on 1st day, 8th day, and 15th day) (Padmanabhan, S., 2009) was administered for three days based on the treatment protocol. D-P treated 30 minutes prior to the DOX administration.

Histopathological investigations were used to detect the toxicity to cardiac tissues. For histopathological studies of the heart, the tissue was dissected. The tissue samples were fixed in formalin solution (10% V/V) and embedded in paraffin (4 mm thick). The slides were stained with hematoxylin and eosin for microscopic examination (Olympus Optical Co., Germany). (Hassan H.F.H., 2017)

Table 1: Treatment Protocol

Group	**Labeled**	**Treatment**
I	Vehicle Control	0.5 ml of 0.9% normal saline
II	Positive Control	Doxorubicin (5 mg/kg), i.p. on 1st, 8th and 15 th days (Positive Control)
III	Test Drugs	D-Pinitol (100 mg/kg), p.o. daily
IV		D-Pinitol (200 mg/kg), p.o. daily
V		D-Pinitol (300 mg/kg), p.o. daily
VI		D-Pinitol (400 mg/kg), p.o. daily
VII		Doxorubicin (5 mg/kg), i.p. on 1st, 8th and 15 th days+ D-Pinitol (100 mg/kg), p.o. daily
VIII		Doxorubicin (5 mg/kg), i.p. 1st, 8th and 15 th days + D-Pinitol (200 mg/kg), p.o. daily
IX		Doxorubicin (5 mg/kg), i.p. 1st, 8th and 15 th days + D-Pinitol (300 mg/kg), p.o. daily
X		Doxorubicin (5 mg/kg), i.p. 1st, 8th and 15 th days + D-Pinitol (400 mg/kg), p.o. daily

Results and Discussion:

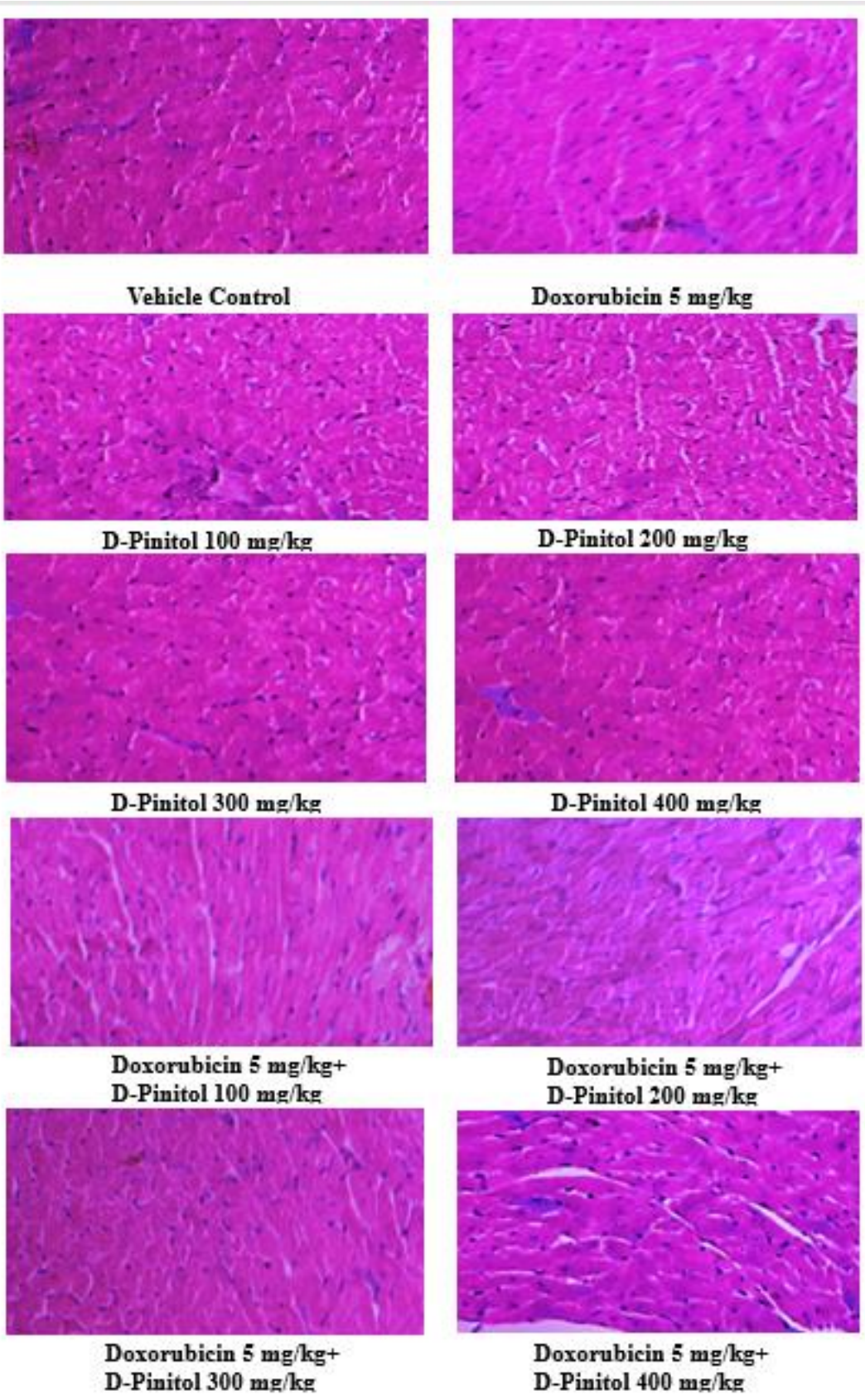

Figure 1: Photomicrograph of a section of heart

In the vehicle control group, the typical histological architecture of heart muscle with cylindrical branching of cardiac myocytes was seen in the histological findings. The majority of the myocytes appeared longitudinally and obliquely cut. The cardiac muscle fiber structures in all D-P alone treated groups (groups III to VI) were normal, as they were in the vehicle control group. Hence, it was revealed that D-P had no adverse effects on cardiac tissue. On the other hand, the typical properties of heart tissue were not present in DOX-treated mice. The fibers of the heart muscle were significantly disturbed. Most of the cardiac myocytes in the tissue had separated and seemed to be deteriorating. The number and severity of necrosis was prominently observed in DOX-alone treated group. When D-P was given to mice that had received DOX treatment, the toxic effects of DOX on cardiac muscle fibers were alleviated with reduction in necrosis and degeneration of myocytes, which showed the cardioprotective effect of D-P.

Prior research concluded that DOX-induced substantial degeneration in heart. (Pugazhendhi, A., 2018; Shivakumar, P., 2012) The current research also found that DOX, when administered for 15 days, caused histopathological degeneration in heart. DOX-induced ROS, oxidative stress (Deavall, D.G., 2012; Shivakumar, P., 2012).) and inflammatory mediators (Hussain, M.A., 2021).) can interact with cell macromolecules to cause cytological damage. D-P has no toxic effects on organs, tissue, or bone marrow when treated alone. Because of its free radical quenching function, antioxidant activity, and anti-inflammatory property, D-P administration could successfully reverse the histological alterations in the bone, bone marrow, and organs examined. As a result of the current research, it can be inferred that DOX caused damage to bone, bone marrow, and visceral organs damage which can be prevented by D-P.

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References:

1. Gürses, İ., Özeren, M., Serin, M., Yücel, N., & Erkal, H. Ş. (2014). Histopathological evaluation of melatonin as a protective agent in heart injury induced by radiation in a rat model. Pathology. *Research and Practice*, 210(12), 863–871. doi:10.1016/j.prp.2014.08.006

2. Remesh, A. (2012). Toxicities of anticancer drugs and its management. *Int J Basic Clin Pharmacol*, 1(1), 2-12.
3. Subburaman, S., Kumaresan, G., & Murugesan. R. (2014). Protective Role of Naringenin Against Doxorubicin-Induced Cardiotoxicity in a Rat Model: Histopathology and mRNA Expression Profile Studies. Journal of Environmental Pathology. *Toxicology and Oncology*, 33(4), 363–376. doi:10.1615/jenvironpatholtoxicoloncol.2014010625
4. Cortés-Funes, H., & Coronado, C. (2007). Role of anthracyclines in the era of targeted therapy. *Cardiovasc Toxicol*, 7(2), 56-60. https://doi.org/10.1007/s12012-007-0015-3
5. Suntres Z.E., (2011). Liposomal antioxidants for protection against oxidant-induced damage. *J Toxicol*.2011, 152474–89.
6. Quiles, J.L., Huertas, J.R., Battino, M., Mataix, J., & Ramerez-Tortosa, M.C. (2002) Antioxidant nutrients and adriamycin toxicity. *Toxicology,* 180, 79–95.
7. Woodley-Cook, J., Shin, L.Y., Swystun, L., Caruso, S., Beaudin, S., & Liaw, P.C. (2006). Effects of the chemotherapeutic agent doxorubicin on the protein C anticoagulant pathway. *Mol. Cancer Ther,* 5, 3303–11.
8. Saalu, L.C., Ajayi, G.O., Adeneye, A.A., Imosemi, I.O., & Osinubi, A.A. (2009). Ethanolic seed extract of grape fruit (Citrus paradisi Macfad) as an effective attenuator of doxorubicin-induced oxidative stress in the rat heart. Int J *Cancer Res,* 5, 44–52.
9. Liu, Y. (2006). Renal fibrosis: new insights into the pathogenesis and therapeutics. *Kidney Int,* 69, 213–7.
10. Mercuro, G., Cadeddu, C., Piras, A., Dessì, M., Madeddu, C., Deidda, M., Serpe, R., Massa, E., & Mantovani, G. (2007). Early epirubicin-induced myocardial dysfunction revealed by serial tissue Doppler echocardiography: correlation with inflammatory and oxidative stress markers. *Oncologist*, 12, 1124–33.
11. Jayasooriya, R.G.P.T., Kang, C-H., Park, S.R., & Choi, Y-H., *et al.,* (2015). Pinitol Suppresses Tumor Necrosis Factor-α-Induced Invasion of Prostate Cancer LNCaP Cells by Inhibiting Nuclear Factor-κB-Mediated Matrix Metalloproteinase-9 Expression. *Trop J Pharm Res*, 14(8), 1357-1364. http://dx.doi.org/10.4314/tjpr.v14i8.6
12. Sripathi, S.K., Poongothai, G. (2013). A review on insulinomimetic Pinitol from plants. *Int J Pharm Bio Sci*, 4(2), 992-1009.

13. López-Domènech, S., Bañuls, C., de Marañón, A.M., & Abab-Jiménez, Z., *et al.,* (2018). Pinitol alleviates systemic inflammatory cytokines in human obesity by a mechanism involving unfolded protein response and sirtuin 1. *Clin Nutr*, 37, 2036-2044. https://doi.org/10.1016/j.clnu.2017.09.015
14. Rengarajan, T., Balasubramanian, MP., Rajendran, P., & Nandakumar, N., *et al.,* (2014). Free radical scavenging and antioxidant activity of D-pinitol against 7, 12-Dimethylbenz(a) Anthracene induced breast cancer in Sprague Dawley rats. *Asian Pac J Trop Dis,* 4(5), 384-390. https://doi.org/10.1016/S2222-1808(14)60592-2
15. Rengarajan, T., Nandakumar, N., Rajendran, P., &Haribabu, L., *et al.,* (2014). D-Pinitol Promotes Apoptosis in MCF-7 Cells via Induction of p53 and Bax and Inhibition of Bcl-2 and NF-κB. *Asian Pac J Cancer Prev,* 15(4), 1757-1762.
16. Yadav, A.R. (2020). CPCSEA guidelines for laboratory animal facility. *Int J Clin Pharmacol Res*, 2(1), 9-13.
17. Padmanabhan, S., Tripathi, D,N., Vikram, A., & Ramarao, P., *et al.,* (2009). Methotrexate-induced cytotoxicity and genotoxicity in germ cells of mice: Intervention of folic and folinic acid. *Mutat Res*, 673(1), 43-52.
18. Hassan HFH, Mansour AM, Abo-Youssef AMH, & Elsadek BEM, *et al.,* (2017) Zinc oxide nanoparticles as a novel anticancer approach *in vitro* and *in vivo* evidence. *Clin Exp Pharmacol Physiol*, 44(2), 235-243. doi: 10.1111/1440-1681.12681.
19. Pugazhendhi, A., Edison, T.N.J.I., Velmurugan, B.K., & Jacob, J.A., *et al.,* (2018). Toxicity of Doxorubicin (Dox) to different experimental organ systems. *Life Sci,* 200, 26-30.
20. Shivakumar, P., Rani, M. U., Reddy, A.G., & Anjaneyulu, Y. (2012). A Study on the Toxic Effects of Doxorubicin on the Histology of Certain Organs. *Toxicol Int,* 19(3), 241-244.
21. Deavall, D.G., Martin, E.A., Horner, J.M., & Roberts, R. (2012). Drug-Induced Oxidative Stress and Toxicity. *J Toxicol*, 2012, 1-13. doi:10.1155/2012/645460.
22. Hussain, M.A., Abogresha, N.M., AbdelKader, G., & Hassan, R., *et al.,* (2021). Antioxidant and Anti-Inflammatory Effects of Crocin Ameliorate Doxorubicin-Induced Nephrotoxicity in Rats. *Oxid Med Cell Longev,* 2021, 1-12.

SUSTAINING SUCCESS: THE EVOLUTION AND CHALLENGES OF AQUACULTURE IN ANDHRA PRADESH

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Abstract:

India's fish production has grown exponentially, especially due to the paradigm shift of the sector's focus from marine to inland fisheries. Aquaculture dominantly contributes to the country's overall landscape of fish production. Andhra Pradesh is a coastal state in India considered an aquaculture hub due to its huge contribution to fish production. Various factors like favorable climatic conditions, diversification of culture fish species, and integrated efforts of individual, institutional, and governmental have led to the growth of the aquaculture sector in the state. Pangasius, Roop Chand, shrimp, and carp production is profitable. Most of the fish and shrimp produced are marketed to other states like West Bengal, Karnataka, and Tamil Nadu, and only a small percentage of the produce is consumed within the state. Strict Quality Standards, Low Price Realization, Trade Barriers, Inadequate Infrastructure Facilities, No Information about Foreign Markets, Poor Government Support, Legal procedures, High Operational Costs, Tough Competition, and Inadequate Value-Added Products are the major problems of the aquaculture industry in Andhra Pradesh. To ensure the consistent growth of this sector, all the practices from seed production to export should be carried out sustainably.

Keywords: Aquaculture, Andhra Pradesh, Marketing, Farm Economics

Introduction:

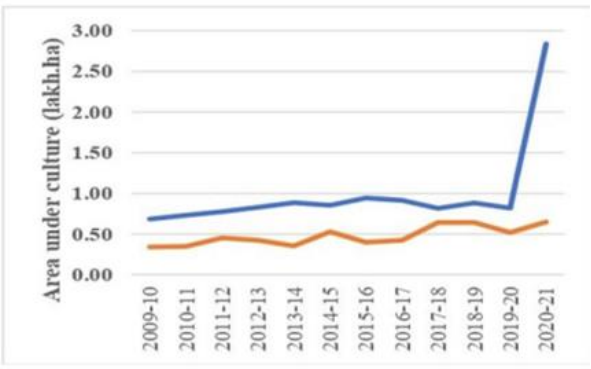
The fisheries sector in India has undergone remarkable development, evolving from a subsistence-based traditional pursuit into a thoroughly developed and diversified commercial enterprise. There has been a significant paradigm shift within the sector, transitioning from focusing on marine fisheries to a greater emphasis on inland fisheries, thereby emerging as a primary contributor to overall fish production. Currently, aquaculture predominantly dominates the fish production landscape in the country, and the total production of the country was 162.48 lakh tonnes, with inland and marine sectors contributing 121.21 lakh tonnes and 41.27 lakh tonnes, respectively (Anonymous, 2022). Andhra Pradesh is renowned as the "aquaculture hub of India" due to its aggressive role in

fish production. The marine fish production in Andhra Pradesh during 2021-22 stood at 5.94 lakh tonnes, whereas inland fish production stood at a remarkable 42.19 lakh tonnes respectively (Anonymous, 2022). A structured and planned expansion of the inland aquaculture farms has led Andhra Pradesh to succeed. The region benefits from favorable climatic conditions, abundant water resources, and an extensive 974 km coastline. It ranked as the leading exporter of marine products in the fiscal year 2021-22. Approximately 1.38 lakh farmers engage in aquaculture across 2.12 lakh ha of land. One hundred eleven cold storage facilities store 2.27 metric tonnes of aquatic products. Additionally, the government now offers subsidized electricity to aquaculture farmers with holdings of less than 10 acres, signaling the further potential for growth in this sector (Siva G, 2023).

History and growth of the aquaculture sector

The growth and success of the aquaculture sector in Andhra Pradesh can be attributed to a combination of factors, including individual, institutional, and governmental efforts. Various stakeholders, such as state and private companies and large and smallholder farmers, have significantly driven the sector's growth. These actors have demonstrated their commitment to aquaculture through investments, risk-taking ability, and strong interlinkages. Fig 1 and Fig 2 shows the growth of area under aquaculture and production in India and Andhra Pradesh and depicts that the production in Andhra Pradesh has surpassed the production of India, despite of its low culture area growth. Diversification of freshwater culture species like exotic striped catfish, pacu, and tilapia in Andhra Pradesh led to aquaculture development in the country. Additionally, the Andhra Pradesh State Fisheries Department has implemented several interventions to support the aquaculture sector, including financial assistance, infrastructure development, and capacity-building programs. These efforts have created an enabling environment for aquaculture development, facilitating increased production, employment generation, and economic growth in Andhra Pradesh's aquaculture sector.

It started primarily with the growth of freshwater aquaculture around the Kolleru Lake region in West Godavari District. This led to the strong growth of forward and backward industry linkages like the production and marketing of fish seed, feed, and other inputs like fertilizers, medicines, icing, packing, transportation, storage, processing, and retailing in this region (Roy *et al.,* 2008). However, this immense growth around the Kolleru Lake region also caused several socio-economic complications.



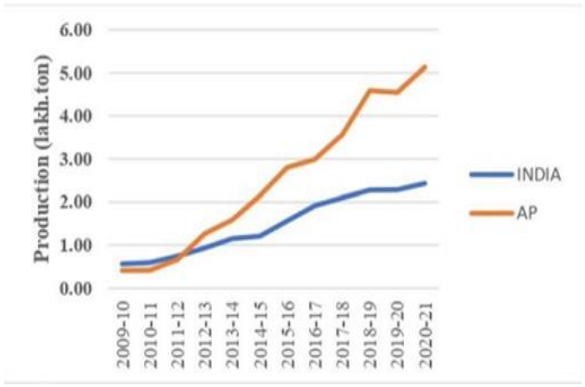


Figure 1: Area under culture (lakh. Ha) **Figure 2: Production (lakh. Ha)**

Source: Dhande *et al.*, 2024

There was a differential impact benefitting some social groups whereas it was detrimental to others. The traditional fishing community population declined around this Kolleru region and certain villages faced enormous deterioration of their economic, health, and living conditions due to the negative externalities of aquaculture (Rao *et al.,* 2018). The major shift in the economic landscape happened after the start of shrimp culture. The early 1990s was considered the golden era of scientific aquaculture. Penaeus monodon was the cultured shrimp at this time, which later, in 1994, was affected by WSSV, leading to considerable losses to all the stakeholders and related industries. A major shift due to the introduction of *Litopenaeus vannamei* again caused a boom in the industry, and until 2017, production of shrimp increased by 83%, making India the second largest shrimp producer in the world. Pangasius (*Pangasianodon hypothalamus*) culture also played a prominent role in growth of this sector in Andhra Pradesh. The huge demand from low-income consumers, lower prices compared to carp, and several such factors led to the growth of the Pangasius culture.

Social profile and culture practices of farmers

The majority of fish farmers of Andhra Pradesh practiced aquaculture mostly in their own ponds (84%), and used their personal savings (42.67%) for aquaculture practice (Abraham *et al.,* 2010). Most of the shrimp (*Litopenaeus vannamei*) farmers in Andhra Pradesh belonged to the age groups of 31-45 years (53.03%), and the majority (27.70%) had a secondary level of education (6th -10th class), 63.3% belonged to the nuclear family type, and nearly 47.22% belonged to the backward class community and the land holding of the farmers was around 2.51- 5 acres (54.44%) (Chittem and Kunda, 2018). In fact, several studies have been conducted covering various aspects of shrimp farming. Factors

like farming experience, mass media exposure, extension agency contact, and landholding affected shrimp farmers' knowledge of better management practices (BMPs). When the Vannamei ponds are infected with various diseases, farmers prefer species diversification to sustain their livelihood. A wide variety of probiotics (almost 45) are used by the shrimp farmers of Andhra Pradesh for the better health of shrimps. Most farmers use only one probiotic, whereas others use up to three probiotics during the culture period.

Farm-level economics and efficiency

Fish farm production Economics and efficiency analysis is required to understand the micro-level health of a particular farm enterprise. It helps in the decision-making and adjusting the farm inputs to maximize the farm output. Polyculture of *Pangasius pangasius* and *Piaractus brachypomus*, along with carps in Andhra Pradesh, is profitable for small and large farmers with net incomes of $5,742 and $5,135. However, a higher percentage of large farmers are more technically efficient than small farmers (Dhande *et al.,* 2023). The farm-specific technical efficiency varied from 75 to 94%, with a mean of 93% for shrimp farmers of Nellore District, whereas for East Godavari District, 32 % of farmers have technical efficiency scores above 95%, while 53.33 % operate between 90 and 95% and 14.67 % of farmers operate at less than 90% (Sivaraman *et al.,* 2015). The Benefit-cost ratio, level of adoption, and average fish yield are higher for Andhra Pradesh farmers than for Odisha farmers. The B:C ratio of Semi-organic shrimp farming and conventional shrimp farming In Krishna and East Godavari districts are 1.88 and 1.57, respectively, indicating that semi-organic farming involving the use of probiotics, antibiotics, and other chemicals is more profitable. For livelihood diversification, the culture of pompano in marine and estuarine cages was successful, with annual net operating income per unit from US$ 1383 to US$ 2917, respectively (Ghosh *et al.,* 2022).

Marketing of harvest from aquaculture

Marketing is another aspect that lays foundation for the economic growth. Only 5% of the harvested fish from Kolleru is marketed within the state, whereas 95% is marketed to other states like Karnataka, Tamil Nadu, Kerala, and Maharashtra, extending way beyond Nepal and North-eastern states. Pangasius is supplied from Andhra Pradesh to West Bengal (73.29%) and Maharashtra (16.4%). Farmers, brokers, transporters, packers, ice providers, wholesalers, secondary wholesalers, and retailers are the actors involved in the Pangasius supply chain (Mugaonkar *et al.,* 2016).

Environmental impacts

Aquaculture, with its economic benefits, also comes with a loss of environmental quality. Important ecosystems are converted and used for aquaculture and later are abandoned after short-term use in Andhra Pradesh. The brackish water aquaculture area in Krishna District increased by 387% from 1990 to 2000. It further decreased by 19% in 2006 leading to the abandonment of 6648 ha of shrimp farms. Later, it again decreased by 47% in 2009, with the number of abandoned shrimp farms rising to 13,493 ha. With the *Litopenaeus vannamei* culture growth from 2009, it led to a revival of 11% of unused, abandoned shrimp farms in 2015 (Jayanthi *et al.,* 2019). Shrimp hatcheries are dependent on the mangroves for various ecosystem services, which implies their threat to the mangroves. The entire Godavari mangrove delta had a partial gross economic value of nearly a million dollars per year for providing shrimp spawners alone.

Disease outbreaks and its economic impacts

In par with the enormous growth, the probability of uncertainty also increased. Several biotic and abiotic factors lead to easy outbreaks of diseases in the culture ponds, leading to huge losses. The output from a fish or shrimp crop depends on the resilience towards various risks. Climate change is the largest risk to coastal aquaculture in the West Godavari District. Nearly, 14% of the aquafarmers are highly vulnerable to climate change, whereas 55% are moderately vulnerable. Environmental, biological, and economic impacts of climate change on aquaculture indicated 20 to 30 % loss due to seasonal variations and 50 to 100% loss due to extremely heavy rainfall, floods, and cyclones (Muralidhar *et al.,* 2021). Crop loss estimation in monetary terms also aids in decision-making for minimizing loss. Health management costs are higher in prawns (Rs. 3136/ acre) than those in carp production systems (Rs.1892/ acre), and also, the physical and financial losses are higher in prawns than in carp culture systems. The revenue loss by Enterocytozoon hepatopenaei (EHP) disease is estimated at Rs. 3977 crores whereas for White spot syndrome virus (WSSV) is Rs. 1670 crores in shrimp farms across India. The significant loss of revenue by EHP is because it was prominent in Andhra Pradesh (Patil *et al.,* 2021). The economic loss in disease-infected Pangasius, IMC's, Roopchand, and Polyculture was estimated in West Godavari and Krishna districts. Economic loss per acre of Pangasius farms is more in Krishna district (Rs. 31633) than the Godavari (Rs. 25488). For IMC farms economic loss per acre is also more in Krishna district (Rs. 24716) than in West Godavari (Rs. 12503). For Roopchand farms economic loss per acre was more in Krishna (Rs. 31375) than in West

Godavari (Rs. 27810). For polyculture systems, the loss per acre in West Godavari (Rs. 30590) is more than in Krishna (Rs. 12811). Covid induced lockdown caused a probable loss of about 40% to the Indian shrimp sector, which in value terms is 1.50 billion USD. Another risk faced by Andhra Pradesh farmers is the menace caused by the Suckermouth armoured catfish (*Pterygoplichthys spp*.) in Krishna and Godavari Districts leading to a decrease in carp production causing economic losses to farmers up to 13.40% and also to fishers to lose their income by 30% by causing extensive damage to their nets and gears (Seshagiri *et al.,* 2021). The recent Covid-19-induced lockdown had an unimaginable impact on all the sector stakeholders. Shortage of labor, input supply disruptions, lack of transportation, and decline in marketing have immensely affected the farmers, causing 10 to 18% losses in production costs.

Challenges and suggestions:

The sector should overcome several challenges like climate change and disease outbreaks, establishing value chains and domestic market linkages, and developing motivated entrepreneurs. Strict Quality Standards, Low Price Realization, Trade Barriers, Inadequate Infrastructure Facilities, No Information about Foreign Markets, Poor Government Support, Legal procedures, High Operational Costs, Tough Competition, and Inadequate Value-Added Products are the major problems of the aquaculture industry in Andhra Pradesh (Chamundeswari and Brindha, 2023). Issues of resource use and distribution of aquaculture practices should be dynamic in planning and execution with the changing environment, particularly climate change issues, continuously changing consumer demand and market environment. To ensure the consistent growth of this sector, all the practices from seed production to export should be carried out sustainably. Environment-friendly farming practices are critical for sustaining the ecological integrity of fragile coastal ecosystems. Emphasis should be laid down for needful development of information and communication technology and precision aquaculture.

Conclusion:

The aquaculture sector in Andhra Pradesh has emerged as a critical contributor to India's fish production, driven by favorable climatic conditions, species diversification, and strong support from stakeholders. The state's strategic shift from marine to inland fisheries has led to significant advancements, particularly in the cultivation of species such as Pangasius, Roop Chand, shrimp, and carp. These efforts have positioned Andhra Pradesh as a leading aquaculture hub with a well-developed supply chain network. However, the

sector faces numerous challenges like disease outbreaks and climate change impacts. Enhancing government support, improving infrastructure, and developing value-added products is imperative to ensure continued growth. Emphasizing sustainable farming practices and improving disease management are crucial for mitigating risks. Additionally, leveraging information and communication technology and fostering innovation in precision aquaculture can drive efficiency and productivity. By addressing these challenges comprehensively, Andhra Pradesh can maintain its leadership in aquaculture production, contributing significantly to the economic development of the state and the nation.

References:

1. Abraham, T.J., Sil, S.K., & Vineetha, P. (2010). A comparative study of the aquaculture practices adopted by fish farmers in Andhra Pradesh and West Bengal. *Indian Journal of Fisheries, 57*, 41–48.
2. Anonymous. (2022). Department of Fisheries, Ministry of Fisheries, Animal Husbandry & Dairying, Government of India, New Delhi. *Handbook of Fisheries Statistics*.
3. Chamundeswari, R., & Brindha, G. (2023). Evaluation of problems aquaculture industry in India—An analytical study. *Journal of Survey in Fisheries Sciences, 10*, 1139–1153.
4. Chittem, P.B., & Kunda, S.K. (2018). Socio-economic condition of the *Litopenaeus vannamei* farmers with implementation of better management practices (BMPs) in Andhra Pradesh, India. *International Journal of Fisheries and Aquatic Studies, 6*(6), 325-331.
5. Dhande, K. K., Sharma, R., Kumar, R. S., & Prasad, G. S. (2024). Inland low saline shrimp culture in Andhra Pradesh: profitability and resource use efficiency. *Aquaculture International, 1*-14.
6. Dhande, K.K., Sharma, R., P. S., A., A., V. (2023). Profitability and resource use efficiency of polyculture system in Andhra Pradesh. *Aquaculture International, 31*, 2905–2918. https://doi.org/10.1007/s10499-023-01115-6
7. Ghosh, S., Jeeva, J.C., Raju, S.S., Megarajan, S., Ranjan, R., Xavier, B., Edward, L., & Gopalakrishnan, A. (2022). Cage Aquaculture of Indian Pompano for Livelihood Diversification of Artisanal Fishers: Insights from Andhra Pradesh, India.
8. Jayanthi, M., Ravisankar, T., Nagaraj, G., Thirumurthy, S., Muralidhar, M., & Saraswathy, R. (2019). Is aquaculture abandonment a threat to sustainable coastal

resource use? – A case study of Andhra Pradesh, India, with options for reuse. *Land Use Policy, 86*, 54-66.

9. Mugaonkar, P., Kumar, N.R., Shelar, G., Biradar, R.S., & Rao, K.G. (2016). Delineation of supply chain of Pangasius in India—A case of Andhra Pradesh. *Current World Environment, 11*, 907–915.
10. Muralidhar, M., Kumaran, M., Jayanthi, M., Syama Dayal, J., Ashok Kumar, J., & Saraswathy, R. (2021). Impacts of climate change and adaptations in shrimp aquaculture: A study in coastal Andhra Pradesh, India. *Aquatic Ecosystem Health & Management, 24*, 28–38.
11. Patil, P.K., Geetha, R., Ravisankar, T., Avunje, S., Solanki, H.G., Abraham, T.J., Vinoth, S.P., Jithendran, K.P., Alavandi, S.V., & Vijayan, K.K. (2021). Economic loss due to diseases in Indian shrimp farming with special reference to *Enterocytozoon hepatopenaei* (EHP) and white spot syndrome virus (WSSV). *Aquaculture, 533*, 736231.
12. Rao, K.N., Demudu, G., & Malini, B.H. (2018). Socioeconomic implications of commercial aquaculture in Kolleru Lake, east coast of India. *Population Geography, 40*(1&2), 09-23.
13. Roy, A.K., Saha, G.S., Kumaraiah, P., & Sarangi, N. (2008). Growth of forward and backward industries linked with aquaculture in Kolleru Lake area, Andhra Pradesh, India. *Aquaculture Asia, 12*, 12–14.
14. Seshagiri, B., Swain, S.K., Pillai, B.R., Satyavati, C., Sravanti, Y., Rangacharyulu, P.V., Rathod, R., & Ratnaprakash, V. (2021). Suckermouth armoured catfish (*Pterygoplichthys* spp.) menace in freshwater aquaculture and natural aquatic systems in Andhra Pradesh, India. *International Journal of Fisheries and Aquatic Studies, 9*(1), 375-384.
15. Siva, G. (2023, March 2). Aquaculture industry set to scale new heights. *The Times of India*. https://timesofindia.indiatimes.com/city/visakhapatnam/aquaculture-industry-set-to-scale-new-heights/articleshow/98350007.cms
16. Sivaraman, I., Krishnan, M., Ananthan, P.S., Satyasai, K.J.S., Krishnan, L., & Haribabu, P. (2015). Technical efficiency of shrimp farming in Andhra Pradesh: Estimation and implications. *Current World Environment, 10*(1), 199.

STUDY ON SEASONAL VARIATION OF ZOOPLANKTON IN PINGALI LAKE NEAR DAHIWADI, DIST. SATARA (M.S.) INDIA

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Abstract:

The present study is carried out during the period of Jan 2023 to Dec 2023 to investigate seasonal variation of zooplankton in Pingali Lake near Dahiwadi, Dist Satara (M.S). During study period total 14 zooplankton species were found belonging to different group Rotifera consist of 6 species, Cladocera consist of 3 species, Copepoda consist of 4 species, and 1 species of Ostracoda. The number of zooplankton was highest in summer season followed by winter and monsoon season.

Keywords: Zooplankton, Lake, Species, Season

Introduction:

Zooplankton are a various group of heterotrophic organisms that consume phytoplankton, regenerate nutrients via their metabolism, and transfer energy to higher trophic levels [16]. Zooplankton are central mechanisms of aquatic food webs and contribute suggestively to aquatic productivity in freshwater ecosystems. Zooplankton is microscopic organisms which do not have the ability of locomotion but move at the compassion of the water movements and wind. They occupy intermediate position in the food web. Zooplanktons are bioindicators in detecting the health and trophic status of aquatic bodies [8].

The Zooplanktons are useful indicator of aquatic community structuring and water condition, [4]. Zooplankton help to regulate the aquatic productivity. During recent years, the diversity, abundance and tolerance of Zooplankton have been used to indicate the deterioration in water quality caused by pollution and eutrophication induced largely by human activity [11]. The study of zooplankton is necessary to evaluate the fresh water reservoir in respect to their ecological and fishery status [7]. These are the most important natural food sources for fish, as they are directly tied to their survival and growth, and they form the foundation of all aquatic ecosystems' food chains and food webs. These are the most important natural food sources for fish, as they are directly tied to their survival and growth, and they form the foundation of all aquatic ecosystems' food chains and food webs

[12]. They are the most important food source for omnivorous and planktivorous fishes, as well as for fish larvae culture [2]. Therefore, the present study deals with the seasonal variation of zooplankton in Pingali Lake near Dahiwadi.

Materials and Methods

Study area:

The Pingali Lake is located the Dahiwadi village in Man Taluka of Satara district. It is situated in Latitude 17°41'11"N and 74°32'1"E Longitude.

Figure 1: Pingali Lake

Sample collection and biological analysis:

The present study was conducted for the period of one year from Jan 2023 to Dec 2023. The water samples were collected monthly between 8 am to 12 am. The data was articulated seasonally as summer, winter, and monsoon. The plankton samples were collected through 50 liters of water by standard plankton net made up bolting silk cloth No. 20 and the collected samples were fixed in 4% formalin. The zooplanktons are identified with the help of standard literature up to generic level by using standard keys of [6, 14]. The qualitative and quantitative analysis of the organism is carried out by 'Sedgwick rafter cell' as per the standard methods [3]

Result and Discussion:

During the present study period seasonal variation of zooplankton in Pingali lake was represented by 14 species consisting of 6 species of rotifera, 3 species cladocera, 4 species copepod, 1 species ostracoda (Table no.1). Among the species that were identified Many researchers across the country have made similar observations. Kar & Kar (2013) identified 26 Zooplankton species in an oxbow lake in Cachar, Assam [9]. Tyor *et al.,* (2014) investigated Zooplankton diversity in a shallow lake in Gurgaon, India [17]. Pawar (2014)

identified 66 species of Zooplankton in certain freshwater bodies near Satara district of Maharashtra, India, with Rotifera having the most diversity, Cladocera having the second highest diversity, and Copepoda having the lowest diversity [13]. In present study area rotifera groups was highly dominated similar result observed by [1, 15].

Table 1: Zooplankton Species recorded in Study area during Jan 2023 to Dec 2023.

Sr. No.	Zooplankton Groups	Family	Species
1	Rotifera	Brachionidae	*Brachionus forficula*
2			*Brachionus calyciflorus*
3			*Brachionus diversicornis*
4			*Keratella chochlearis*
5			*Keratella tropica*
6			*Keratella crassa*
7	Cladocera	Moinidae	*Moina micrura*
8		Chydoridae	*Chydrous sphaericus*
9		Daphniidae	*Cerodaphnia corcuta*
10	Copepoda	Diaptomidae	*Mesocyclops*
11			*Thermocyclops*
12			*Microcyclops*
13			*Nauplius larva*
14	Ostracoda	Cyprididae	*Stenocypris*

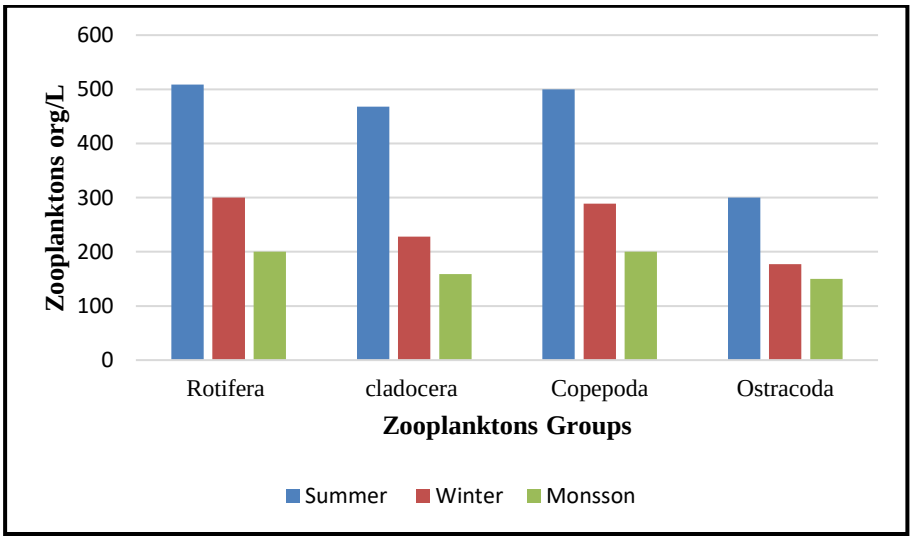

Figure 1: Seasonal variation of zooplanktons at study area during Jan 2023 to Dec 2023

In the present investigation population density was maximum in summer season and minimum in monsoon (Fig. no.1). Summer population maxima of zooplankton is correlated with higher temperature, lower transparency, high standing crop of primary producers leading to greater availability of food supported by a number of workers [5]. Kaur *et al.,* (2018) also reported that the temperature was most important factor that affects the copepods density and diversity. Their production increased with increase in temperature. This may be due to the fact that the higher temperature increased the biochemical & biological activities and increased the production of microorganisms [10].

Conclusion:

Therefore, it may be concluded that during the study period, rotifer appeared to be the most dominant community. The lake zooplankton population was more diverse, indicating that the water body was rich in nutrients and beneficial to the fish population. Thus, keeping in understanding the importance of the study, steps should be taken for the conservation and maintenance of the Pingali Lake.

Acknowledgement:

The authors are thankful to Principal and Head of Department of Zoology Dahiwadi College Dahiwadi for providing Laboratory and library facility for the present research work.

References:

1. Aasidhara Darvekar and Vijay Raut (2022): Studies on diversity, seasonal variation and zooplankton community of freshwater, ramadeshwar lake (hirwa talav) of tehsil –ramtek, district- nagpur, maharashtra, India. *World Journal of Pharmaceutical and Life Sciences*. Vol. 8, Issue 10, 91-99
2. Alam, A. K. M. N, Islam, M. A, Mollah, M. F. A. and Haque, M. S. (1987). Status of zooplankton in newly constructed ponds and their relation to some meteorological and limnological factors. Bangladesh Journal of Fisheries, 14(1): 83- 88.
3. APHA (1998). Standard methods for examination of water and waste water. (20th edition), American public health Association, Washington D.C.
4. Bhat. N. A, Wanganeo.A and Raina. R (2014); The composition and diversity of net zooplankton species in a tropical water body (Bhoj wetland) of Bhopal India, *International journal of biodiversity and conservation* 6(5); 373-381.
5. Bhati, D.P.S. and Rana, K.S. (1987): Zooplankton in relation to aboitic components in the fort moat of Bharatpur, Proc. Nat. Acad. Sci. India, 57(B), 237-242

6. Edmondson, W. T. (1959). Fresh water biology, Edward and hipple, 2 nd Edn. John willy son. Inc; Newyork, pp. 95-189.
7. Goswami, A.P., Mankodi, P.C. (2012): Study on zooplankton of freshwater reservoir Nyari II, Rajkot Dist, Gujarat, India, ISCA. J. Biol. Sci., 1(1): 30 34
8. Jakhar. P. (2013); Role of phytoplankton and zooplankton as health indicator of aquatic ecosystem, International Journal of innovative research and studies 2(12); 490-500.
9. Kar, S. and Kar, D. (2013). Studies on zooplankton diversity of an oxbow lake of South Assam, India. International Journal of Current Research, 5(12):3652-3655.
10. Kaur A, Hundal SS and Aulakh RK (2018) Seasonal study of zooplankton diversity in the polluted water stretch of Buddha Nullah, Ludhiana. Journal of Entomology and Zoology Studies 2018; 6(5): 2241- 2245.
11. Mekong River Commission (2015): Technical report Identification of Freshwater Zooplankton of the Mekong River and its Tributaries, Cambodia, Lao PDR, Thailand & VietNam. MRC technical paper; 45: 1-197.
12. Miah, Md. F., Roy, S., Jinnat, E. and Khan, Z. K. (2013). Assessment of Daphnia, Moina and Cylops in Freshwater Ecosystems and the Evaluation of Mixed Culture in Laboratory. American International Journal of Research in Formal, Applied & Natural Sciences, 4(1): 1-7
13. Pawar, S.M. (2014). Zooplankton Diversity and Density in Some Freshwater Bodies around Satara (M.S) India. Journal of Environments, 1(2): 64-67.
14. Reddy, Y. R. (1994). Copepod, Cladocera Diaptomidous Guide to the identification of microinvertibrate of the continental water of the world, Vol.5, SPB. Publisher the Hague, Netherlan
15. Seema Singh, Veena Kumari, Monalisa, Basant Kumar Gupta and Mohommad Arif (2021): Study on Zooplankton Diversity in A Fresh Water Pond (Raja Bandh) of Jamtara, Jharkhand, India. Int J Adv Life Sci Res. Volume 4(2) 05- 13
16. Steinberg D K and Robert H (2009). Zooplankton of the York River. Journal of Coastal Research. 57: 66-79.
17. Tyor, A.K., Chopra, G. and Kumari, S. (2014). Zooplankton diversity in shallow lake of Sultanpur National Park, Gurgaon (Haryana). International Journal of Applied Biology and Pharmaceutical technology, 5(1): 35- 40.

SYSTEM OF BREEDING OR SYSTEM OF MATING (BASED ON GENETIC RELATIONSHIP)

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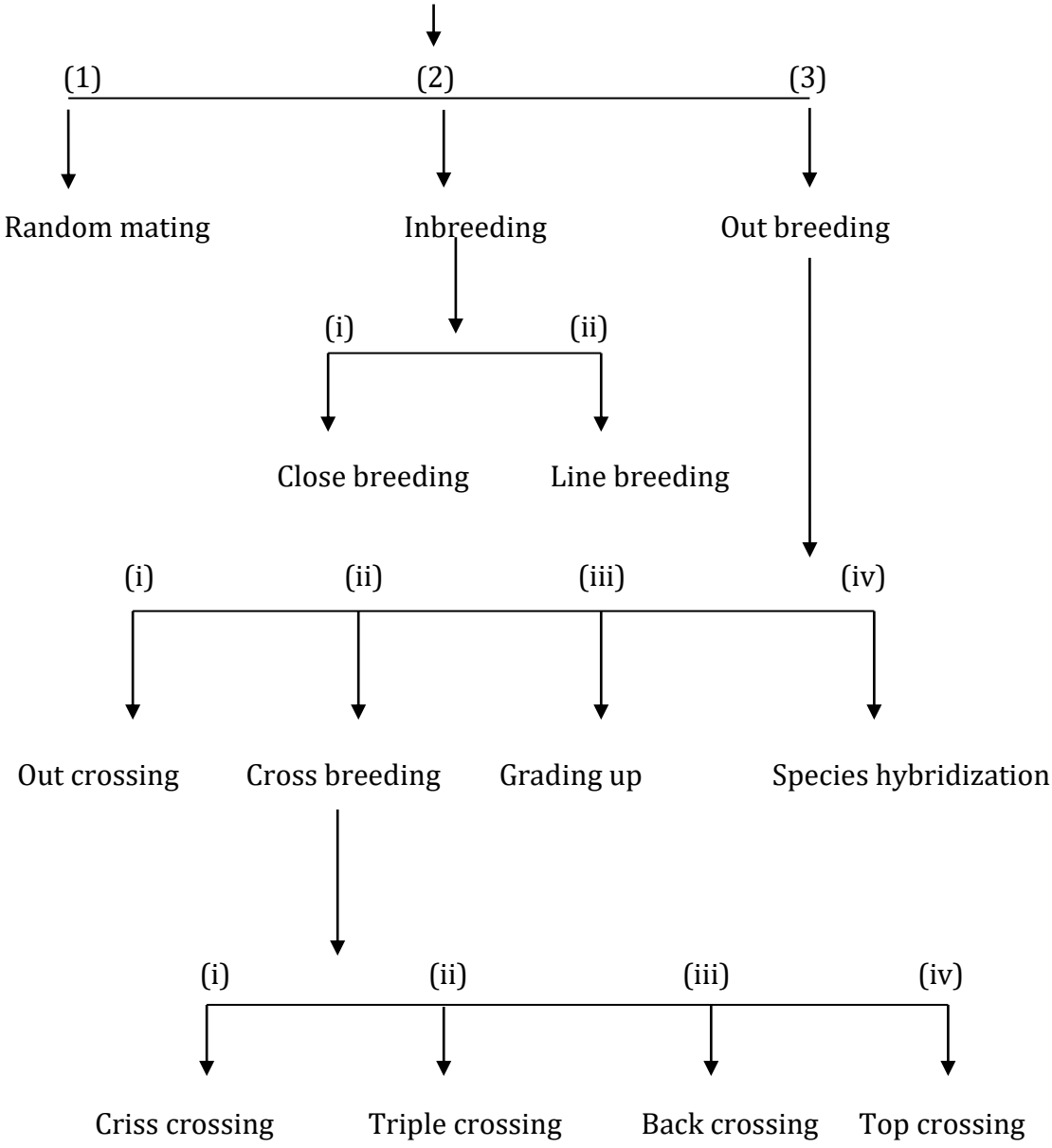
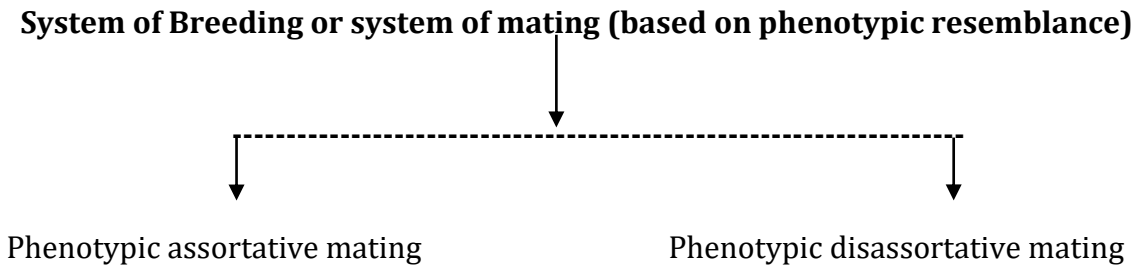
Animal breeding:

Animal breeding is the intentional mating of male and female animals for a specific purpose.

System of Breeding or system of mating (based on genetic relationship)





Random mating:

It is also called panmixia, which refers to the random mating of individuals within a population. In this system, each individual has an equal opportunity to mate with the opposite sex, meaning there is no mate selection. For instance, if a group of cows and bulls is left to roam freely on a pasture, their mating would occur randomly.

Inbreeding or genetic assortative mating:

Mating of related males and females that are more closely related than the average relationship within the population is known as inbreeding. This involves mating individuals that are related within 4-6 generations or have a common ancestor within this range. Inbreeding is also referred to as 'genetic assortative mating'.

Type of Inbreeding:

Depending on the closeness of the relationship among mated individuals, or the degree or intensity of closeness between mates, inbreeding is classified into two subclasses:

1. Close breeding
2. Line breeding

(i) Close breeding:

Mating of more closely related males and females is called close breeding. Examples include parent-offspring mating (such as sire-daughter or dam-son) and full sibling mating (full brother and full sister).

Aims or purpose of close breeding:

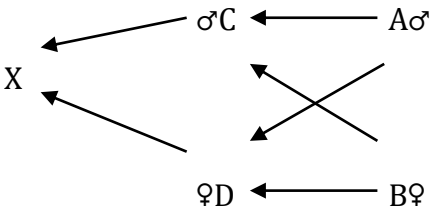
1. To develop highly inbred lines.
2. To identify undesirable recessive genes through parent-offspring mating.
3. To produce more uniform progeny or to increase homozygosity.

Disadvantages of close breeding:

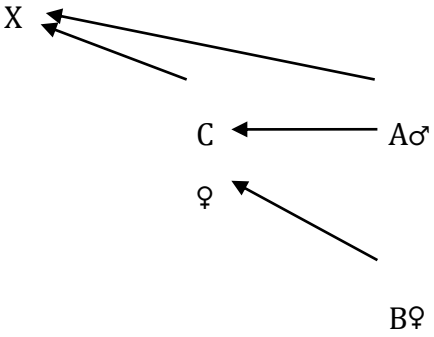
1. Inbreeding depression
2. Intensification of undesirable characteristics

Note: Close breeding should only be practiced or recommended when both parents are outstanding individuals.

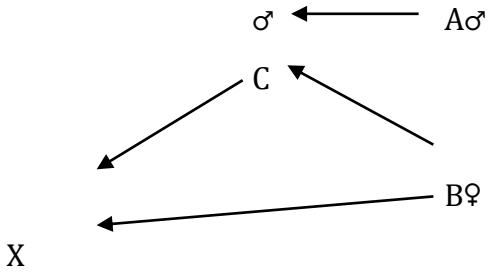
Examples of close breeding:



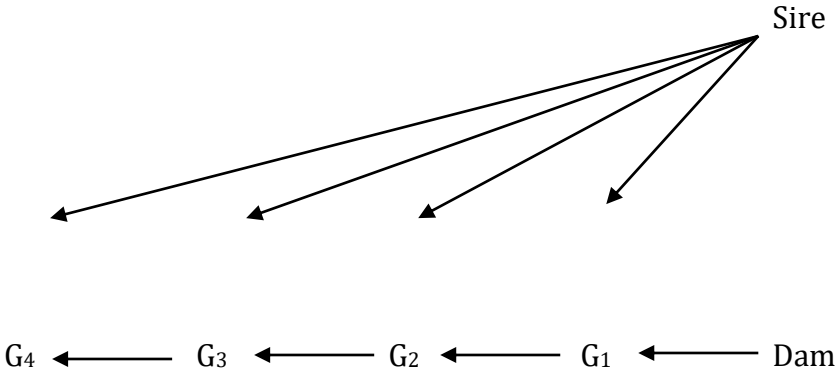
Mating of full brother and full sister, also known as real brother and sister mating.



Mating of father to his daughter



Mating of mother to her son



This is close breeding, which involves the mating of a sire to his offspring across successive generations. Such matings can be used to develop sire lines.

(II) Line breeding:

Mating of less closely related individuals or distantly related individuals is called line breeding. Examples include mating of cousins, half-siblings, grandparents and their grandchildren.

Effects of line breeding:

The effects of line breeding are similar to close breeding, but the intensity of these effects is less compared to close breeding.

Aims of line breeding:

The primary aim of line breeding is to perpetuate the special characteristics of outstanding animals or strains.

Effects of Inbreeding: (A) Genetics effects (B) Phenotypic effects

(A) Genetic Effects:

i. Inbreeding increases homozygosity and decreases heterozygosity without affecting gene frequencies.
ii. It enhances heritability and reduces variation.
iii. Due to increased homozygosity, inbreeding fixes characters in an inbred population, whether favorable or unfavorable. This necessitates culling of animals in which unfavorable traits are fixed.
iv. Inbreeding alters the genetic structure of the population by changing genotypic frequencies while leaving gene frequencies unchanged.
v. It increases genetic uniformity among animals.
vi. Inbreeding is an effective method to obtain true strains from unknown stock, as it fixes characters in the homozygous condition, facilitating selection of desirable and elimination of undesirable individuals.
vii. Inbreeding enhances the prepotency or the ability of an individual to pass on its characteristics to its progeny. Prepotency depends on the homozygosity of dominant genes.

(B) Phenotypic effects of inbreeding:

1. **Effect on growth:** Inbreeding tends to depress the growth rate in farm animals.

2. **Effect on reproductive performance:** Inbreeding reduces reproductive efficiency in farm animals. It adversely affects puberty and testicular development delay, reduces gametogenesis, and increases embryonic losses.

3. **Effect on vigor:** Inbreeding results in loss of vigor.

4. **Effect on production:** Productive traits show inbreeding depression, which refers to a decrease in productive performance of inbred animals. This decrease is moderate and increases with continued inbreeding.

5. **Effect on phenotypic uniformity:** Inbred animals within a line show increased phenotypic uniformity for single-factorial traits. However, this is not the case for polygenic traits, where variations within inbred lines are more due to environmental rather than genetic causes.

6. **Appearance of lethal factors and abnormalities**: Inbred animals are more likely to exhibit hereditary abnormalities or lethal factors compared to outbred animals. These abnormalities appear more frequently in inbred populations.

Recommendation for Inbreeding:

Inbreeding is recommended for seed stock breeds in the following situations:

1. **In better than average herd:** Inbreeding can be considered in herds that are above average in performance.
2. **Herd with two or more sires:** In herds where there are multiple sires, inbreeding can help to keep the level of inbreeding under control.
3. **To develop inbred lines as seed stock:** Inbreeding can be used to develop inbred lines that can then be crossed according to their combining ability to produce superior offspring.

Recommendation for never subjecting to inbreeding:

Inbreeding should never be practiced in the following situations:

1. **In commercial herd or graded herd:** Inbreeding should be avoided in herds that are maintained for commercial purposes or in graded herds where performance varies widely.
2. **Poor performance herd below average:** Herds with below-average performance should not be subjected to inbreeding as it can further exacerbate poor traits.
3. **Herd with one sire or without knowledge about genetics:** Inbreeding should be avoided in herds with a single sire or where there is insufficient knowledge about the genetics of the animals.

These recommendations help to manage the potential risks associated with inbreeding and ensure that it is used appropriately to improve specific genetic traits in controlled breeding programs.

Outbreeding:

Out breeding refers to the mating of unrelated male and female animals which are not related within 4-6 generations or do not share any common ancestors within that range. It is also known as genetic disassortative mating.

Types of outbreeding:

There are four different types or forms of out breeding:

(i)**Outcrossing:**

Outcrossing involves mating unrelated male and female animals within the same breed, or mating unrelated male and female animals that belong to the same breed.

Example: Mating a Hariana cow with a Hariana bull, or a Sahiwal cow with a Sahiwal bull (unrelated superior sire of the same breed).

Out crossing within a herd using selected sires is also referred to as selective breeding.

Main purposes or uses of outcrossing:

1. To utilize genetic variability within a breed.
2. To use selected superior sires on females within a herd.
3. To improve the performance of purebreds.

Selected animals, which are superior in performance, are expected to be less homozygous. Therefore, outcrossing with selection is capable of genetic change and improvement.

Advantages of outcrossing:

(i) **Brings a new combination of desirable characteristics:**

Outcrossing introduces new genetic material into a population, which can result in a new combination of desirable traits.

(ii) **Effective system for genetic improvement, if combined with selection:**

When outcrossing is carefully combined with selection, it can be a highly effective method for genetic improvement. Selection helps to retain desirable traits while introducing new genetic variability through outcrossing.

(iii) **Highly effective for characters controlled by additive gene effects:**

Outcrossing is particularly effective for traits that are influenced by the additive effects of genes. Examples include milk production in dairy cattle and growth rate in beef cattle, where the introduction of new genetic material can lead to improved performance. These advantages highlight the potential of outcrossing as a tool in breeding programs to enhance genetic diversity and improve overall performance in livestock populations.

(ii) Crossbreeding:

Crossbreeding refers to the mating of male and female animals from two different established breeds. The progeny produced by crossbreeding is called crossbred. Crossbreeding plays a major role in livestock improvement by developing new breeds of livestock. It is often said that the pure breeds of today were the crossbreds of yesterday.

Main purposes or uses of crossbreeding:

1. **To take advantage of heterosis (hybrid vigor):** Crossbreeding exploits genetic differences between two breeds, resulting in offspring that exhibit heterosis or hybrid vigor. This heterosis makes the crossbred animals more productive and superior to either of the parental breeds.
2. **To combine the good qualities of two or more breeds:** Crossbreeding allows the combination of desirable traits from different breeds into a single individual, leading to improved overall performance.
3. **To achieve maximum heterozygosity in commercial animals:** Crossbreeding increases genetic variability in commercial animals, which can lead to improved health, productivity, and adaptability.
4. **Effective for traits with low genetic variability:** Crossbreeding is particularly effective for improving traits that have low genetic variability within a breed. It allows for the creation of new breeds of livestock that possess desired characteristics from both parental breeds.

Crossbreeding has been instrumental in the development and improvement of livestock populations worldwide, contributing significantly to agricultural productivity and sustainability.

Examples:

Holstein Friesian x Sahiwal ; H.F. x T.P., Brwon swiss x Sahiwal/Red sindhi

↓ ↓ ↓

Frieswal Karan fries Karan swiss

Jersey x Red sindhi; B.S. x Local breed of Kerala. Jersey x Zebu

↓ ↓ ↓

Jersindh Sunandini Taylor

(A) Criss crossing:

Criss crossing occurs when two breeds (A and B) are crossed alternatively in a systematic pattern. Here's how it works:

1. **First generation (F1):**
    - Breed A is crossed with Breed B.
    - Offspring are denoted as AB.
2. **Second generation (F2):**
    - The F1 individuals (AB) are then crossed with individuals of the opposite breed.
    - For instance, AB individuals are crossed with B individuals and also with A individuals.
    - This results in offspring like ABB, ABA, BAB, and BAA

(B) Triple crossing:

Triple crossing involves the crossing of three different breeds in a rotational manner. Here's how it works with three breeds (A, B, and C):

1. **First generation (F1):**
    - Breed A is crossed with Breed B.
    - Offspring are denoted as AB.
2. **Second generation (F2):**
    - The AB individuals are then crossed with Breed C.
    - This results in offspring like ABC.
3. **Third generation (F3):**
    - The ABC individuals are then crossed back with Breed A (or another systematic rotational pattern involving all three breeds).
    - This can result in offspring like ABA, BAC, CAB, etc.

Triple crossing is used to achieve specific breeding goals by combining the desirable traits of three different breeds in a systematic and rotational manner. It is a strategy often used in livestock improvement programs to enhance genetic diversity and to develop new lines or breeds of animals with improved performance characteristics.

(C) Back crossing:

Back crossing refers to the mating of an F1 hybrid offspring with one of its own parents. This is done to introduce the genetic traits of that parent back into the offspring. Here's an example to illustrate back crossing:

Let's use Breed A and Breed B to explain back crossing:

1. **First generation (F1):**
    - Breed A is crossed with Breed B to produce F1 offspring, denoted as AB.
2. **Back crossing example:**
    - In back crossing, one of the F1 offspring (AB) is then mated with one of its parents.
    - For example, AB (F1) is mated with Breed A.

This results in the following offspring:

- If AB (F1) is mated with Breed A:
    - Offspring would be AAB (back crossed with Breed A).
- Alternatively, AB (F1) could be mated with Breed B:
    - Offspring would be ABB (back crossed with Breed B).

Back crossing is used to reinforce the desirable traits of one parent (either Breed A or Breed B in this example) in the offspring, while still maintaining some level of hybrid vigor from the initial F1 cross. It can be a useful breeding strategy to fix specific traits in subsequent generations while utilizing the benefits of hybrid vigor in the initial cross.

(D) Top crossing:

Top crossing is a breeding strategy where superior sires of one breed are crossed with females of another breed to produce crossbred offspring. This technique is often used to introduce desirable traits from one breed into another breed, typically in commercial settings. Here’s how top crossing works:

1. **Selection of superior sires:**
    - High-performing sires (males) from Breed A are selected based on specific desirable traits such as growth rate, milk production, or disease resistance.
2. **Crossing with females of breed B:**
    - These selected sires (from Breed A) are then mated with females (cows) of Breed B.

3. **Purpose:**
    - The purpose of top crossing is to improve certain aspects of Breed B using the genetics of Breed A, thereby enhancing productivity, performance, or other desirable traits.
4. **Examples:**
    - For instance, if Breed A is known for its high milk production and Breed B is known for its adaptability to local conditions, top crossing could result in crossbred offspring that exhibit improved milk production while maintaining the adaptability of Breed B.

Top crossing can be an effective method to capitalize on the strengths of different breeds and is commonly used in commercial livestock production to optimize genetic potential and performance traits.

Use of crossbreeding:

Crossbreeding has several advantages that make it a valuable tool in livestock improvement:

1. **Increased production performance:** Crossbreeding often leads to an immediate increase in production performance, such as milk or meat yield, due to the combination of favorable traits from different breeds.
2. **Increased reproductive efficiency:** Crossbreeding can enhance reproductive efficiency, including earlier sexual maturity, improved fertility, and reduced embryonic loss.
3. **Increased growth rate:** Crossbred animals frequently exhibit faster growth rates compared to purebred animals, due to heterosis.
4. **Increased survivability:** Crossbreeding can improve the overall health and survivability of offspring, including disease resistance and adaptation to local conditions.
5. **Advantage of heterosis:** Crossbreeding takes advantage of heterosis (hybrid vigor), resulting in offspring that are superior in performance to their purebred parents.
6. **Development of new breeds and strains:** Crossbreeding can be used to develop new breeds or strains of animals with specific desirable traits that may not exist in purebred lines.

Disadvantages of crossbreeding:

Despite its benefits, crossbreeding also has some limitations and challenges:

1. **Breakup of established characters:** Crossbreeding can disrupt established breed characteristics and combinations of traits, potentially leading to loss of specific traits that were present in the purebred lines.
2. **Maintenance of pure breeds:** Crossbreeding requires the maintenance of two or more purebred lines to produce crossbred offspring. This can increase complexity and costs associated with breeding programs.

In summary, while crossbreeding offers significant advantages in terms of performance improvement and heterosis, breeders must carefully consider the potential disadvantages, such as the loss of established breed traits and the challenges of maintaining purebred lines. Proper management and selection are essential to successfully utilize crossbreeding in livestock improvement programs.

(iii) Grading up:

Grading up is a systematic crossbreeding method used to upgrade the quality of animals within a herd or population. This process involves breeding purebred sires (usually of a superior breed) with females of a lower quality or less pure breed. Here's how grading up works:

1. **Initial step:**
    - Start with females of a native or commercial breed that may have desirable traits but are of lower quality or productivity.
2. **Introduction of purebred sires:**
    - Purebred sires of a superior breed are introduced into the herd.
    - These purebred sires are selected for their superior performance in desired traits such as growth rate, milk production, or disease resistance.
3. **Crossbreeding:**
    - The purebred sires are mated with the native or commercial breed females over several generations.
    - The resulting offspring are progressively more and more of the superior breed as each generation is crossed with the purebred sires.
4. **Purpose:**
    - The purpose of grading up is to improve the overall quality and productivity of the herd or population.

- Over time, the percentage of genes from the superior breed increases in the population, resulting in improved performance and consistency in desirable traits.

5. **Examples:**
    - For example, if a commercial herd of cattle has low milk production, a breeder might use purebred sires of a high-yielding dairy breed (like Holstein) to breed with the native cows over successive generations.
    - This process results in crossbred offspring that have progressively more Holstein genetics, improving milk production in the herd.

Grading up is a gradual and systematic method of crossbreeding that allows for the improvement of less productive or lower-quality herds by introducing superior genetics from purebred animals. It is commonly used in commercial livestock production to upgrade herds and increase overall productivity.

Species hybridization:

Species hybridization refers to the crossing of individuals from two different species. This can occur naturally or can be facilitated by humans in controlled breeding programs. Here's an overview of species hybridization:

1. **Definition:**
    - Species hybridization involves mating individuals from two different species, resulting in offspring with a mixed genetic background.

Example:

1. Male ass (Jack) x Female horse (Mare) = Mule
2. Male horse (stallion) x Female ass (Jannet) = Hinny
3. Stallion x Female Zebra = Zebroid
4. Yak x Cow = Pienniu
5. American buffalo bull x American cow = Cattalo
   (Bos bison) (Bos taurus)
6. Mithun x cow → = Jatsa (F_1 male)
   → = Jatsamin (F_1 female)

7. Mithun x siri cow → = Jechha (F_1 males)
   → = Jessan (F_1 female)

References:

1. Falconer, D. S., & Mackay, T. F. C. (1996). *Introduction to Quantitative Genetics* (4th ed.). Longman.
2. Henderson, C. R. (1984). *Applications of Linear Models in Animal Breeding*. University of Guelph Press.
3. Nicholas, F. W. (2010). *Introduction to Veterinary Genetics* (3rd ed.). Wiley-Blackwell.
4. Lynch, M., & Walsh, B. (1998). *Genetics and Analysis of Quantitative Traits*. Sinauer Associates.
5. Bourdon, R. M. (2000). *Understanding Animal Breeding* (2nd ed.). Prentice Hall.
6. Mrode, R. A. (2014). *Linear Models for the Prediction of Animal Breeding Values* (3rd ed.). CABI.
7. Weir, B. S. (1996). *Genetic Data Analysis II: Methods for Discrete Population Genetic Data*. Sinauer Associates.
8. Falconer, D. S. (1981). *Introduction to Quantitative Genetics* (2nd ed.). Longman.
9. Hill, W. G. (1974). Estimation of genetic change. *Animal Breeding Abstracts*, *42*(1), 1-15.
10. Bulmer, M. G. (1980). *The Mathematical Theory of Quantitative Genetics*. Clarendon Press.

SEAWEED AND SEAWEED EXTRACTS HEALTH BENEFITS ON HUMAN HEALTH AND NUTRITION

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Abstract:

Seaweeds, which are crucial marine resources in food chains, are increasingly being acknowledged for their nutritional and medicinal advantages. With a high content of vitamins, proteins, minerals, omega-3 fatty acids, and carbohydrates, these foods are commonly referred to as the "medicinal food" of the 21st century. Their bioactive components possess antiviral, anti-inflammatory, antibacterial, and anti-carcinogenic effects, which help in the prevention of chronic diseases like as cardiovascular ailments and cancer. Seaweeds, which were traditionally consumed as food, used for medicinal purposes, and utilized as fertilizers, now have a vital function in the field of nutraceuticals. Seaweeds of the green, red, and brown varieties show potential in the treatment of neurological illnesses. They are also crucial to Western diets for their health advantages, assisting in weight management and enhancing metabolic health. Their significance in environmental sustainability, sustaining biodiversity, and functioning as carbon sinks show their ecological value. Research continues to broaden their uses in food systems and healthcare, bringing answers to critical health and environmental concerns.

Introduction:

Seaweeds are important marine resources and major producers in marine food chains and are increasingly recognized for their nutritional and therapeutic benefits. Rich in bioactive elements such as vitamins, proteins, carbs, minerals, etc. Seaweeds are consumed fresh, dried, and processed, earning the label "medicinal food" of the 21st century. Their medicinal qualities include antibacterial, antiviral, anti-inflammatory, anti-carcinogenic, antithrombotic, anti-tumor, anti-stress, and anti-carcinogenic actions. Epidemiological studies imply that frequent seaweed consumption is connected to

decreased occurrences of chronic illnesses including cancer and cardiovascular disorders, attributing this to their high fiber, antioxidant, and mineral content.

Historically utilized as food, folk medicines, dyes, and fertilizers, seaweeds are increasingly prominent as nutraceuticals or functional foods. These algae, particularly *Chlorophyta*, *Rhodophyta*, and *Phaeophyta* types, have shown potential effects in the prevention and treatment of neurological disorders such as Alzheimer's, Parkinson's, and Multiple Sclerosis. Moreover, seaweeds are gaining popularity in Western diets due to their health advantages and their impact on Asian cuisine. The incorporation of seaweed isolates in food items has exhibited better satiety and decreased postprandial glucose and fat absorption, highlighting its potential in producing anti-obesity meals. This study shows the expanding relevance of seaweeds in promoting human health and their application in the food and pharmaceutical sectors.

The rising usage of seaweeds in modern diets and industrial applications emphasizes their adaptability and importance. Beyond their traditional uses, seaweeds are currently being examined for their potential in the cosmetic and pharmaceutical sectors due to their extensive bioactive profiles. The polysaccharides included in seaweeds, which operate as low-calorie soluble fibers, give extra advantages by maintaining gastrointestinal health and assisting in weight management. In locations where seaweed intake is popular, there is a substantial decline in lifestyle-related illnesses, demonstrating a clear association between seaweed-rich diets and improved public health outcomes.

Numerous research has shown the health advantages of frequent intake and supplementation with seaweed in addition to a regular diet. Regular seaweed consumption reduces depression in Japanese pregnant women and reduces the risk of adult suicide. Seaweed is low in calories and abundant in nutrients, including proteins, polysaccharides, dietary fiber, vitamins, bioactive metabolites, minerals, etc. Regular use of dietary seaweeds decreased the incidence of diabetes mellitus in the Korean population (Ganesan *et al.,* 2019).

Additionally, seaweeds help with environmental sustainability. They are crucial to the health of marine ecosystems because they preserve biodiversity and act as carbon sinks. The cultivation of seaweeds can help minimize the consequences of ocean acidification and create sustainable livelihoods for coastal populations. As research continues to reveal the various benefits of seaweeds, their integration into global food

systems and healthcare regimens is set to grow, giving promising solutions to some of the most serious health and environmental concerns of our day.

Classification:

1. Green Seaweeds (*Chlorophyta*)

   Phylum: *Chlorophyta*

   Classes: *Dasycladophyceae, Bryopsidophyceae, Prasinophyceae, Ulvophyceae and Chlorophyceae.*

   Examples: *Ulva* (Sea Lettuce), *Monostroma*

2. Red Seaweeds (*Rhodophyta*)

   Phylum: *Rhodophyta*

   Classes: *Rhodophyceae*

   Examples: *Gracilaria* (Cultivated for food), *Porphyra* (Intertidal zones in temperate climate and cultivated for food)

3. Brown Seaweeds (*Phaeophyta*)

   Phylum: *Ochrophyta*

   Class: *Phaeophyceae*

   Examples: *Fucus* (Wracks), *Laminaria* and *Saccharina* (Kelps), *Sargassum* (Pelagic especially in the Sargasso Sea)

Edible seaweeds genus list:

Data Collected from MacArtain *et al.,* 2007; Gómez-Ordóñez *et al.,* 2010; Romarís-Hortas *et al.,* 2010; Bittner *et al.,* 2008; Watanabe *et al.,* 1999.

A. Green algae (*Chlorophyta*):

   Ulvaria / Enteromorpha.

B. Brown Algae (*Phaeophyta*):

   Alaria sp., Hizikia sp., Himanthalia/ Laminaria, Saccharina, Fucus, Sargassum, Sargassum, Ascophyllum, Bifurcaria, Dictyotales, Undaria, Eisenia.

C. Red algae (*Rhodophyta*):

   Chondrus, Asparagopsis, Grateloupia, Mastocarpus/ Gigartina, Porphyra, Gracilaria, Rhodymenia/ Palmaria.

Application of different seaweed species in human health and disease prevention

- *Alvarezii Kappaphycus*

  It has been discovered that this species raises HDL-C levels and lowers total cholesterol, LDL, and triacylglycerol levels, all of which improve cardiovascular health (Matanjun, 2010).

- *Mekabu's* (*U. pinnatifida*)

  According to research conducted in vitro and in vivo, U. pinnatifida demonstrates strong anticancer potential, especially against breast cancer cells (Déléris *et al.,* 2016).

- *Sargassum wightii*

  Sargassum wightii has long been utilized by Chinese for its medicinal use and is well-known for its effectiveness in treating goiter (Mohapatra *et al.,* 2016).

- *S. Horneri, C. Hakodatensi,* and *U. Pinnatifida*

  According to Grasa-López *et al.,* (2016), these species have been demonstrated to reduce lipid hydroperoxide levels, which may be advantageous in the management of oxidative stress and associated disorders.

- *Sarconema sp.* and *Asparagopsis taxiformis*.

  These species are useful in managing goiter, a condition brought on by an enlarged thyroid gland (Reddy, 2012).

- *Caulerpa racemose*

  An alga rich in minerals, vitamins, fiber, and antioxidants called *Caulerpa racemosa* can be added to diet items to help promote weight reduction. *Caulerpa racemose* is used as traditional medicine to treat rheumatism and to decrease blood pressure (Fithrian, 2015).

- *Gelidium amansii*

  Gelidium amansii reduces the expression of adipogenic genes and suppresses adipogenesis, which may aid in the management of obesity (Kang *et al.,* 2017).

- Brown algae

  According to studies, brown seaweed helps to maintain the balance of cholesterol and prevents the development of triglycerides in HepG2 cells, which is good for metabolic health (Nagappan *et al.,* 2017).

- *Porphyra dentata*

  This seaweed possesses beneficial properties for the management of diabetes as it effectively reduces levels of glucose in the bloodstream and enhances the release of insulin in the bloodstream (Chao *et al.,* 2014).

- *Laurencia papillosa, Gracilaria crassa,* and *Turbinaria ornata*

  These seaweeds have hepatoprotective, wound-healing, and anti-ulcer qualities. According to research, ranitidine (90%) and *Gracilaria crassa* (76%) provide the highest protection (81%) against stomach ulcers (Pati *et al.,* 2016).

Biological activity of seaweed compounds

1. Antioxidant activity: Polyphenols, amino acids, sulphated polysaccharides, unsaturated fats, peptides (Peñalver *et al.,* 2020).
2. Anticancer activity: Carotenoid, phenolic compounds, sulphated polysaccharides, fucoxanthin (Cotas *et al.,* 2021; Farghali *et al.,* 2023).
3. Antimicrobial activity: Fucoidan, sulfoquinovosyldiacylglycerols, caulerpin (Bhowmick *et al.,* 2020; Polat *et al.,* 2023)

Nutritional and bioactive composition of indian coastal seaweeds

- Protein and Amino Acids

Protein concentration in diverse seaweeds ranges from 1.8% to 18.9%, with Phaeophyceae species displaying the highest levels of protein and Chlorophyta members the lowest (Kasimala *et al.,* 2015). Sixteen amino acids are found in seaweeds such as *Caulerpa racemosa, Ulva lactuca, Chnoospora minimum, Padina gymnospora,* and *Acanthophora spicifera* from India's east coast. *Acanthophora spicifera* displays the greatest amounts of glutamic acid (17.4%) and aspartic acid (15.7%) (Kumar *et al.,* 2013). Moreover, the protein content differences are affected by nearby water quality (Dhargalkar *et al.,* 1980).

- Minerals

When compared to terrestrial plants, seaweeds have a higher bioavailability and a greater supply of nutrients (Bergner, 1997). They provide nearly all necessary minerals, comprising around 7% to 38% of their weight when dried. Elements present in seaweeds include all major macro and micronutrients. In general, brown seaweeds have higher mineral levels than red seaweeds (Vijay *et al.,* 2017). *Sargassum odontocarpum* and *Padina tenuis* are notably high in macrominerals, with reduced iron concentration (Kasimala *et al.,* 2015).

- Lipids and Fatty Acids

Lipid content of seaweeds varies between 1.5 and 5%. Members of Chlorophyta have the highest lipid content, whereas members of Rhodophyta have the lowest (Kasimala *et al.,* 2015). Seaweeds contain a lot of important fatty acids. For example, the highest concentration of α-linolenic acid is found in green seaweeds, whereas arachidonic acid, eicosapentaenoic acid, octadecatetraenoic acid, DHA and DPA, are found in brown seaweeds and red seaweeds (Oucif *et al.,* 2020; Burtin, 2003). Around 24.4% palmitic acid is found in the brown algae *Dictyota ceylanica* (Chakraborty and Santra, 2008). Omega 6 and omega 3 fatty acids of seaweed, have been shown to help prevent a number of ailments, including diabetes, arthritis, and cardiovascular disease (Burtin, 2003).

- Vitamins

The Phaeophyceae family is notably rich in vitamins B1, B2, B6, and nicotinic acid (Kasimala *et al.,* 2015). These features emphasize the potential of seaweeds to address iodine and other mineral and vitamin shortages, playing a significant role in designing functional diets that prevent numerous illnesses (Kasimala *et al.,* 2015).

- Carbohydrates and Dietary Fiber

Carbohydrate content in seaweeds ranges from 12% to 65%, with the greatest amounts in Chlorophyceae about 76%, followed by Rhodophyceae between 20% to 76% and Phaeophyceae with about 20% to 65% (Kasimala *et al.,* 2015; Lu *et al.,* 2022).

Seaweed as a supplement to food products

Seaweeds has gained popularity as a dietary supplement due to its high nutritional value and multiple health advantages. Here are several seaweeds used as a dietary supplement (Kumar *et al.,* 2021).

- Nori: It has a high mineral content, is high in protein, dietary fiber, and vitamins B6 and B12. Although nori was originally used to make sushi, it is now used with other dishes to give extra nourishment. It promotes the production of new red blood cells, lowers the risk of anemia, and stimulates the growth of the body and brain.
- Kombu: Kombu is a type of large brown seaweed belongs to the class Phaeophyceae. Common species used for kombu mostly include *Saccharina species* like *Saccharina japonica*, etc. Kombu is processed dry and rehydrated before usage, typically used to soups, salads, and sauces. *Saccharina japonica*, for example, is rich in nutrients including iron, salt, calcium, phosphate minerals and iodine along with glutamic acid for the "umami" taste.

- Wakame: Wakame is a good source of Iodine and several vitamins like Vitamin A, K, E, C etc. It belongs to brown seaweed and is mostly utilized in Japan and China. A 2 tablespoon of wakame serving provides around 5% of daily folate intake. Wakame is rich in polysaccharides like fucoidan, xanthophyll like fucoxanthin, and soluble dietary fiber, making it a popular option for its nutritional advantages, including weight reduction assistance.
- Sea Lettuce: Sea lettuce belong to Class Chlorophyta. *Ulva Lactuca* is one of the most widely consumed sea lettuce species because of its high nutritious value and is also considered a rich source of Vitamin B12. It contains a high protein level, a high soluble fiber content, and a moderate amount of iron and minerals.
- Seaweed Chocolate: *Ulva reticulata* belong to Class Chlorophyta is richest source of iron contain. Around 40–50% of the iron content is found in this seaweed which can be combined with chocolate to provide a high iron content (Tahira Banu *et al.,* 2015). Thus, chocolate with extra value can be created to help anemic adolescents.
- Seaweed Coffee: Brown seaweed (*Sargassum whightii*) was utilized to make seaweed-infused coffee by Kumar *et al.,* 2019, using seaweed powder at 1%, 3%, and 5% concentrations. They found that there is a positive correlation between the amount of seaweed present in coffee and its antioxidant activity. In addition, the thermal, spectral, and rheological properties of seaweed coffee were examined (Kumar *et al.,* 2019).

Seaweeds for potential drug development

- Kahalalide F, a depsipeptide obtained from the Chlorophyta seaweed *Bryopsis sp.*, is now undergoing advanced clinical studies for its potential application in the treatment of liver and lung cancer in humans (Cotas *et al.,* 2021; Brown *et al.,* 2014; Martín-Algarra *et al.,* 2009)
- China's medicine safety regulator has said that Oligomannate, a therapy derived from seaweed, is effective for treating mild to moderate cases of Alzheimer's disease (Zaugg *et al.,* 2019).
- A cancer therapy utilized Algasol T331, an old chemical derived from the polymer of brown seaweed. This chemical has been proven to be efficacious in the treatment of cancer patients following surgery and radiation, this Compounds offer significant advantages by providing protection to the body and accelerating its recuperation

from highly effective and harmful cancer therapies. Specifically, they help alleviate asthenia and restore hematic function (Claudio *et al.,* 1966).

Conclusion:

Seaweeds are increasingly regarded for their nutritional and medicinal properties, rich in minerals, proteins, vitamins, carbohydrates, and omega-3 fatty acids. They are ingested fresh, dried, or processed and are regarded the "medicinal food" of the 21st century. Seaweeds display antibacterial, antiviral, anti-stress, antithrombotic effects, anti-inflammatory, anti-tumor, and anti-carcinogenic. Studies show that frequent seaweed eating may lower the risk of chronic illnesses including cancer and cardiovascular problems, attributable to their high fiber, antioxidant, and mineral content.

Historically utilized for food, folk remedies, dyes, and fertilizers, seaweeds are increasingly important as nutraceuticals. Green, red, and brown seaweeds show potential in preventing and treating neurological problems. They are gaining popularity in Western diets and are integrated into numerous food products for their health advantages, including assisting with weight management. Seaweeds are also being researched for their potential in cosmetics and medicines. As research on seaweeds continues, their integration into global food systems and healthcare is projected to rise, giving answers to health and environmental issues.

References:

1. Amudha S., B. S. Mamatha, V. Prema, K. K. Bhat, and G. A. Ravishankar, “Studies on development and storage stability of instant spice adjunct mix from seaweed (Eucheuma),” Journal of Food Science and Technology, vol. 48, no. 6, pp. 712–717, 2010.
2. Bergner P., Healing Power of Minerals, Special Nutrients, and Trace Elements, Prima Pub, Kolkata, India, 1997.
3. Bhowmick, S.; Mazumdar, A.; Moulick, A.; Adam, V. Algal Metabolites: An Inevitable Substitute for Antibiotics. Biotechnol. Adv. 2020, 43, 107571.
4. Bittner L, Payri CE, Couloux A, Cruaud C, de Reviers B, Rousseau F. Mol. Phylogenet. Evol. (2008); 49: 211.
5. Brown, E.M.; Allsopp, P.J.; Magee, P.J.; Gill, C.I.; Nitecki, S.; Strain, C.R.; McSorley, E.M. Seaweed and Human Health. Nutr. Rev.2014, 72, 205–216.
6. Burtin P., “Nutritional value of seaweeds,” Electronic Journalof Environmental, Agricultural and Food Chemistry, vol. 2, no. 4, pp. 498–503, 2003.

7. Chakraborty S. and S. C. Santra, "Biochemical composition of eight benthic algae collected from Sunderban," Indian Journal of Geo-Marine Sciences, vol. 37, no. 3, pp. 329– 332,2008.
8. Chao, P. C.; Hsu, C. C.; Liu, W. H. Renal protective effects of Porphyra dentate aqueous extract in diabetic mice. Biomedicine, 4(3), 2014.
9. Claudio, F.; Stendardo, B. An Experimental Contribution to the Clinical Use of an Algal Phytocolloid (Algasol T331) in Oncology. In Proceedings of the Fifth International Seaweed Symposium, Halifax, NS, Canada, 25–28 August 1965; Elsevier: Amsterdam, The Netherlands, 1966; p. 369.
10. Cotas, J.; Pacheco, D.; Gonçalves, A.M.M.; Silva, P.; Carvalho, L.G.; Pereira, L. Seaweeds' Nutraceutical and Biomedical Potential in Cancer Therapy: A Concise Review. J. Cancer Metastasis Treat. 2021, 7, 13.
11. Déléris, P.; Nazih, H.; Bard, J. M. Seaweeds in human health. In Seaweed in health and disease prevention. Academic Press, 319-367. 2016.
12. Dhargalkar V. K., T. G. Jagtap, and A. G. Untawale, "Biochemical constituents of seaweeds along the Maharashtra coast," Indian Journal of Marine Sciences, vol. 9, no. 4, pp. 297– 299, 1980.
13. Farghali, M.; Mohamed, I.M.A.; Osman, A.I.; Rooney, D.W. Seaweed for Climate Mitigation, Wastewater Treatment, Bioenergy, Bioplastic, Biochar, Food, Pharmaceuticals, and Cosmetics: A Review. Environ. Chem. Lett. 2023, 21, 97–152.
14. Fithriani, D. Opportunities and Challenges for Developing Caulerpa Racemosa as Functional Foods. KnE 9 Life Sciences, 85-96. 2015.
15. Ganesan, A. R.; Tiwari, U.; Rajauria, G. Seaweed nutraceuticals and their therapeutic role in disease prevention. Food Science and Human Wellness, 2019.
16. Gómez-Ordóñez E, Jiménez-Escrig A, Rupérez P. Food Res. Int. (2010);43:2289.
17. Gomez-Zavaglia, A.; Prieto Lage, M.A.; Jimenez-Lopez, C.; Mejuto, J. C.; Simal-Gandara, J. The Potential of Seaweeds as a Source of Functional Ingredients of Prebiotic and Antioxidant Value. Antioxidants, 8(9),406. 2019.
18. Grasa-López, A.; Miliar-García, A.; Quevedo-Corona, L.; Paniagua-Castro, N.; Escalona-Cardoso, G.; Reyes-Maldonado, E.; Jaramillo, M E. Flores Undaria pinnatifida and fucoxanthin ameliorate lipogenesis and markers of both inflammation and cardiovascular dysfunction in an animal model of diet-induced obesity. Mar Drugs, 14 (8), 148. 2016.

19. Hamed, I.; Özogul, F.; Özogul, Y.; Regenstein, J.M. Marine Bioactive Compounds and Their Health Benefits: A Review. Compr. Rev. Food Sci. Food Saf. 2015, 14, 446–465.
20. Kang, J. H.; Lee H. A.; Kim, H. J.; Han, J. S. Gelidium amansii extract ameliorates obesity by downregulating adipogenic transcription factors in diet-induced obese mice. Nutrition Research and Practice,11(1),17-24. 2017.
21. Kasimala M. B., L. Mebrahtu, P. P. Magoha, G. Asgedom,and M. B. Kasimala, "A review on biochemical compositionJournal of Food Quality 13and nutritional aspects of seaweeds," Caribbean Journal of Science Technology, vol. 3, pp. 789–797, 2015.
22. Kumar, Yogesh & Tarafdar, Ayon & Badgujar, Prarabdh. (2021). Seaweed as a Source of Natural Antioxidants: Therapeutic Activity and Food Applications. Journal of Food Quality. 10.1155/2021/5753391.
23. Kumar S. R., C. M. Ramakritinan, and M. Yokeshbabu, "Proximate composition of some selected seaweeds from Palkbay and Gulf of Mannar, Tamil Nadu, India," Asian Journal of Biomedical and Pharmaceutical Sciences, vol. 3, no. 16, pp. 1–5, 2013.
24. Kumar Y., A. Tarafdar, D. Kumar, and P. C. Badgujar, "Effect of Indian brown seaweed *Sargassum wightii* as a functional ingredient on the phytochemical content and antioxidant activity of coffee beverage," Journal of Food Science and Technology, vol. 56, no. 10, pp. 4516–4525, 2019.
25. Lu LW, Chen J-H. Seaweeds as Ingredients to Lower Glycemic Potency of Cereal Foods Synergistically—A Perspective. Foods. 2022; 11(5):714. https://doi.org/10.3390/foods11050714
26. MacArtain P, Gill CIR, Brooks M, Campbell R, Rowland IR. Nutr. Rev. (2007); 65: 535.
27. Martín-Algarra, S.; Espinosa, E.; Rubió, J.; López, J.J.L.; Manzano, J.L.; Carrión, L.A.; Plazaola, A.; Tanovic, A.; Paz-Ares, L. Phase II Study of Weekly Kahalalide F in Patients with Advanced Malignant Melanoma. Eur. J. Cancer 2009, 45, 732–735.
28. Matanjun, P.; Mohamed, S.; Muhammad, K.; Mustapha, N. M. Comparison of Cardiovascular Protective Effects of Tropical Seaweeds, Kappaphycus alvarezii, Caulerpa lentillifera, and Sargassum polycystum, on High-Cholesterol/High-Fat Diet in Rats. Journal of Medicinal Food, 3(4), 792-800. 2010.
29. Mohapatra, L.; Bhattamishra, S. K.; Panigrahy, R.; Parida, S.; Pati, P. Antidiabetic effect of Sargassum wightii and Ulva fasciata in high fat diet and multi low dose streptozotocin induced type 2 diabetic mice. U K Journal of Pharmaceutical and Biosciences, 4(2), 13- 23. 2016.

30. Nagappan, H.; Pee, P. P.; Kee, S. H.; Ow J. T.; Yan, S.W.; Chew, L. Y.; Kong, K. W. Malaysian brown seaweeds Sargassum siliquosum and Sargassum polycystum: Low density lipoprotein (LDL) oxidation, angiotensin converting enzyme (ACE), α-amylase, and α-glucosidase inhibition activities. Food Research International, 99, 950-958. 2017.
31. Oucif H., M. Benaissa, S. Ali Mehidi, R. Prego, S. P. Aubourg, and S. M. A. Abi-Ayad, "Chemical composition and nutritional value of different seaweeds from the west Algeriancoast," Journal of Aquatic Food Product Technology, vol. 29,no. 1, pp. 90–104, 2020.
32. Pati, M. P.; Sharma, S. D.; Nayak, L. A.; Panda, C. R. Uses of seaweed and its application to human welfare: A review. Int. J. Pharm. Pharm. Sci, 8, 12-20. 2016.
33. Peñalver, R.; Lorenzo, J.M.; Ros, G.; Amarowicz, R.; Pateiro, M.; Nieto, G. Seaweeds as a Functional Ingredient for a Healthy Diet. Mar. Drugs 2020, 18, 301.
34. Polat, S.; Trif, M.; Rusu, A.; Šimat, V.; Cagalj, M.; Alak, G.; Meral, R.; Özogul, Y.; Polat, A.; Özogul, F. Recent Advances in Industrial Applications of Seaweeds. Crit. Rev. Food Sci. Nutr. 2023, 63, 4979–5008.
35. Reddy, V.S. medicinal value of seaweeds and its applications–a review.2012.
36. Romarís-Hortas V, García-Sartal C, Barciela-Alonso MC, Moreda-Piñeiro A, Bermejo Barrera P. J. Agric. Food. Chem. (2010);58: 1986.
37. Tahira Banu A. and S. Uma Mageswari, "Nutritional status and effect of seaweed chocolate on anemic adolescent girls, Food Science and Human Wellness, vol. 4, no. 1, pp. 28– 34,2015.
38. Vijay K., S. Balasundari, R. Jeyashakila, P. Velayathum,K. Masilan, and R. Reshma, "Proximate and mineral composition of brown seaweed from Gulf of Mannar," International Journal of Fisheries and Aquatic Studies, vol. 5, no. 5,pp. 106–112, 2017.
39. Watanabe F, Takenaka S, Katsura H, Masumder SAMZH, Abe K, Tamura Y, *et al.,* J. Agric. Food Chem. (1999); 47: 2341.
40. Zaugg, J.; Peng, J. China Approves Seaweed-Based Alzheimer's Drug. It's the First New One in 17 Years. CNN. 2019. Available online: https://edition.cnn.com/2019/11/03/health/china-alzheimers-drug-intl-hnk scli/index.html

CANCER - A THREAT TO LIFE

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What is cancer?

Cancer is a group of disease where rapid creation of abnormal cells that grow beyond their usual limits & then invade adjacent cells of body and organs.

Cell is basic & fundamental unit of life. In the human body about 10^{13} cells of different types are found organs. To make an organ, cell needs nutrients to divide & grow. These cell division happens in most controlled manner. If this control over cell division is lost or deviate from normal rule will result in chaos in respective areas. This loss of control over cell division & unnecessary production of contagious mass of cells is called as "Tumor".

Tumors are of two types such as 'benign tumor' & 'malignant tumor'

Benign tumor:

Benign tumor are not cancerous tumors. Benign tumor forms if cell divides when it should not, but stays within normal location. It do not spread to other parts of body & are surrounded by a containing membrane. Benign tumors can be removed by surgeries if it becomes larger. Usually these tumors are not threat to life. But sometimes can cause serious symptoms or be life threatening such as benign tumor in a brain E.g. Warts, Mole (a small tumor on variety of tissue).

Malignant tumors:

"Malignant tumors are cancerous". Cells of cancer begins to divide and also acquire the ability to invade & damage tissue or organs nearby and also sometimes these cells can break away from malignant tumor and enter lymphatic system or blood stream and spread to other body organs (Commonly this process is called as "Metastasis"). The main feature of these cells is cell's ability to grow rapidly, uncontrollably & independently from the tissue where it started. There are over 200 different types of cancer which are named after cell type & origin. E.g. Hepatomas are cancer of liver. Carcinomas are cancer of epithelial cells.

Characteristics	Benign Tumors	Malignant Tumors
Differentiation	Tumors cells are similar to original mature cells	Tumor cells might not be similar to original mature cells.
Growth Rate	Slow, might stop or regress	Rapid, autonomous; usually does not stop or regress
Type of Growth	Expand and displace	Invade, destroy and replace
Metastasis	Not seen	Seen in each case
Health Effect	Usually does not cause death	May cause death if not diagnosed and treated.

History of cancer

Cancer has existed for all human history. Though cancer existed in animal in prehistoric time, even before men appeared on earth. The earliest written information regarding cancer can be found in Egyptian Edwin Smith Papyrus dated circa 1500 BC & it refers to breast cancer. Father of medicine and Greek physician Hippocrates referred cancer with Greek word 'Karkinos' (Crab) as appearance of cut surface of malignant tumor was resembled to Crab with its feet. Later Celsius, a Roman physician translated Greek term Karkinos into a Cancer, a Latin word for crab. Another roman physician Galen used term 'Oncos', a Greek word for swelling to describe tumor & so nowadays it is used as name for cancer specialist i.e. "Oncologist"

Common types of cancer:

- **Breast cancer:**

  Breast cancer is the most common type of cancer. Breast cancer is the cancer that forms in cells of breast. It can occur in both, but women are most likely to be affected by this type of cancer. About 1% of all breast cancer cases affected by men.

- **Lung cancer:**

  Lung cancer is the type of cancer that begins in the lungs. Lung cancer is of two types; non-small cell lung cancer & small cell lung cancer. Smoking causes most of lung causes, but nonsmoker can also develop lung cancer.

- **Prostate cancer:**

  Prostate cancer is cancer of prostate gland, a small walnut shaped gland in men that produce seminal fluid that nourishes and transport cells. Prostate cancer usually

grows slowly and are confined to prostate gland, where they may not cause serious harm. However, while some types of prostate cancer grow slowly and may need minimal or even no treatment, other types are aggressive and can spread quickly.

- **Colorectal cancer:**

  Colorectal cancer is that cancer that develops in tissues of colon and rectum, parts of large intestine. Colorectal cancer typically affects older adult, though it can happen at any age.

- **Blood cancer:**

  Blood cancer is type of cancer that affect your blood cells. Leukemia, Lymphoma & Myeloma are most common types of blood cancer. leukemia occurs most often in adult older than 55, but it is common in children younger than 15.

Causes of cancer:

The following are the most well studied known or suspected causes of cancer. By limiting the exposure to such factors can reduces the chances of developing cancer.

1. Age factor:

The major risk for many specific types of cancer, is growing older. Overall, cancer incidence rates rise consistently with age, from fewer than 25 cases per 1,00,000 people in age groups under 20 to around 350 cases per 1,00,000 people in age groups 45-49, and more than 1,000 cases per 1,00,000 people in age group 60 and older.

According to most recent statistical research data, the half of total cancer cases occurs in age group above 50 years.

2. Obesity:

It is been observed that, obese people are more likely to develop cancer of breast, rectum, endometrium (uterine lining), esophagus, kidney, pancreas and gall bladder. There are some measures that can help minimize the risk of certain cancers like consuming a good diet, physical fitness, maintaining a healthy weight etc.

3. Tobacco:

Tobacco is one of the major causes of cancer and cancer related mortality. The tobacco contains many carcinogenic chemicals that damages the structure of DNA. So, the people who use tobacco products or regular smoking of tobacco products are at greater risk of cancer.

Tobacco is associated with many types of cancer including lung cancer, tracheal cancer, mouth cancer, throat cancer, bladder cancer, kidney cancer, liver cancer, cervical cancer, pancreatic cancer, colon cancer etc.

4. Infectious agents:

Many microorganisms are often considered as a cause of different diseases in human beings. The microorganisms like viruses, bacteria and parasites are the infectious agents, which can raise the risk of cancer development. Especially viruses have been designated as the major cause of cancer, accounting about 16-20% of total human cancer.

The oncogenic viruses can be transmitted from one person to another via blood or other body fluids or can also be transferred vertically from infected mother to fetus.

5. Radiations:

Ionizing radiation is a type of radiation that has enough energy to damage DNA and causes cancer. X-rays, gamma rays are some examples of ionizing radiations. These types of radiations can be emitted in nuclear power plant accidents as well as during development, testing and use of atomic weapons.

Who's affected by cancer?

The exact cause of cancer is yet to be known. However, certain risk factors have been demonstrated to raise a person's chances of developing cancer in research studies.

The exposure to certain chemicals or other substances are all cancer risk factors. Some factors which can not be controlled like age and family history can also be responsible for cancer development. A family history of some malignancies may indicate the presence of an inherited cancer.

The development of cancer depends upon several factors like habits, family history, health conditions and surrounding environment.

a. **Age:** Cancer can take decades to develop. That's why most people are diagnosed with cancer are of 65 or older. It is more common in older adults, but cancer isn't exclusively an adult disease. It can be diagnosed at any age.
b. **Habits:** Certain lifestyle choices are also responsible for increased risk of cancer. Smoking, drinking more than one drink a day for women and up to two drinks a day for men, excessive exposure to sun or blistering sunburn, being obese and having unsafe sex can increase chances of cancer.
c. **Family history:** Cancer is not a disease which is inherited commonly. Having family history with cancer does not necessarily cause cancer in all generations. It in

necessary to get genetic testing to see whether you have inherited mutations that might increase risk of certain cancers.

Why are cancer cases rising in India?

The total number of cancer cases in India is projected to go up from 14.6 lakh in 2022 to 15.7 lakh in 2025, according to the Indian Council of Medical Research- National Cancer Registry Program (ICMR-NCRP). The statistical report by NCRP in 2020, the cancer incidence in men is estimated to be 679,421 in 2020 and 763,575 in 2025, while among women, it is estimated to be 712,758 in 2020 and 806,218 in 2025. The report further highlighted that oral, lung and colorectal cancers were the most common cancers among men.

In India tobacco was found to be responsible for 25-40% of cancers and dietary habits may be responsible for about 10-20% of cancers. Dr. Mehul Bhansali, Director Surgical Oncology, Jaslok Hospital and research Center highlights that the increased cases of breast cancer, which is a major type of cancer in India. According to Dr. Bhansali poor lifestyle, longer working hours, increasingly stressful lives, smoking, alcohol consumption, use of contraception, are all contributing to breast cancer cases.

The major concern about cancer in India is that, many cancer cases go undetected until they reach advanced stages. According to Dr. Vijay Patil, Consultant (Medical oncology) at PD Hinduja Hospital and Medical Research Center, Khar, the risk factors which are high in Indians that can lead to cancer are:

a. Alcohol
b. Obesity
c. Infection
d. Tobacco

Preventive measures

- Exercise regularly
- Eliminate or reduce stress and enhance the ability of effectively cope with stress
- Go to annual health check up
- Learn to practice self-examination (Breast & testicular)
- Eat a balanced diet that includes vegetables, fresh fruit, whole grains & adequate amount of fibres.
- Take at least 6-8 hours of rest per night
- Reduce exposure to known or suspected carcinogen.

- Quit smoking & drinking.
- Avoid eating preserved food.

References:

1. "Cancer". World Health Organization. 12 September 2018. Retrieved 19 December 2018
2. "What Is Cancer?". National Cancer Institute. *17 September 2007*. Retrieved 28 March 2018.
3. Cancer Fact Sheet". Agency for Toxic Substances & Disease Registry. 30 August 2002. Archived from the original on 13 August 2009. Retrieved 17 August 2009
4. Prostate cancer - Symptoms and causes - Mayo Clinic
5. Colon cancer - Symptoms and causes - Mayo Clinic

BLENDING NATURAL HERBS WITH MODERN MEDICAL APPROACHES

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Abstract:

This chapter explores the burgeoning trend of incorporating natural herbs into modern medical practices, examining the historical roots, scientific validation, current trends, and challenges associated with this integration. As we navigate through the pages, we unravel the potential benefits of combining ancient herbal wisdom with cutting-edge medical advancements, fostering a holistic and patient-centric approach to healthcare.

Keywords: Herbal Remedies, Commonly Employed

Introduction:

In an era dominated by synthetic pharmaceuticals, the resurgence of natural herbs in contemporary medicine signals a shift towards embracing the healing powers inherent in nature [1-2]. This chapter aims to illuminate the path that has led to the renewed interest in herbal remedies. By examining historical practices, scientific breakthroughs, current trends, and challenges, we hope to provide a comprehensive understanding of the integration of natural herbs into modern medical paradigms. Raw plants or extracts made from various sections of plants are the two main forms these herbal remedies may be found in stores. Herbal components that are physiologically active are extracted by boiling or percolating the plant in a solvent such as water, alcohol, or another solvent [3-5]. Concentrated pastes, powders, or liquids may be made from these initial liquid extracts by heating or drying them. There are a wide variety of organic compounds present in both the unprocessed plant and its extract, such as sterols, alkaloids, glycosides, tannins, terpenes, flavonoids, and fatty acids. Sometimes it's hard to pin down exactly what it is that causes biological activity in people [6-9]. The pharmacological activity of the organic components may also be changed by processing procedures such as heating or boiling [6].

The amounts of components in a certain batch of herb might be affected by environmental variables such soil conditions, altitude, seasonal fluctuations, humidity, and more. The development of consistent herbal products is influenced by a multitude of variables, including diseases, insects, planting density, competition from other plants, timing of sowing, and genetics. Some producers strive for product consistency by

standardising, as there are many variables that impact the concentration of active substances. The first step is to determine which chemical components will serve as markers, and then to modify the manufacturing process such that each batch has the same amount of markers [10-13]. Nevertheless, there are unknown consequences for non-standardized components since this procedure of standardisation may include mixing various batches of the same plant [14-16]. To solve this problem, some producers add purified active markers to the extraction, which changes the original organic ingredient balance but produces a consistent quantity of the standardised component. Standardization becomes even more complex when dealing with products that include multiple herbs [17].

Historical roots:

The use of natural herbs for medicinal purposes is deeply woven into the fabric of human history. Across cultures and continents, ancient healing traditions have relied on the therapeutic properties of plants [18]. Traditional Chinese Medicine, Ayurveda, and Indigenous healing practices are rich reservoirs of knowledge that have stood the test of time [19-20]. The historical roots of herbal medicine provide a cultural and empirical foundation that modern medicine seeks to integrate into its framework [19].

Scientific validation:

Advancements in scientific research have cast a spotlight on the chemical intricacies of natural herbs, elucidating their therapeutic potential. From the isolation of active compounds to understanding their mechanisms of action, the scientific community is unlocking the secrets of herbal remedies [21]. Numerous studies highlight the anti-inflammatory, antioxidant, and antimicrobial properties of herbs, supporting their efficacy in treating various conditions. This scientific validation serves as a bridge between traditional wisdom and evidence-based medicine [22].

Current trends:

The integration of natural herbs into contemporary medicine is evident in the current trends shaping the healthcare landscape [23]. Herbal supplements, once relegated to the fringes, are now finding their place on pharmacy shelves. The rise of integrative medicine, blending conventional treatments with alternative therapies, reflects a growing acceptance of holistic approaches. Healthcare practitioners are increasingly open to incorporating herbal remedies into treatment plans, acknowledging the potential synergies between natural and pharmaceutical interventions [24].

The market is witnessing a surge in botanical extracts, phytochemical-based drugs, and personalized herbal formulations. Patients, too, are seeking natural alternatives, leading to the popularity of herbal teas, tinctures, and dietary supplements. The current trends underscore a cultural shift towards a more sustainable and patient-centered model of healthcare.

Challenges and considerations:

As the integration of natural herbs gains momentum, it is not without challenges. Standardization of herbal products, ensuring consistent quality, and addressing potential interactions with conventional medications are crucial considerations. The effectiveness and safety of herbal products depend on improving regulatory systems. It is crucial to have interdisciplinary teams consisting of both traditional herbalists and contemporary healthcare experts in order to overcome these obstacles [25].

The ethical use of herbal treatments relies heavily on education and knowledge. For the general public and medical practitioners alike to make educated judgements on the use of natural herbs in treatment plans, reliable information is essential. Overcoming these challenges requires a concerted effort from the scientific, medical, and herbal communities. Herbal medicines are sometimes falsely believed to be harmless because of their ''natural'' nature. However, herbs really contain powerful bioactive chemicals, and plants are the source of over one-third of the pharmaceutical pharmaceuticals on the market [26]. Many harmful and sometimes fatal side effects have been linked to the use of herbal products. These problems may be a result of direct toxicity, allergies, contamination, or combinations with other herbs and medications. Even healthy people looking for answers to problems like obesity might have serious implications due to the adverse effects of certain herbal remedies, according to recent articles. As an example, a case study revealed that 104 women had nephrotoxicity, end-stage renal illness, and even carcinogenic consequences after using herbal weight-loss items containing Aristolochia fangchi, a Chinese plant. Yohimbine was the active ingredient of a dietary supplement that caused acute hepatotoxicity in seven individuals in another case series [27]. There have been reports linking the popular herb kava to liver damage, liver transplantation, and even death, according to warnings issued by regulatory agencies. There have been reports of adverse outcomes associated with the plant ephedra, including fatalities and long-term disabilities.

Potential adverse effects may be exacerbated by contaminants included in herbal remedies. After reviewing 260 Asian patent medications, researchers discovered that 25% of them had high amounts of heavy metals and 7% included hidden drugs that were added

for therapeutic purposes. A herbal medicine called PC-SPES was thought to have potential in treating prostate cancer. However, research into its actual anticancer benefits was hindered by the discovery that it included synthetic chemicals. Extra dangers could arise if herbs mix with medications or other herbs. Some herbs that were shown to interact with one another in a comprehensive study were St. John's wort, ginseng, Ginkgo biloba, garlic, and kava. Some herbs may increase the risk of bleeding or the sedative effects of anaesthetics, according to another study, which might lead to interactions during the perioperative period. It is difficult to firmly attribute reported adverse effects to the combination or other variables since most probable herb-drug interactions are established via case reporting. Given that 16% of individuals in the US who use prescription pharmaceuticals also use herbs or supplements, the lack of research on herb-drug interactions is concerning and adds to the uncertainty around the risks of taking herbal products with prescription drugs. The lack of comprehensive monitoring methods for herbal products makes it difficult to ascertain the actual frequency of adverse effects. This is in contrast to pharmaceutical treatments. It should be noted that the FDA is not required to receive reports of adverse events from herbal product makers, and patients often refrain from informing their doctors about their herbal usage, which further restricts the reporting of such incidents.

Conclusion:

The integration of natural herbs into contemporary medicine signifies a harmonious marriage between ancient wisdom and modern science. As we embrace the therapeutic potential of nature, we pave the way for a more comprehensive and patient-centric approach to healthcare. The journey from historical roots to scientific validation and current trends reflects a collective realization that the healing power of natural herbs holds promise in addressing the complexities of modern health challenges. By navigating challenges and fostering collaboration, we can usher in an era where the integration of natural herbs becomes a cornerstone of a holistic and sustainable healthcare system.

References:

1. Awang D. V. Quality control and good manufacturing practices: Safety and efficacy of commercial herbs. Food Drug Law J. 1997;52:341–4.
2. Awang D. V, Fugh-Berman A. Herbal interactions with cardiovascular drugs. J Cardiovasc Nurs. 2002;16(4):64–70.
3. Barrett B. Alternative, complementary, and conventional medicine: Is integration upon us? J Altern Complement Med. 2003;9(3):417–27.

4. Blumenthal M. Guest editorial: The rise and fall of PC-SPES: New generation of herbal supplement, adulterated product, or new drug? Integr Cancer Ther. 2002;1(3):266–70.
5. Boyd D. B. Integrative oncology: The last ten years-a personal retrospective. Altern Ther Health Med. 2007;13(1):56–64.
6. Brinker F. Managing and interpreting the complexities of botanical research. Herbal Gram. 2009;82:42–9.
7. Bruno J. J, Ellis J. J. Herbal use among U.S. elderly: 2002 National Health Interview Survey. Ann Pharmacother. 2005;39(4):643–8.
8. Chadwick L, Fong H. H. S. Herb quality assurance and standardization in herb-drug interaction evaluation and documentation. In: Lam Y. W. F, Huang S. M, Hall S. D, editors. Herbal Supplement-Drug Interactions. New York: Taylor & Francis; 2006. pp. 191–203.
9. Cochrane Collaboration. 2009. The Cochrane Library. (accessed June 30, 2009).
10. Cordell G. A. PCSPES: A brief overview. Integr Cancer Ther. 2002;1(3):271–86.
11. Efficace F, Horneber M, Lejeune S, Van Dam F, Leering S, Rottmann M, Aaronson N. K. Methodological quality of patient-reported outcome research was low in complementary and alternative medicine in oncology. J Clin Epidemiol. 2006;59(12):1257–65.
12. Ernst E. Methodological aspects of traditional Chinese medicine (TCM). Ann Acad Med Singapore. 2006;35(11):773–4.
13. Ernst E, Thompson Coon J. Heavy m in traditional Chinese medicines: A systematic review. Clin Pharmacol Ther. 2001;70(6):497–504.
14. Evans S. Changing the knowledge base in Western herbal medicine. Soc Sci Med. 2008;67:2098–106.
15. Farnsworth N. R. Relative safety of herbal medicines. Herbal Gram. 1993;29:36A–367H.
16. Fitzloff J, Yat P, Lu Z. Z. Perspectives on the quality assurance of ginseng products in North America. Seoul, Korea.: Advances in Ginseng Research - Proceedings of the 7th International Symposium on Ginseng. 1998
17. Fong H. H. S. Integration of herbal medicine into modern medical practices: Issues and prospects. Integr Cancer Ther. 2002;1(3):287–93.
18. Fong H. H. S, Pauli G. F, Bolton J. L, van Breemen R. N, Banuvar S, Shulman L, Geller S. E, Farnsworth N. R. Evidence-based herbal medicine: challenges in efficacy and safety

assessments. In: Leung P. C, Fong H. H. S, Xue C. C, editors. Annals of Traditional Chinese Medicine Vol 2: Current Review of Chinese Medicine. Singapore: World Scientific; 2006. pp. 11–26.

19. Food and Drug Administration. 2007. Dietary supplement current good manufacturing practices (CGMPs) and interim final rule (IFR) facts. Accessed August 2, 2009.
20. Gagnier J. J, Boon H, Rochon P, Moher D, Barnes J, Bombardier C. Recommendations for reporting randomized controlled trials of herbal interventions: Explanation and elaboration. J Clin Epidemiol. 2006a;59(11):1134–49.
21. Gagnier J. J, Boon H, Rochon P, Moher D, Barnes J, Bombardier C. Reporting randomized, controlled trials of herbal interventions: An elaborated CONSORT statement. Ann Intern Med. 2006b;144(5):364–7.
22. Gagnier J. J, DeMelo J, Boon H, Rochon P, Bombardier C. Quality of reporting of randomized controlled trials of herbal medicine interventions. Am J Med. 2006;119(9):800.e1–11..
23. Geffen J. R. From integrative to multidimensional medicine. Altern Ther Health Med. 2007;13(1):14–8.
24. Gilroy C. M, Steiner J. F, Byers T, Shapiro H, Georgian W. Echinacea and truth in labeling. Arch Intern Med. 2003;163(6):699–704.
25. Giordano J, Garcia M. K, Strickland G. Integrating Chinese traditional medicine into a U.S. public health paradigm. J Altern Complement Med. 2004;10(4):706–10.
26. Grimaldi D. Integration of complementary and alternative medicine into mainstream health care. J Psychoso Nurs Ment Health Serv. 2008;46(10):8–9.
27. Harkey M. R, Henderson G. L, Gershwin M. E, Stern J. S, Hackman R. M. Variability in commercial ginseng products: An analysis of 25 preparations. Am J Clin Nutr. 2001;73(6):1101–6.

From Cells to Ecosystems: Exploring Life Science Research Volume I

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