THEORETICAL AND APPLIED RESEARCH IN AGRICULTURAL, FORESTRY, AND AQUATIC SCIENCES

Editor
Prof. Dr. Muzaffer Mustafa HARLIOĞLU

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Chapter 1

Application of Genome Editing Systems in Farm Animals: ZFNs and TALEN

Muhammet Mücahit SARI[1], Ayşe SARI [2], Neffel Kürşat AKBULUT[3]

Introduction

Selective breeding, conducted to better adapt animals to specific environmental conditions, management systems, and market demands, has been a fundamental practice in the livestock industry for centuries and has played a key role in the diversity of farm animal genetic resources observed today. In the last century, in particular, several technological advances have accelerated the sector's development and have been critical in shaping modern animal husbandry. Animals with high genetic merit can increase both meat and milk yields. However, yields are affected by many factors. Adequate and balanced nutrition, in particular, can optimize the yield of animals with high genetic merit (Ayaş, 2023). For example, choosing high-quality forages such as alfalfa hay and silage (Tasdelen et al., 2024), homogeneous mixing of TMR (Trusted Source), anionic feeding, which has gained popularity in recent years (Ayaş, 2023), and reducing harmful substances such as toxins, molds, and nitrates in feed (Ayaş, 2024), can all optimize the effect of genetics on yield. These advances in reproductive technologies initially enabled the widespread adoption of animals with high genetic merit and the implementation of more intensive selection practices. With the introduction of quantitative genetic approaches and the subsequent integration of genomic tools, advances in breeding value estimation, computational algorithms, and selection accuracy have evolved to sustain the increased rate of genetic improvement (Jones & Wilson, 2022). These technological advances have directly contributed to increased productivity in animal production. For example, between 1957 and 2005, an approximately 400% increase in growth rate of broiler chickens was observed, while a 50% improvement in feed conversion ratio was achieved (Zuidhof et al., 2014). Similarly, during the same period, average milk yield of dairy cattle in North America increased by approximately 400% thanks to both genetic selection and improved management

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practices; the effect of genetic improvement has been decisive in this increase (Knapp et al., 2014). International genetic evaluation systems for dairy cattle have been regularly implemented for the last 25 years, which has allowed for similar productivity increases to be achieved worldwide. These developments have also contributed significantly to the reduction of greenhouse gas emissions per unit of animal product in different production systems (Pryce & Bell, 2017; Pryce & Haile-Mariam, 2020).

With the global population expected to approach nearly 10 billion by 2050, the sustainable governance of finite natural resources emerges as a critical challenge of worldwide concern. This demographic increase increases pressure on food production and necessitates increasing the efficiency of agricultural production systems (Proudfoot & Tait-Burkard, 2017). Consequently, enhancing animal welfare and production efficiency, while maintaining the quality of animal-derived products, has become an essential strategy for advancing sustainable development objectives (Wani et al., 2023). In recent years, the combined use of traditional breeding methods and controlled genetic modification of farm animals has led to significant improvements in production parameters (Hallerman et al., 2022; Tait-Burkard et al., 2018).

Genetic engineering applications were first reported in animal husbandry in 1985 (Hammer et al., 1985). This technology entails modifying an organism's genetic composition by randomly integrating recombinant DNA (rDNA) sequences into its genome to generate a desired phenotype. Organisms produced through this process are commonly termed genetically modified organisms (GMOs). Despite substantial progress in genetic engineering over the past forty years, only two genetically modified animal-derived products have thus far received approval for human consumption worldwide. A primary factor restricting the broader commercialization of genetically modified animal products is the protracted and costly nature of regulatory assessment procedures in many jurisdictions (Van Eenennaam et al., 2021).

1. Genome Editing Technologies

Genome editing technologies refer to a biotechnological toolkit that allows for high-fidelity nuclease-based modifications to an organism's genome. Programmable nuclease-based approaches are classified into four main groups: meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the clustered regularly interspaced short palindromic repeats-Cas9 system (CRISPR-Cas9). These nucleases introduce precise cuts in the genome by generating double-strand breaks (DSBs) within specific nuclear DNA regions. In the absence of an external repair template, such breaks are predominantly resolved through non-homologous end joining (NHEJ). This repair pathway enables the direct ligation of

DNA ends through the insertion or deletion of nucleotides, irrespective of the target sequence. When occurring within coding regions, such stochastic modifications can induce frameshifts, thereby abolishing gene expression and resulting in the irreversible loss of the corresponding protein's function. Moreover, the simultaneous induction of DSBs at two distinct genomic loci may lead to sequence deletions spanning the nuclease recognition sites, or even larger-scale chromosomal rearrangements (Cox et al., 2015; Raza et al., 2022).

Figure 1 provides an overview of the distinctions among meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR-Cas systems, highlighting their respective strengths and limitations. The meganuclease, ZFN, and TALEN platforms induce double-strand breaks at specific DNA loci through protein–DNA recognition mechanisms (Hsu et al., 2014; Li et al., 2021; Ng et al., 2020). These approaches necessitate the design of specifically engineered proteins for each target DNA sequence, and editing multiple loci requires distinct protein modifications for every site. Consequently, the procedures are both time-consuming and financially demanding. In contrast, the CRISPR-Cas9 system relies on base-pairing complementarity between a guide RNA (gRNA) and the target genomic DNA sequence. This mechanism affords a more streamlined, adaptable, and highly efficient platform for programmable genome editing (Raza et al., 2022).

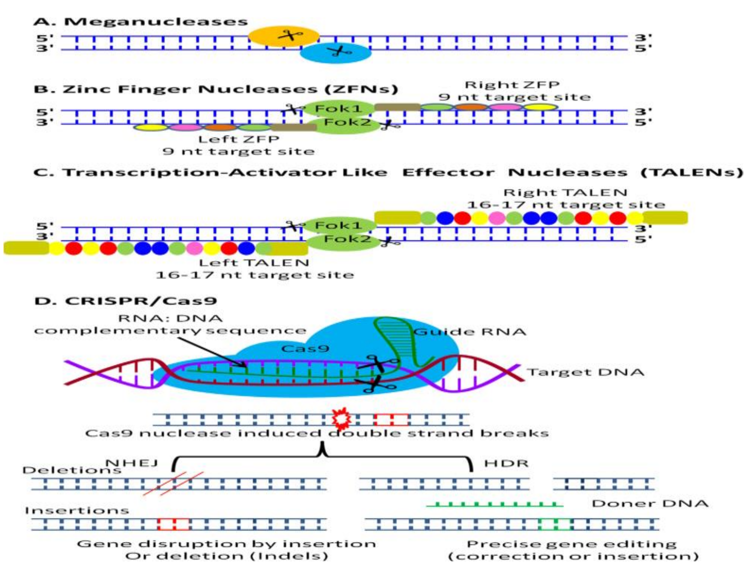

Figure 1. Nuclease-based genome editing approaches. Schematic diagram showing the genome editing process using (A) Meganucleases, (B) Zinc Finger Nucleases (ZFN), (C) Transcription Activator-Like Effector Nucleases (TALEN), and (D) the CRISPR/Cas9 system (Raza et al., 2022).

Genome editing technologies have been utilized within the livestock sector to pursue a range of objectives. These applications include the development of disease-resistant animal breeds, the enhancement of productive traits, the alteration of milk composition, and the creation of polled (hornless) cattle (Alberio & Wolf, 2021; Carlson et al., 2016; Koloskova et al., 2021). Among the various genome-editing tools, the CRISPR system has emerged as the predominant technology, extensively employed for targeted gene disruption in both biomedical research and therapeutic development (Butler et al., 2016).

Genome editing technologies present substantial potential for augmenting livestock productivity and strengthening the economic viability of related agricultural sectors. Traditionally, gene editing efforts in livestock primarily depended on knockout and knock-in strategies, both of which proved inefficient and technically challenging, largely due to the absence of germline embryonic stem cells (Oishi et al., 2016). Moreover, because many traits of economic significance in livestock are quantitative being regulated by multiple genes genetic improvement typically necessitates editing several loci across the genome, a task that is prohibitively complex and costly with conventional gene manipulation methods. Thus, to achieve a meaningful translational impact on livestock productivity and profitability, advanced genome editing platforms capable of precisely and efficiently targeting multiple loci in diverse host species are required (Raza et al., 2022).

2. Meganucleases

Meganucleases represent a distinct class of endodeoxyribonucleases, defined by their distinguishing feature of recognizing and cleaving particularly long DNA recognition sequences, which typically span 14 to 40 base pairs (bp). This catalytic activity is demonstrated effectively in both laboratory (in vitro) and physiological (in vivo) environments (Figure 1A) (Raza et al., 2022). This protein family, comprising hundreds of distinct variants, has been discovered across a diverse spectrum of organisms, encompassing eukaryotes, bacteria, and archaea. These enzymes are mostly encoded within mobile class I introns and inteins and can be synthesized not only by the nuclear genome but also by mitochondrial and chloroplast genomes. Meganucleases have a homodimeric structure consisting of two identical subunits, each 160–200 amino acids (aa). These enzymes recognize specific DNA sequences between 12 and 40 base pairs (bp) in length, creating double-strand breaks. This long recognition sequence allows meganucleases to exhibit high specificity and significantly reduces the number of potential cutting sites on the genome (Tufan, 2019). Although many meganucleases have been identified to date, it is not possible to find a naturally occurring enzyme suitable for every desired site. Therefore, enzymes suitable for the specific site can be produced by modifying the recognition sites of existing meganucleases using various techniques or by creating chimeric proteins. The initial phase of genome editing utilized naturally occurring meganucleases, which recognize and cleave target sequences of approximately 18 base pairs. The targeting capacity of these enzymes can be expanded through the engineering of homodimeric or heterodimeric forms, thereby increasing the number of genomic sites accessible for modification. A significant limitation of this approach, however, is the requirement for the de novo design of a unique enzyme for each specific target sequence under investigation (Smith et al., 2006).

The relatively long recognition sequences allow these enzymes to bind and cleave target sites with high efficiency despite the presence of polymorphisms. Of the various meganuclease families, the LAGLIDADG group is among the most extensively characterized. These enzymes are defined by the presence of a conserved LAGLIDADG amino acid motif, which is indispensable for their catalytic function. Members of this family exhibit variation in the number of these motifs; for instance, the I-CreI meganuclease possesses a single motif, whereas others, such as I-SceI, incorporate multiple copies. The enzymatic activity and DNA recognition specificity of these endonucleases are highly sensitive to mutations within amino acid residues that constitute the DNA-binding interface (Doyon et al., 2006; Rosen et al., 2006).

The integrity of genomic DNA within the nucleus is continuously threatened by various endogenous factors, particularly reactive oxygen species and other metabolic byproducts generated through normal cellular activities. In response to such damage, cells activate evolutionarily conserved DNA repair pathways, with the primary mechanisms being nonhomologous end joining (NHEJ) and homologous recombination (HR). The choice between these two pathways is critical for maintaining genomic stability, as NHEJ is error-prone while HR provides a template-dependent, high-fidelity repair mechanism. Among meganucleases, I-SceI is particularly recognized for its efficacy in inducing the HR repair pathway. This specific function, initially characterized in yeast (Jacquier & Dujon, 1985), has facilitated its adoption as a tool for targeted genome manipulation. Research during the 1990s established the utility of I-SceI meganucleases for directed cleavage of the neomycin resistance gene in murine cell lines. However, a significant constraint for their direct application in animal models is the general absence of their native recognition sequences within animal genomes. Consequently, the use of meganucleases for genetic engineering in livestock necessitates the preliminary introduction of their target sites into the desired genomic loci via transfection. Despite this limitation for direct use in animal husbandry, meganucleases have been successfully employed to introduce a range of genetic alterations, including point mutations and targeted recombinations, across various species (Horzempa et al., 2010; Maggert et al., 2008; Windbichler et al., 2007; Yu et al., 2008). For instance, Ménoret et al. (2013) microinjected a meganuclease-coding plasmid targeting the RAG1 gene into the pronuclei of mouse zygotes to generate a severe immunodeficiency model through suppression of RAG1 expression.

3. Zinc finger nucleases (ZFNs)

A landmark achievement in genome engineering was the creation of Zinc Finger Nucleases (ZFNs), which provided the first programmable means to generate site-specific double-strand breaks (DSBs) in DNA (Bibikova et al., 2002). The conceptual foundation for ZFNs emanated from the earlier identification of zinc finger (ZF) domains modular protein motifs that facilitate sequence-specific DNA recognition within the transcription factor IIIA of Xenopus laevis oocytes (Miller et al., 1985; Figure 1B). A prototypical ZF domain adopts a Cys2-His2 architecture, wherein a centrally coordinated zinc ion stabilizes a compact protein fold. This structure, approximately 30 amino acids in length, is composed of two antiparallel β-sheets packed against an α-helix, with the zinc ion chelated by conserved cysteine and histidine residues. This specific configuration is essential for the domain's DNA-binding function. The primary function of the zinc finger domain is DNA recognition; each individual domain specifically binds to a triplet nucleotide sequence through amino acid interactions within its α-helical "recognition helix." Consequently, a ZF array composed of three such domains exhibits binding specificity for a 9-base pair DNA sequence (Zafar et al., 2019).

A pioneering strategy in targeted genome engineering involved the creation of a chimeric enzyme by fusing the catalytic domain of FokI a type IIS restriction endonuclease isolated from Flavobacterium okeanokoites to a customized zinc finger DNA-binding module. This hybrid protein functions as a synthetic nuclease designed to cleave predetermined genomic sequences with high precision. A critical requirement for efficient double-strand break formation is the dimerization of the FokI cleavage domains, a process that occurs when two ZFN subunits bind to adjacent nucleotide sequences flanking the target site (Kim et al., 1996). Structurally, ZFNs are modular proteins consisting of a sequence-specific zinc finger array linked to the non-specific DNA cleavage domain derived from FokI (Kim et al., 1997). Fundamental work by Chandrasegaran's group revealed that the DNA-binding and cleavage functions of FokI reside in distinct domains.

This key insight paved the way for engineering chimeric nucleases with customized specificities by combining the FokI nuclease domain with diverse zinc finger DNA-binding arrays (Kim et al., 1996), representing the first successful generation and implementation of ZFNs as programmable genomic tools.

Zinc Finger Nucleases (ZFNs) function as obligate dimers, a structural requirement that effectively doubles the length of the recognized DNA sequence and enhances targeting specificity. For instance, a dimer composed of two three-finger ZFN subunits recognizes a combined 18-base pair sequence, a length theoretically sufficient to specify a unique genomic address. The two ZFN monomers bind to opposing DNA strands, with their recognition sites separated by a short intervening sequence, or "spacer," typically 5–7 nucleotides in length. The double-strand break is subsequently introduced within this spacer region by the dimerized FokI nuclease domains (Zafar et al., 2019; Smith et al., 2000).

Although Zinc Finger Nuclease (ZFN) technology was initially reported in 1995 (Shi & Berg, 1995), its application in livestock species was not realized until 2011, with demonstrations in rabbits (Flisikowska et al., 2011) and pigs (Hauschild et al., 2011). These pioneering studies highlighted the technology's dual potential for biomedical and agricultural applications. In rabbits, ZFNs were used to disrupt the endogenous IgM locus to enable the production of human polyclonal antibodies. Concurrently, work in pigs achieved a knockout of the α1,3-galactosyltransferase (GGTA1) gene. A significant subsequent advance was reported by Liu et al. (2014), who employed a ZFN-assisted homology-directed repair strategy in bovine fetal fibroblasts to generate transgenic cows. These animals expressed lysostaphin a protein conferring resistance to Staphylococcus aureus, the causative agent of mastitis and human lysozyme in their mammary glands. Milk from these cows exhibited significant antibacterial activity in vitro, and the study marked the first successful creation of both knockout (KO) and knock-in (KI) modifications in a large mammal using ZFNs. Despite these successes, the broader application of ZFNs in transgenic animal production is constrained by the complexity, duration, and high cost associated with the design and manufacturing of effective proteins (Liu et al., 2013).

Zinc Finger Nuclease (ZFN) technology has proven highly efficient across diverse cell types and livestock species. A notable application was reported by Yu et al. (2011), who achieved targeted mutagenesis of the beta-lactoglobulin (BLG) gene locus in cattle. The researchers delivered ZFNs into bovine fetal fibroblast cells, resulting in a high proportion of modified cells. Genetic analysis of 100 mutant clones revealed that most contained short fragment deletions or insertions at the target site. These engineered fibroblasts were then utilized as nuclear donors in somatic cell nuclear transfer (SCNT). From 209 reconstructed embryos transferred to recipient animals, eight viable calves were born. Sequencing confirmation demonstrated that all offspring carried biallelic modifications at the BLG locus, validating the successful integration of ZFN-induced mutations into the bovine genome.

In a related study, Song et al. (2015) employed ZFNs to target the beta-lactoglobulin (BLG) gene in goats. The researchers performed the recombinant expression and purification of ZFN proteins designed for the caprine BLG locus, subsequently evaluating their cleavage efficacy through both in vitro and cell-based assays. The study demonstrated that the prokaryotic expression plasmids pET-BLG-LFN and pET-BLG-RFN could be accurately constructed and efficiently expressed in Escherichia coli. Furthermore, it was determined that the purified ZFN proteins exhibited cleavage activity by targeting the BLG gene both in vitro and in fibroblast cells, making them an effective gene modification tool.

A study conducted by Fan and colleagues (2011) demonstrated a methodology for targeted transgene integration into the 3' untranslated region of the chicken ovalbumin gene using custom ZFN expression vectors developed with Zinc Finger Vector Kits. Their systematic evaluation revealed that a single-chain ZFN configuration featuring two FokI nuclease domains connected by an elongated, flexible linker preserved efficient double-stranded DNA cleavage capability without compromising target specificity. The investigation further established that the functional efficiency of such single-chain ZFNs is closely dependent on the structural properties of the inter-domain linker, emphasizing that optimized linker design can substantially improve the precision and effectiveness of genome editing operations.

4. Transcription activator-like effector nucleases (TALENs)

A significant advancement in targeted genome editing emerged with the development of transcription activator-like effector nucleases (TALENs), which were engineered to overcome the design constraints and reduced specificity associated with zinc finger nuclease (ZFN) platforms (Becker & Boch, 2021). TALENs function as programmable nucleases that can be rationally engineered for precise binding to designated DNA sequences, facilitating the introduction of double-strand breaks with exceptional accuracy. Owing to their modular design and enhanced targeting flexibility, TALENs were quickly established as a versatile tool for genetic manipulation, finding successful application across diverse taxonomic groups from plants and livestock to various model and non-model organisms. A landmark achievement for this technology was its application in clinical medicine, becoming the first genome-editing platform to be successfully employed in a human cancer therapy, culminating in a life-saving intervention in 2015 (Qasim et al., 2017). Additionally, TALENs facilitated the development of the first commercially available genome-edited agricultural product, which entered the market in 2019 (Menz et al., 2020).

Genome editing forms the core of this approach, allowing for the direct modification of genetic material within living cells, thus enabling an expansion from in vitro methods to in vivo applications. While molecular cloning processes rely on multiple restriction enzymes, genome editing can be achieved by combining the strengths and weaknesses of different techniques (Becker & Boch, 2021).

During the 1990s, pioneering technologies such as meganucleases and zinc finger nucleases (ZFNs) established the conceptual and technical foundations for targeted genome editing. These early efforts culminated in a definitive milestone in 2002 with the generation of the world's first organism modified via designed genomic editors (Carroll, 2021). Although ZFNs and meganucleases are associated with practical constraints such as design complexity and technical challenges limiting their widespread adoption they continue to be utilized within biotechnology firms that possess the specialized expertise required for their application.

The year 2010 marked a breakthrough in genetic engineering with the development of Transcription Activator-Like Effector Nucleases (TALENs), created by combining the DNA-binding domains of transcription activator-like effectors (TALEs) with the catalytic subunit of the FokI restriction enzyme. Unlike previous technologies, TALENs' straightforward design framework made sophisticated genome editing accessible to a wider range of researchers. This trajectory of innovation accelerated dramatically in 2012 with the emergence of the CRISPR/Cas9 system, which revolutionized the field through its exceptional targeting flexibility, high efficiency, and operational simplicity. A principal advantage of the CRISPR/Cas9 system over TALEN technology resides in its streamlined design and implementation process, which has enabled research laboratories across the globe to integrate precision genome editing into their standard methodologies. Moreover, continuous innovation since its discovery has yielded a diverse array of Cas9 variants and alternative CRISPR-based systems, underscoring the technology's remarkable adaptability and expanding its potential applications across biological research and therapeutics (Pickar-Oliver & Gersbach, 2019).

Transcription activator-like effectors (TALEs) are virulence proteins secreted by phytopathogenic bacteria from the genus Xanthomonas. Upon delivery into plant cells, these proteins localize to the nucleus where they bind promoter sequences with high specificity, leading to the transcriptional manipulation of host genes. The modular architecture of TALEs comprises three primary domains. The N-terminal region contains a type III secretion signal and facilitates general DNA binding through non-specific interactions (Szurek et al., 2002). In contrast, the C-terminal domain houses an acidic activation domain, nuclear localization signals, and a segment that interfaces with the host transcription factor IIA (Yuan et al., 2016). Specific DNA recognition is mediated by a central repeat domain consisting of multiple tandem repeats, each typically 33–35 amino acids long. Within each repeat, two hypervariable residues at positions 12 and 13 termed repeat variable di-residues (RVDs) determine nucleotide specificity (Boch et al., 2009). Empirical studies of RVD-DNA interactions have identified both highly specific combinations that bind a single nucleotide and more degenerate motifs capable of recognizing multiple nucleotides. This modular architecture allows for the rational design of custom TALE and TALEN proteins through the systematic assembly of these repeat modules to target desired DNA sequences (Miller et al., 2015).

The programmable nature of TALE repeat domains allows for the rational design of synthetic DNA-binding proteins that can be targeted to specific genomic sequences in mammalian cells. These engineered domains are functionally converted into site-specific nucleases, termed transcription activator-like effector nucleases (TALENs), by fusion with the non-specific DNA cleavage domain of the FokI endonuclease. The resulting TALEN pairs generate targeted double-strand breaks (DSBs), harnessing the cell's endogenous repair mechanisms primarily non-homologous end joining (NHEJ) or homology-directed repair (HDR) to achieve genome modifications (Cermak et al., 2011; Mahfouz et al., 2011). The efficacy of TALENs for generating gene knockouts has been validated in numerous species, spanning traditional model organisms such as mice, rats, and zebrafish to agriculturally significant livestock (Huang et al., 2011; Tesson et al., 2011).

While ZFNs allow for a variety of genetic modifications, the technical challenges associated with primary cell cultures, limited production of low-cost and reliable reagents, and embryo injection have slowed the applicability of this technology to genome engineering in farm animals (Gaj et al., 2013). In contrast, TALENs stand out as tools that exhibit higher efficiency and can be produced more efficiently in primary cells. Indeed, injection of TALEN mRNAs into the cytoplasm of farm animal zygotes allows for efficient knockout (KO) of the target gene (Yang & Wu, 2018).

This approach has been exemplified in swine models, where TALEN-edited fetal fibroblasts were employed as nuclear donors to generate miniature pigs possessing both monoallelic and biallelic modifications in the low-density lipoprotein receptor (LDLR) gene (Carlson et al., 2012). In a related application, the Sry gene integral to male sexual development was targeted for knock-in via TALEN-mediated editing in cattle. Wang et al. (2021) successfully introduced a genetic construct into the Sry locus using TALENs and subsequently generated sex-reversed, monozygotic infertile cattle through somatic cell nuclear transfer (SCNT).

A TALEN-based gene targeting strategy was successfully employed by Park et al. (2014) to generate genetically modified chickens. The researchers achieved a targeted knockout of the ovalbumin (OV) gene in chicken primordial germ cells, with test-cross analyses subsequently confirming germline transmission of the introduced mutations to the resulting progeny. TALEN technology successfully induced nucleotide deletion mutations that resulted in a shift in the reading frame, resulting in loss of function of the OV gene. These findings demonstrate that the use of TALENs in primary germ cell lines offers a powerful strategy for the safe and effective production of chickens with specific gene editing.

Dehorning is widely practiced in cattle farming to reduce the risk of injury to both animals and producers. However, this method carries significant disadvantages due to its high cost and the pain and stress it inflicts on animals. As an alternative solution to this problem, Carlson et al. (2016) used genome editing mediated by transcription activator-like effector nucleases (TALENs) to transfer the PC Celtic POLLED allele into the genomes of two bulls. Whole-genome sequencing (WGS) analyses revealed that this editing was accomplished without introducing off-target mutations. Furthermore, both bulls were reported to reach maturity polled, demonstrating the potential for both improving animal welfare and applying this approach to cattle breeding.

The allergenic β-Lactoglobulin (BLG) protein found in goat milk was successfully eliminated in goats using the TALEN-mediated homologous recombination (HR) method by Cui et al. (2015). Milk analyses from cloned goats revealed high levels of human lactroferrin (hLF) expression and/or low BLG production in heterozygous individuals, while complete loss of BLG protein was reported in homozygous goats.

The Myostatin (MSTN) gene, a key negative regulator of muscle growth, has been a frequent target in genome editing research due to its significant role in controlling muscular development. Yu et al. (2016) designed a TALEN pair (MTAL-2) capable of binding and disrupting the MSTN gene, demonstrating that this system could successfully introduce mutations in somatic cells without inducing developmental abnormalities. In a complementary approach, Proudfoot et al. (2015) produced genetically edited cattle and sheep through direct microinjection of TALEN mRNA into zygotes. Collectively, these investigations demonstrated a viable alternative to somatic cell nuclear transfer (SCNT), enabling the generation of gene knockouts or the introduction of specific alleles through precise editing of the MSTN locus.

5. Conclusion

Precise genome editing platforms have revolutionized animal biotechnology by providing powerful tools to augment livestock productivity and operational sustainability. Programmable nucleases including meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the CRISPR-Cas9 system allow for targeted genetic modifications in agricultural species. These technologies facilitate the development of advanced animal lines with optimized traits, such as enhanced disease resilience, superior production qualities, and other economically valuable characteristics. When integrated with conventional breeding strategies, these tools contribute to improved production parameters and product quality in animal agriculture.

Although ZFNs and TALENs have proven effective for generating transgenic animals and facilitating targeted gene modifications, their widespread adoption has been constrained by complexities in protein design and elevated costs. Nonetheless, both systems have been successfully employed to introduce functional mutations in large mammalian species and livestock. The continued advancement and application of genome editing technologies are expected to play an increasingly vital role in meeting growing global food demands, promoting animal welfare, and mitigating the environmental footprint of animal production.

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Chapter 2

Ocean Acidification: Chemical Mechanisms, Biological Responses, and Ecosystem Consequences

Fikriye ALTUNKAYNAK[1], Ertan KARAHANLI[2]

1. Introduction

Oceans absorb a significant portion of human-induced carbon dioxide (CO_2) emissions, playing a critical role in the global carbon cycle (Sabine et al., 2004). This process leads to increased CO_2 levels in the atmosphere and, consequently, significant changes in the chemical properties of seawater. The decrease in pH resulting from this absorption process by the oceans is called 'ocean acidification.' The average pH of the ocean surface has decreased by approximately 0.1 units since the Industrial Revolution (Raven et al., 2005). If this trend continues, pH levels are expected to drop by another 0.3 units by the end of the century (Chen, 2025).

Recent studies indicate that ocean acidification is beginning to affect not only individual marine organisms but also large-scale ecosystem structures. For example, an analysis by the Plymouth Marine Laboratory (2024) revealed that the world's oceans have exceeded the established 'safe operating area' limits in four major basins. This phenomenon has been detected in approximately 40% of surface waters and approximately 60% of water bodies down to 200 m depth, according to NOAA (2025) data. This finding suggests that the rate of change in ocean chemistry is faster than previously estimated.

Hsiao et al. (2025) demonstrated that ocean acidification rates accelerated between 1985 and 2022, threatening the structural integrity of coral reefs in particular. Similarly, Pansini (2025) emphasized that short- and long-term acidification impacts disrupt biochemical processes, reduce biomass, and weaken ecosystem functions. Joshi and Warrior (2022), in their modeling study of the Bay of Bengal, demonstrated that seasonal variability and anthropogenic emissions significantly impact surface pH values.

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A 2023 study published in the journal Oceanography indicated that, in addition to the observed decline in global surface ocean pH values, carbonate ion and calcium carbonate saturation states are also near "critical thresholds." This suggests that the resilience of marine organisms that rely on calcification may be at greater risk in the coming years. Furthermore, Zeng, He, and Zhan (2025) demonstrated that thermal stresses due to climate change could cause an irreversible decline in coral reefs. These findings demonstrate that ocean acidification is not merely a chemical process but an ecosystem crisis.

Therefore, ocean acidification must be addressed beyond its classical definition and as a holistic threat to ecosystems. In this context, this book chapter will discuss the mechanisms, chemical processes, effects on marine organisms, and long-term ecosystem consequences of ocean acidification in light of scientific findings.

2. Ocean Acidification

Ocean acidification is a chemical process resulting from the absorption of anthropogenic carbon dioxide (CO_2) from the atmosphere into seawater, leading to a decrease in pH. While sea surface pH was approximately 8.18 before the Industrial Revolution, it has decreased to an average of around 8.05 today (Amiraux et al., 2022). While this value may seem small, due to the logarithmic nature of the pH scale, it represents an approximately 30% increase in hydrogen ion concentration. This acidification trend directly impacts marine calcification processes.

The most significant consequence of ocean acidification is the decrease in the concentration of carbonate ions (CO_3^{2-}). These ions, calcium carbonate ($CaCO_3$), are the fundamental building blocks for organisms that form skeletons and shells. As pH decreases, carbonate ions convert to bicarbonate, reducing the shell-forming capacity of marine organisms (Zhang et al., 2022). This process is particularly related to the saturation ratios of calcite and aragonite; The decrease in these rates reduces the survival rates of marine crustaceans, corals, and planktonic organisms.

Post-2020 studies have shown that the acidification trend is progressing more rapidly in cold seas such as the North Pacific and Southern Ocean (Qiao et al., 2025). Due to the lower temperatures and higher CO_2 solubility in these regions, the effects of ocean acidification reach the deep water layers from the surface more quickly. It is also reported that acidification interacts with the global oxygen cycle and accelerates deoxygenation processes (Ford et al., 2025). Figure 2.1 shows the trend of pH change in ocean surface waters, both in past periods (A) and future projections (B). Data based on paleoclimatic records reveal that pH values have fluctuated between 8.2 and 8.3 over the past several hundred thousand years, indicating that a long-term chemical balance has been maintained. However, the rapid increase in atmospheric CO_2 with the industrial revolution disrupted this balance and initiated a significant downward trend in pH values. Models extending from today to 2100 project that pH could decline from 8.1 to 7.7. This trend translates into an approximately 60% increase in hydrogen ion concentration, weakening the buffering capacity of seawater and indicating that ocean acidification will continue to accelerate. The figure therefore demonstrates that the impact of anthropogenic CO_2 emissions on marine chemistry is occurring at an unprecedented rate on geological timescales.

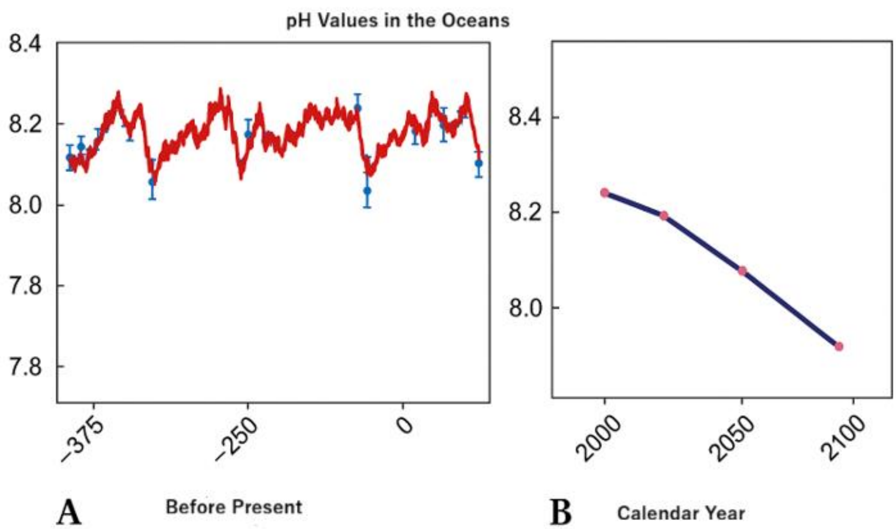

Figure 2.1. Change in pH value in the ocean from past to present

These findings demonstrate that ocean acidification is no longer just a chemical problem but also an ecological crisis. Therefore, continuous monitoring of seawater pH changes is critical for sustainable conservation policies, especially in regions with high carbon sink capacity.

3. Chemical Processes and Anthropogenic Impacts

Carbon dioxide in the atmosphere interacts with water to form carbonic acid (H_2CO_3), which then dissociates into bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions. In this equilibrium system, as the pH decreases, the bicarbonate content increases and the carbonate content decreases. This disrupts the saturation conditions with calcium carbonate (Wei et al., 2024). These changes in seawater chemistry directly affect the mineralization processes of calcifying organisms.

Figure 3.1 shows the pH-dependent concentration changes of the main inorganic carbon species (CO_2, HCO_3^-, CO_3^{2-}) and borate species ($B(OH)_3$, $B(OH)_4^-$) in seawater. CO_2 predominates in dissolved form in the pH range of 5–6 and decreases rapidly as pH increases. At approximately pH 7, the bicarbonate (HCO_3^-) ion reaches its highest level and constitutes the majority of the total inorganic carbon in seawater. Above pH 8, the carbonate (CO_3^{2-}) ion becomes dominant, representing favorable chemical conditions for calcifying organisms. Similarly, in the borate system, $B(OH)_3$ predominates at low pH conditions and converts to the $B(OH)_4^-$ form as pH increases. These curves demonstrate that the buffering capacity of seawater is based on a pH-sensitive equilibrium system, and that CO_2 increases with decreasing pH, reducing the amount of carbonate ions. Therefore, the figure reveals that during ocean acidification, the carbonate balance shifts toward bicarbonate, and this shift directly impacts marine calcification processes.

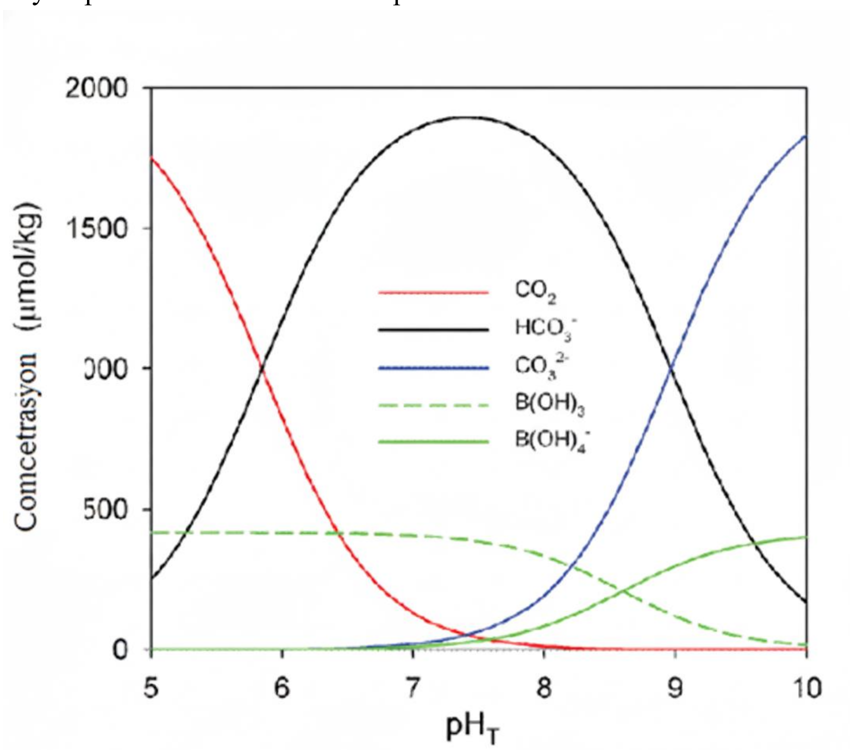

Figure 3.1. Seawater carbonate balance system

Figure 3.2 shows the changes in seawater chemical parameters with depth. As seen in the graph, dissolved inorganic carbon (DIC) increases with depth, while temperature decreases accordingly, and cold deep waters increase CO_2 solubility. pH, which is high at the surface, decreases with depth, reflecting the vertical nature of ocean acidification associated with increasing CO_2 accumulation. Carbonate ion (CO_3^{2-}) decreases significantly at depth, while dissolved CO_2 concentration increases, suggesting a shift in the carbonate balance toward bicarbonate. This shift leads to a decrease in the aragonite and calcite saturation ratios (Ω) with depth, increasing the risk of dissolution for calcifying organisms. Therefore, the figure clearly demonstrates that the chemical processes of ocean acidification weaken the calcium carbonate stabilization capacity of deepwater layers.

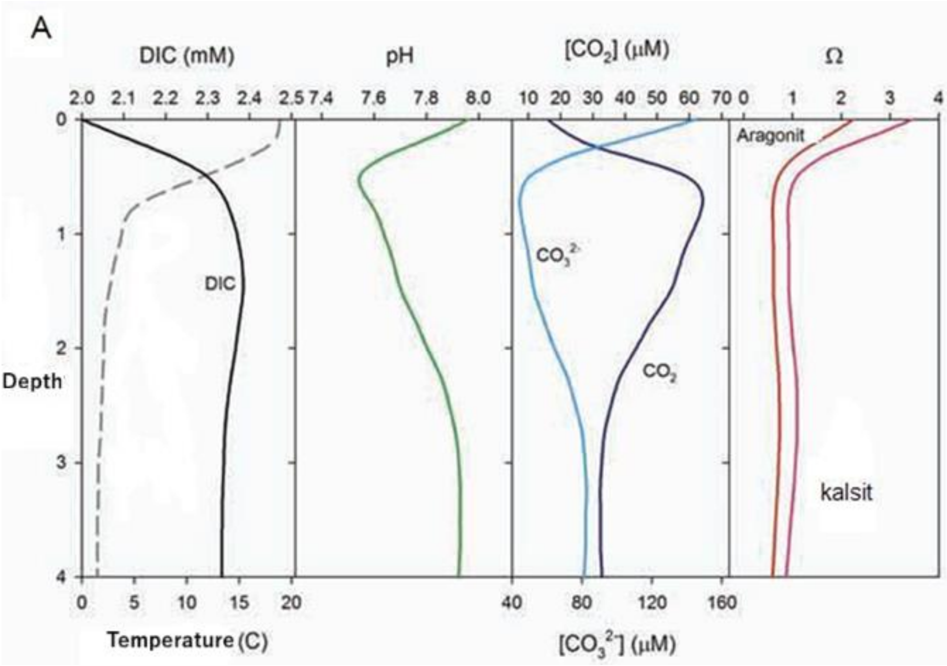

Figure 3.2. Changes in seawater chemical parameters with depth

Figure 3.3 shows the distribution of pH values with latitude and depth. The figure shows that pH values in surface waters are around 8.1, decreasing to 7.3 with depth. This suggests that alkaline conditions dominate at the surface due to the consumption of CO_2 through constant gas exchange with the atmosphere and photosynthetic activities, while CO_2 accumulation increases in deep waters through respiration and organic matter decomposition. The significant decrease in pH values between 250 and 1000 m depth indicates that ocean acidification is concentrated in the intermediate water layers. The decrease in pH values observed from equatorial regions to higher latitudes can be explained by differences in temperature, dissolved CO_2, and water mass circulation. Overall, the figure reveals that ocean acidification is distributed both vertically and horizontally, with a decrease in chemical buffering capacity, particularly in deep water layers.

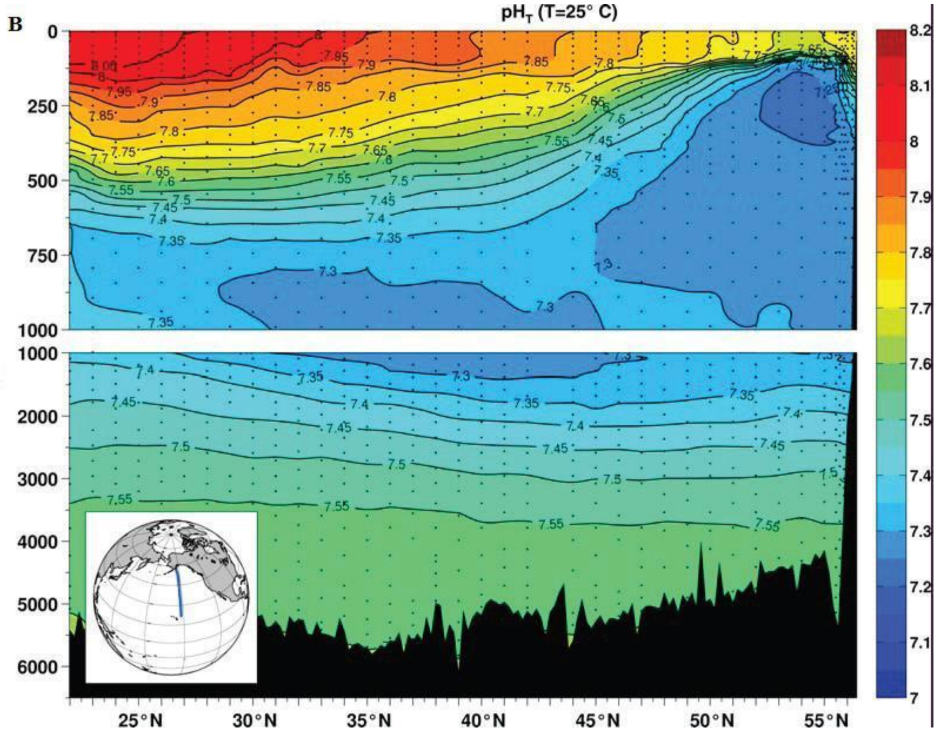

Figure 3.3. Distribution of pH values according to latitude and depth

Studies conducted over the last two decades have revealed that this chain reaction, initiated by the entry of anthropogenic CO_2 into the ocean surface, causes permanent changes not only in the surface layers but also in deep waters (Mongwe et al., 2024; Ford et al., 2025). One of the most important parameters in this process is the "Revelle factor," which determines the oceans' capacity to buffer additional CO_2. An increase in the Revelle factor above 10 indicates that the system can no longer buffer the additional carbon (Alekseeva et al., 2023). Figure 3.4 shows the trends in pCO_2, pH, dissolved inorganic carbon (DIC), and carbonate ion (CO_3^{2-}) concentrations between 1800 and 2200. The graph shows that the partial pressure of atmospheric CO_2 (pCO_2) increased rapidly with the industrial revolution, and a corresponding significant decrease in seawater pH occurred. This decrease in pH leads to an increase in dissolved inorganic carbon and a sharp decrease in the amount of carbonate ions. Specifically, the decrease in pH from 8.1 to 7.6 in the post-2000 period corresponds to a roughly halving of CO_3^{2-} concentration. This trend suggests that increased CO_2 absorption disrupts the carbonate balance in seawater, weakening calcification processes. Therefore, the figure suggests that if anthropogenic carbon emissions continue, the chemical buffering capacity of the oceans will gradually decrease, and acidification will place significant pressure on ecosystems by the end of the 22nd century.

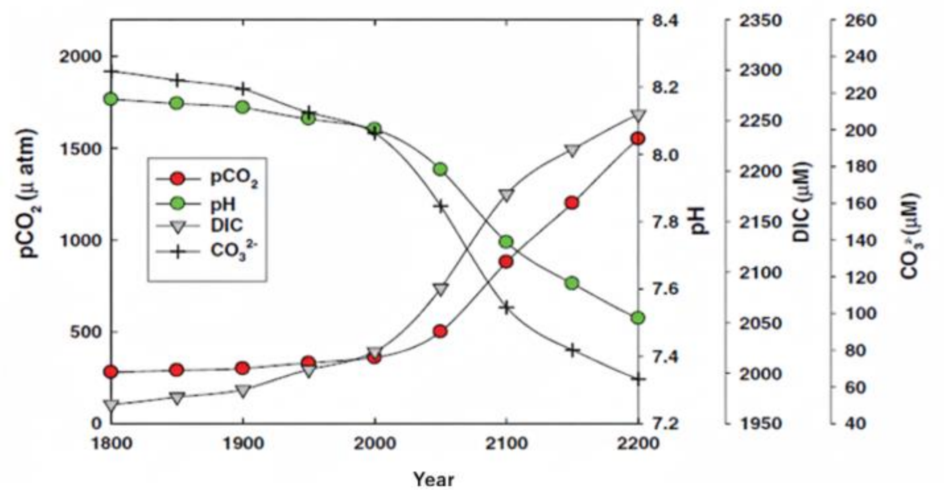

Figure 3.4. Trends in pCO_2, pH, dissolved inorganic carbon (DIC) and carbonate ion (CO_3^{2-}) concentrations between 1800 and 2200

Anthropogenic carbon increase also affects carbonate ion saturation depth. The calcite and aragonite saturation depth ($\Omega = 1$) is increasingly approaching the surface, posing a risk to calcification-based organisms living in shallow marine habitats. For example, the "aragonite saturation limit" at a depth of 1,000 m in the Southern Ocean has moved approximately 200 m upwards in the last 30 years (Zhang et al., 2022). This situation also threatens the long-term balance of the carbon cycle.

Consequently, the disruption of chemical processes has not only biochemical but also economic consequences. The shellfish industry, reef tourism, and fishing activities face serious economic losses due to ocean acidification (Hassoun et al., 2025). Therefore, controlling ocean acidification has become not only an environmental but also a socio-economic imperative. 4. Physiological Responses in Marine Organisms

Ocean acidification is one of the most significant environmental stressors that directly impacts the physiological balance of marine organisms. With increasing acidification, vital processes such as calcification, metabolism, nutrient uptake, and respiration are disrupted (Shi & Li, 2024; Iglesias-Rodríguez et al., 2023). These changes are particularly pronounced in organisms with calcium carbonate ($CaCO_3$) structures.

Calcification is a fundamental biological process for crustaceans (e.g., mussels, oysters, corals). Low pH conditions slow the rate of shell formation by reducing $CaCO_3$ precipitation. Laboratory experiments have shown that a decrease in pH by 0.3 units reduces the calcification rate by 15–40% (Ries et al., 2009; Rhoden, 2025). et al., 2021). Figure 4.1 shows the relationship between the calcification rate and aragonite saturation (Ω_{ara}g) and atmospheric CO_2 levels (pCO_2) in different groups of marine organisms. In panel A, the general trend is for the calcification rate to decrease rapidly as aragonite saturation decreases, suggesting that calcium carbonate precipitation becomes more difficult at low Ω_{ara}g values. In panel B, calcification in mussels (Mytilus edulis) and oysters (Crassostrea gigas) decreases significantly with increasing pCO_2; particularly at CO_2 levels above 1000 ppm, the calcification rate drops below zero, indicating the onset of shell dissolution. In panel C, calciferous plankton (Emiliania huxleyi, Gephyrocapsa oceanica) exhibit a similar trend, with calcification rates decreasing sharply as atmospheric CO_2 concentrations increase. Considering all panels together, it is concluded that increasing CO_2 concentrations suppress calcification processes by reducing aragonite saturation, thus posing a serious physiological stressor for crustaceans and planktonic calcifiers.

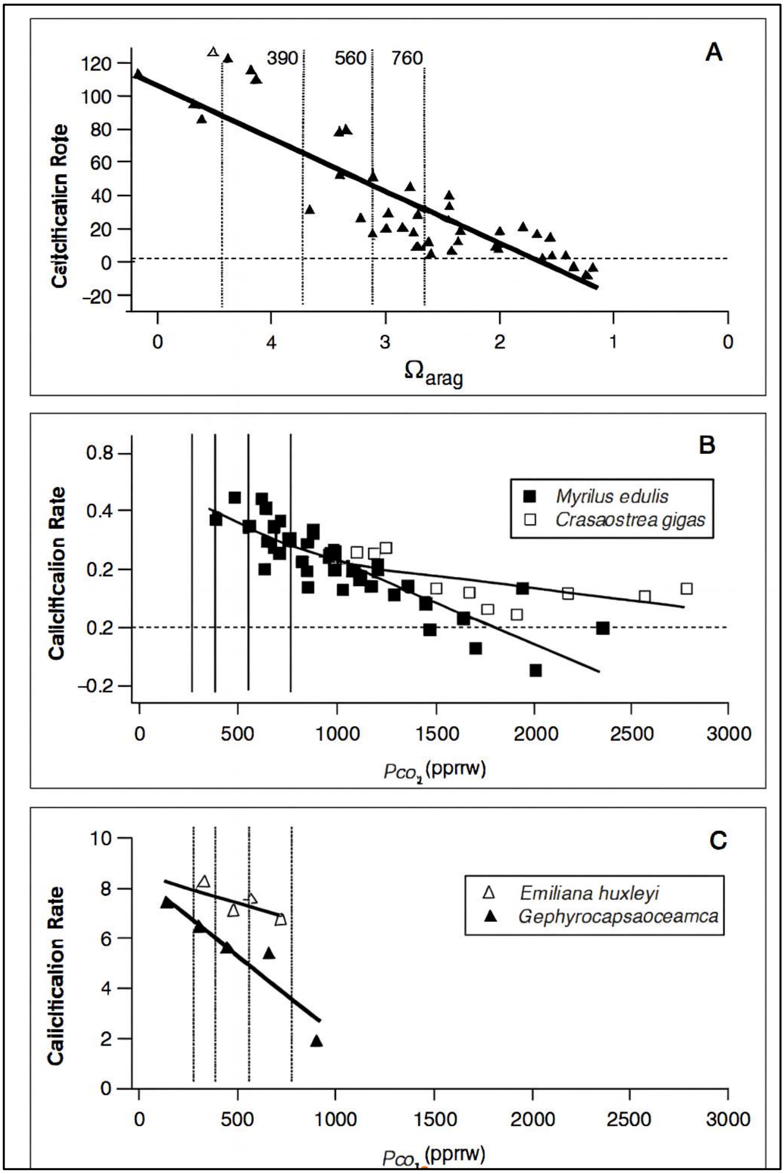

Figure 4.1. Relationship of calcification rate with aragonite saturation (Ω_{ara}g) and atmospheric CO_2 levels (pCO_2) in different groups of marine organisms

Recent genomic and physiological studies have shown that acidification challenges internal pH regulation mechanisms in marine organisms. In species such as mollusks and sea urchins, ion exchange systems in the cell membrane (Na^+/H^+ pump, HCO_3^- transporters) are particularly inadequate against acidification (Iskandar et al., 2024). This results in decreased protein synthesis, decreased growth rate, and decreased reproductive success.

However, adaptive physiological responses have been observed in some species. For example, seagrasses (Posidonia oceanica) can temporarily raise local pH through increased photosynthesis, creating a micro-scale buffering effect for calcifying organisms in the environment (Van Dam et al., 2021). Similarly, some coral species (e.g., Porites lutea) show partial resistance by increasing carbonate ion production through their symbiotic algae (Wang et al., 2021). The effects of ocean acidification vary depending on the organism's life stage. Low pH conditions experienced during the larval stage cause shell deformations and reduced survival rates (Spencer et al., 2020). This threatens the regenerative capacity of populations.

Consequently, ocean acidification impairs not only biochemical processes but also ecological functions in marine organisms. There are significant differences between species' physiological responses to acidification, suggesting that species composition will change in the future.

5. Impacts on the Ecosystem

The ecosystem-level consequences of ocean acidification affect the entire structure of marine food webs and biological productivity. Acidification reduces ecosystem stability by altering species' trophic relationships (Hassoun et al., 2025). Coral reefs, planktonic ecosystems, and crustacean communities are particularly vulnerable to this impact.

5.1 Coral Reefs

Coral reefs are the ecosystems where we can most clearly observe the effects of ocean acidification. Low pH reduces the calcification rate of corals and slows the growth rate of reefs. Between 1990 and 2020, the average calcification rate in coral reefs decreased by 30% (Davis et al., 2021; Hughes et al., 2023).

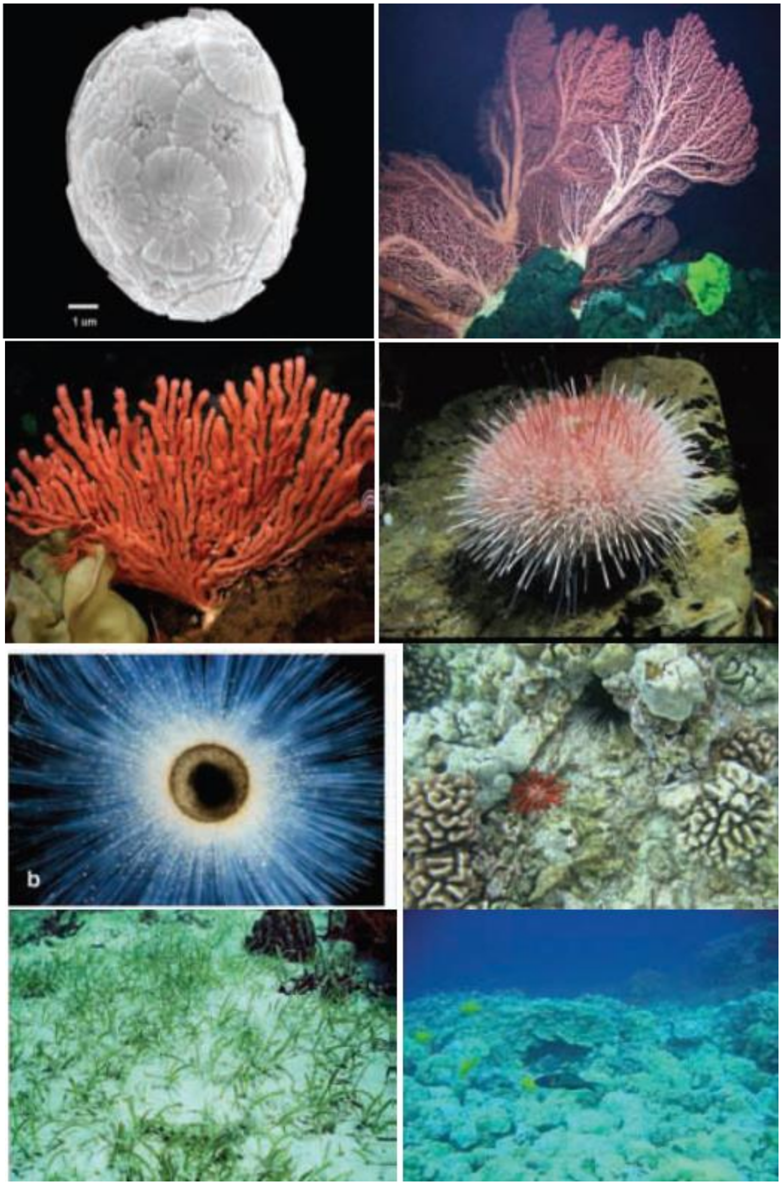

Figure 5.1. Some examples of organisms affected by ocean acidification

Increasing CO_2 levels also affect the photosynthetic capacity of symbiotic algae, increasing the frequency of "bleaching" events. This disrupts the coral-zooxanthellae relationship, reducing reef biodiversity (Helgoe et al., 2024).

5.2 Planktonic Ecosystems

Phytoplankton form the basis of primary production in marine ecosystems. Ocean acidification affects phytoplankton's photosynthetic efficiency and carbon fixation rate (Jin & Gao, 2021). While some species (e.g., diatoms) may respond favorably to high CO_2 concentrations in the short term, long-term declines in species diversity have been observed. At the zooplankton level, pH declines reduce digestive efficiency and impair energy transfer in the food chain (Ratnarajah et al., 2023).

5.3 Ecosystem Services

Ocean acidification affects not only biodiversity but also ecosystem services. Fisheries, aquaculture, coastal protection, and carbon sink services are directly affected by this process (Sura et al., 2025). Coral reef degradation increases coastal erosion, and the decline of crustacean species indirectly reduces commercial fish stocks.

Conversely, it has been noted that some macroalgae (e.g., Ulva spp.) can grow faster under increasing CO_2 conditions, thus providing a partial ecological offset (Van Dam et al., 2021).

Consequently, the effects of acidification are felt at both the biological and socioeconomic levels at the ecosystem level. This process is one of the most critical indicators of the marine impacts of climate change.

6. Adaptability and Sensitivity by Species

Ocean acidification is one of the most significant environmental processes that alters ecological balance by pushing species to their biochemical and physiological limits. Species' responses to these conditions are closely linked to their life strategies, respiratory patterns, ion regulation capacities, and metabolic buffering abilities (Iskandar et al., 2024). While some marine organisms can withstand high CO_2 conditions, others face lethal effects. For example, calcium carbonate-derived microorganisms such as pteropods and foraminifera are experiencing rapid population losses due to shell erosion (Bednaršek et al., 2024). At the same time, the decrease in species diversity in phytoplankton communities disrupts the balance of the food web.

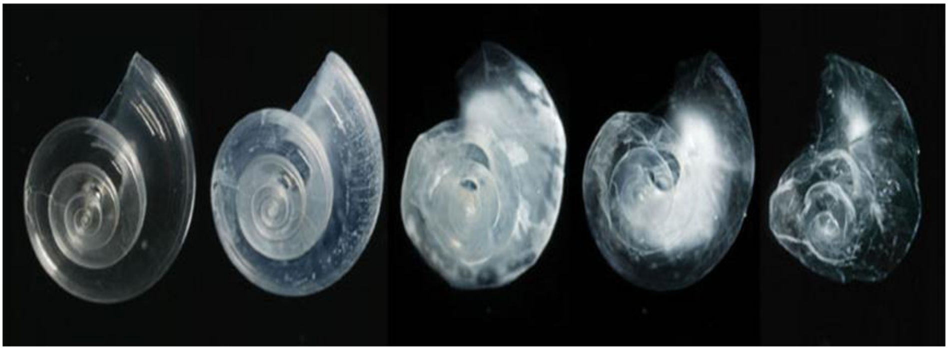

Figure 6.1. Microorganisms sensitive to acidification (pteropod, foraminifera)

Some species have evolved physiological tolerance mechanisms. For example, in fish such as Anarhichas minor (spotted wolffish) and Gadus morhua (Atlantic cod), plasma bicarbonate buffer systems can counteract acidification (Mariu et al., 2023). In contrast, in crustacean invertebrates, ion-balancing mechanisms are weak, so pH reductions suppress protein synthesis (Baag et al., 2023).

Figure 6.2. Physiological buffering mechanisms against acidification (Heliocidaris erythrogramma)

In the larval stage, the effects are more dramatic. Sea urchins, sea stars, and mollusks exhibit shell deformation and developmental delays under low pH conditions (Spencer et al., 2021). Furthermore, the development of the nervous system and sensory cells in fish embryos is negatively affected under low pH conditions (Roberts et al., 2024).

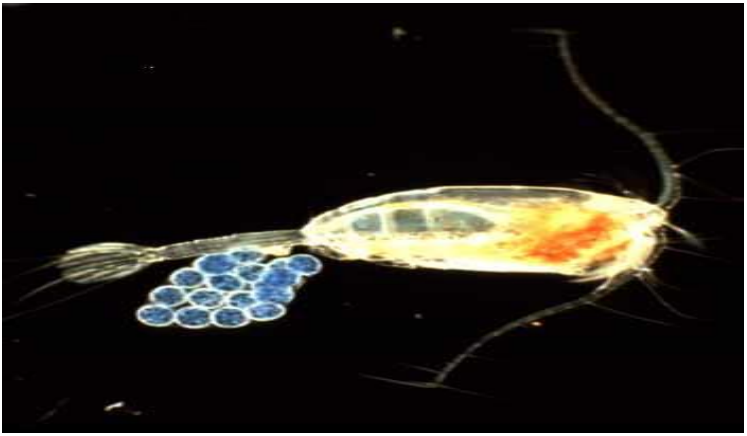

Figure 6.3. Larval development and pH change

As a result, species responses to ocean acidification are quite diverse, and these differences may lead to future reshaping of ecosystem structures. The decline of calcification-based organisms leads to a cascade of biodiversity declines in reef ecosystems (Iglesias-Rodríguez et al., 2023).

7. Conclusion and Evaluation

Ocean acidification is one of the quietest yet most profound effects of climate change. Increasing CO_2 emissions from fossil fuel combustion, industrial activities, and land use changes disrupt the chemical balance of seawater, and the resulting decrease in pH radically impacts marine life. Over the last 250 years, the oceans have absorbed half of the increase in atmospheric CO_2, resulting in a decrease in surface pH of 0.1 units (Amiraux et al., 2022). While this change may seem small, it has knock-on effects on biogeochemical cycles, calcification processes, and food chains. Recent modeling studies indicate that if current CO_2 emissions continue, the average pH could drop to 7.7 by 2100 (IPCC, 2021). This represents an irreversible ecological threshold for many marine species. In this context, three key strategies stand out for mitigating the impacts of ocean acidification:

- Emission reduction: Restricting fossil fuel use and transitioning to renewable energy are essential to controlling the primary source of acidification.
- Local buffering practices: Increasing seagrass meadows and macroalgal habitats can provide a natural buffer thanks to their local pH-raising effects (Van Dam et al., 2021).

- Ecosystem-based management: Conservation policies that increase ecological resilience in fisheries and coastal planning should be developed (Hassoun et al., 2025).
- In conclusion, ocean acidification is a process that threatens not only marine species but also the future of humanity. Unless carbon management policies, scientific monitoring, and ecosystem restoration are addressed holistically, the biological and economic value of the seas will be permanently diminished.

Combating ocean acidification requires emission control and ecosystem-based management approaches, as well as strengthening global monitoring networks, increasing data sharing, and supporting innovative solutions for carbon capture and increasing marine alkalinity. The socioeconomic impacts of this process are also profound; declining fish stocks and the destruction of coral reefs threaten food security, coastal economies, and livelihoods. Therefore, ocean acidification must be integrated into the broader framework of climate and marine management policies, with mitigation and adaptation strategies implemented in concert. Raising public awareness and improving ocean literacy will strengthen behavioral change and policy support. Ultimately, ocean acidification is not only an environmental crisis but also a test of humanity's capacity for cooperation, innovation, and long-term responsibility for the planet's future.

Ocean acidification profoundly impacts not only marine ecosystems but also the ecological connections between land and sea. Increasing acidification disrupts the physiological balance of organisms living in estuaries and coastal deltas and reduces biodiversity in freshwater and seawater mixing zones. Furthermore, acidic conditions lead to the dissolution of heavy metals from sediments into the water column, increasing the risk of toxicity in both marine and fluvial ecosystems. Carbon, nutrients, and pollutants transported through rivers also accelerate the acidification process by impacting the chemical balance of the oceans. Therefore, marine and fluvial systems must be addressed together, prioritizing basin-based management, pollution control, and holistic monitoring of the carbon cycle for sustainable solutions.

Ocean acidification poses serious threats to the life cycles and ecological balances of aquatic organisms. Decreasing pH levels lead to dissolution and growth retardation, particularly in species that form calcium carbonate shells or skeletons (e.g., corals, mussels, oysters, and plankton). This weakens the fundamental links in the food chain, indirectly impacting higher-trophic fish and marine mammals. Larval and juvenile organisms are more sensitive to acidic environments, leading to reduced population recovery rates. Furthermore, acidic conditions affect navigation, hunting, and escape behaviors in some fish species, reducing their chances of survival. Therefore, establishing protected areas to strengthen the adaptive capacity of aquatic organisms, preserving genetic diversity, and expanding ecosystem-based management practices are crucial.

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Chapter 3

Fishing Grounds Footprint- A Review

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Introduction

In recent decades, rapid industrialization, excessive population growth, rapid urbanization, intensive agricultural activities, rapid technological advancement, excessive consumption of natural resources, and dependence on non-renewable energy have increased pressure on the environment. As a result, since the beginning of the 21st century, humans have been on the verge of an irreversible environmental crisis, including loss of biodiversity, global climate change, and destruction of ecosystems. This situation has accelerated researchers' focus on studies related to the global processes of environmental problems. The most important goal of this research is to understand natural processes, as well as to develop sustainability-based plans, overcome ecological challenges, and develop solutions to problems.

Human activities have both direct and indirect effects on the functioning of ecosystems. Increased carbon emissions associated with the use of fossil fuels are one of the most significant causes of climate change (IPCC, 2021). Pollution caused by heavy metals and various chemicals released into the environment as a result of industrial activities threatens the sustainable use of both terrestrial and aquatic ecosystems.

The protection of ecosystems is not only an ecological necessity, but also a critical requirement from an economic and social perspective. Humanity's ability to build a sustainable future is possible through understanding ecological processes and observing environmental boundaries.

This review article aims to provide a brief overview of the Ecological Footprint (EF) and information on the concept of the Fishing Grounds Footprint (FGF) based on relevant literature.

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A General Overview of the Ecological Footprint

The human economy depends on the planet's natural capital, which provides all ecological services and natural resources. Humans have had a significant impact on the world in connection with population growth and economic development. Population growth, resource scarcity, and environmental degradation are natural resources that have a significant relationship between the environment and the economy. The meaning of this ecological principle is clear: To be sustainable, people must live within nature's carrying capacity. Since the early 1970s, successive reports have warned that the unlimited growth of the human population and consumption is unsustainable (Zhao et al., 2005).

The Ecological Footprint (EF) is a quantitative indicator developed to measure the environmental impact of human activities. This concept was first proposed by William Rees (1992) and later systematized in collaboration with his doctoral student Mathis Wackernagel. These researchers defined the ecological footprint as the demand on biologically productive land and sea areas provided by nature to meet human consumption and dispose of waste (Rees, 1992; Wackernagel and Rees, 1996). Wackernagel and Rees systematized this approach in their 1996 book, Our Ecological Footprint: Reducing Human Impact on the Earth (Wackernagel and Rees, 1996).

The EF expresses how the annual resource demand of a given population unit (individual, city, country, world) compares to the biocapacity that ecosystems can provide in that year. Both demand (resource production + waste absorption) and supply (biocapacity) are normalized to the global hectare (gha) unit; thus, different types of land such as agricultural land, pasture, forest, fishing area, and built-up area are converted to a single scale. The carbon component is generally included by calculating the equivalent area that can be absorbed by the atmosphere (based on the carbon absorption capacity of forests), (Kitzes et al., 2007).

Researchers have provided different definitions of the EF. Some of these definitions are given below.

The EF is a measure of the demand that human activities place on the biosphere. More precisely, it measures the amount of biologically productive land and water area required to produce all the resources consumed and absorb all the waste produced by an individual, population, or activity, given current technology and resource management practices (Fig. 1), (Ewing et al., 2010).

The EF represents the pressure of human activities on the environment and can be used to identify terrestrial and marine areas that provide global bioproductivity and to show the proportion of these areas under human use (Adalı et al. 2023). The EF is also an important indicator that reflects sustainable development by making ecological losses in the biosphere visible and allows for the monitoring of the demand for natural resources (Wackernagel and Rees, 1998; McDonald and Patterson, 2004; Solarin and Bello, 2018).

Although EF analyses are conducted on scales ranging from a single product to the entire world, country-level EF assessments are generally considered the most comprehensive evaluations. (Kitzes et al., 2009).

The EF is a calculation-based indicator system whose fundamental premise is the fact that the Earth has a limited amount of biological production to support all life on it (Wackernagel et al., 2018 a,b). A widely accepted measure of sustainability, the EF provides an integrated, multi-scale approach to tracking the use and overuse of natural resources and its impacts on ecosystems (Mancini et al., 2018) and biodiversity (Galli et al., 2014).

The EF has become a widely used tool in sustainability research and policy development. One of its strengths is that it provides a metric that is understandable and comparable, particularly for decision-makers (GFN, 2023). However, some researchers have criticized the method for its limited reflection of energy and technology factors (Rees, 2000).

The total EF of an individual or community consists of subcomponents covering different types of consumption. These are;

1. Carbon footprint (forest area required to absorb emissions from fossil fuel use),
2. Agricultural land footprint (area required for food and fiber production),
3. Rangeland footprint (livestock area for meat and dairy products),
4. Forest footprint (for lumber, wood, and paper products),
5. Fishing Groundssfootprint (for seafood production),
6. Built-up area footprint (for urbanization, infrastructure, roads, etc.) (Monfreda et al., 2004; Wackernagel and Rees, 1996), (Fig. 2 and Fig. 3).

Figure 1. Ecological footprint=the amount of material consumed (https://www.tommiemedia.com/what-is-an-ecological-footprint/)

The sum of these components indicates the ecological demand of an individual or society (Wackernagel and Rees, 1996). The unit used is the global hectare (gha).

Figure 2. Ecological footprint components (https://overshoot.footprintnetwork.org/kids-and-teachers-corner/what-is-an-ecological-footprint/)

Figure 3. Breakdown of the Ecological Footprint components (https://footprint.info.yorku.ca/about-ecological-footprint-and-biocapacity/)

A General Overview of the Fishing Grounds Footprint (FGF)

Throughout human history, fishing has been one of the most important activities in terms of both livelihood and food security (FAO, 2022). Today, fishing plays a vital role in the global fisheries sector, providing a direct livelihood for approximately 59.5 million people worldwide through both capture fisheries and aquaculture (FAO, 2020, 2022). Fishing has not only economic but also cultural and social dimensions; especially for coastal communities, fishing is not merely a source of income but also an integral part of their way of life (Teh and Sumaila, 2013).

Fish is an important source of protein, particularly in regions where alternative food sources are unavailable or economically unfeasible (Edwards et al., 2019). Beyond its health benefits, fishing contributes significantly to income generation and employment opportunities, both directly and through ancillary service activities (Solarin et al., 2024). Furthermore, the fishing industry is recognized as a growth engine in many countries, and more than 12% of the world's population depends on fishing income for their livelihood (WWF Report, 2010). This sector contributes significantly to food security, nutritional well-being, and economic stability by serving as a source of staple foods, animal protein, and employment opportunities (Naylor et al., 2021). Despite the importance of the fishing industry, increasing human activities are seen to have negative impacts on the environmental status of habitats and species (Naddafi et al., 2022). Climate change, in particular, threatens fishing both directly and indirectly (Nilsson et al., 2019).

The Food and Agriculture Organization (FAO), (2020) reports that per capita fish consumption has doubled globally since 1960. Given the expectation that the world population will exceed 9 billion by 2050, it is easy to predict that global fish consumption will continue to increase. Therefore, the need for reformist policy measures and technological developments that will encourage sustainable fisheries production has become more urgent, not only due to increasing consumption but also due to existing challenges such as water pollution and damage to biodiversity resulting from overfishing. The FGF is an appropriate environmental indicator for monitoring such risks and avoiding potential pitfalls (Yilanci et al., 2022). However, driven by population growth, technological advances, and global market demands, fishing activities have expanded at an unprecedented rate since the 20th century. This process has placed serious pressure on both marine and freshwater ecosystems. Overfishing, habitat destruction, bycatch, and ghost fishing have led to depleted stocks, reduced biodiversity, and losses in ecosystem services (Pauly and Zeller, 2016; Sala et al., 2021). According to FAO data, 35.4% of the world's fish stocks are overexploited, and only 7.2% are fished within biologically sustainable limits (FAO, 2022). These developments have highlighted the need for new tools to measure the environmental and socio-economic impacts of fishing activities. At this point, the concept of "Fishing Groundss Footprint" has emerged as an important indicator for measuring the multidimensional pressures of fishing on ecosystems and guiding management strategies (Coll et al., 2008; Kroodsma et al., 2018). The FAO's 1995 Principles of Responsible Fisheries have also facilitated the integration of the footprint approach into the international policy framework. In particular, the emphasis on "ecosystem-based management" has paved the way for the use of the FGF as a management indicator (FAO, 1995).

Research on FGF is quite limited in the literature. Instead, studies exist on the sustainability of related environmental indices such as CO_2 emissions, ecological footprint, and carbon footprint. Between 1961 and 2016, the FGF (in global hectares) increased by more than 80% (Global Footprint Network, 2019).

The FGF has been defined in various ways by many researchers. These definitions are provided below.

The FGF is an important indicator of the state of the environmental ecosystem and is a concept that defines the water area required to meet the demand for aquatic products. (Yilanci et al., 2022).

It is defined as the equivalent surface area that supports fishing methods that protect aquatic ecosystems in order to meet countries' fishing demands (Lin et al., 2019).

The FGF is an ecology-focused concept used to calculate the area required in freshwater, seas, and oceans to meet the demand for aquatic products (Solarin et al., 2021; Yilanci et al., 2022).

The FGF is an indicator of the conservation of freshwater and saltwater fisheries resources and the creation of a sustainable aquatic environment for future generations. Examining the characteristics of the FGF provides insights and important findings that indicate whether environmental policies related to fisheries and marine ecosystems will be effective (Adalı et al., 2023).

The FGF is expressed in global hectares per thousand people, while total fishing production and its components are expressed in tons (Solarin et al., 2024).

The FGF can generally be defined in terms of four main components. These are:

1. **Spatial Footprint:** This refers to the geographical extent of the areas where fishing fleets operate. Globally, the area of operation of industrial fishing fleets has reached approximately 55 million km^2, covering 55% of the world's oceans (Kroodsma et al., 2018).
2. **Ecological Footprint:** It encompasses the effects of hunting pressure on stocks, changes in species trophic levels, and biodiversity losses. Pauly et

al. (2002) explained this situation with the concept of "fishing down the food web" and showed that there is a shift in hunting from large predator species to smaller, lower trophic level species.

3. **Carbon Footprint:** Includes greenhouse gas emissions caused by fuel and energy consumption in fishing activities. For example, shrimp trawling is one of the methods with the highest carbon footprint per unit of fish due to its high energy consumption (Parker et al., 2018).
4. **Social Footprint:** This encompasses the economic and social impacts of fishing on local communities. In communities dependent on small-scale fishing in particular, declining fish stocks directly threaten food security and livelihoods (Teh and Sumaila, 2013).

These four dimensions move the FGF beyond being merely an environmental indicator, integrating it with perspectives of sustainable

Research shows that the FGF area has expanded and intensified over time.

- Demersal species (caught with bottom trawls) are associated with high habitat destruction and energy intensity.
- Pelagic species (e.g., sardines, anchovies) are characterized by a lower ecological and carbon footprint.
- Shellfish (mussels, oysters) have a low EF because they are naturally filter feeders, but aquaculture areas can exert limited pressure on water quality and local ecosystems (Coll et al., 2008).

Research shows that the FGF has expanded and intensified over time. Technological advances following World War II (cooling systems, satellite navigation, more powerful vessels) have enabled fishing fleets to venture into distant waters and target deeper and new habitats (Watson et al., 2017).

- Early 20th century: Fishing was mostly limited to coastal ecosystems.
- 1950-1980 period: Industrial fishing became widespread, extending to larger areas of the oceans.
- 2000s and beyond: Increased demand due to the impact of global trade and the rise of IUU (Illegal, Unreported, Unregulated) fishing made human impact visible in most oceans (Kroodsma et al., 2018).

During this process, significant differences have emerged between small-scale and industrial fishing. Small-scale fishing is generally associated with a low carbon footprint and contributions to local food security, while industrial fishing is characterized by high energy consumption, habitat destruction, and fishing for global markets (Jacquet and Pauly, 2008).

Examples of Research Conducted on Fishing Grounds Footprints

Research shows that a large proportion of fishing activities in the world's oceans occur in specific areas of high intensity. Kroodsma et al. (2018) used satellite data in a global analysis to reveal that approximately 55% of marine areas are subject to fishing pressure. The coasts of Europe, North America, and East Asia, in particular, are the centers of intensive fishing activities.

Yilanci et al. (2019b) examined EF and its six components in 25 OECD countries between 1961 and 2013, revealing the effects of changes on EF.

Jennings and Kaiser (1998) noted that fishing activities cause habitat loss, biomass decline, and disruptions in species interactions in marine ecosystems.

Teh and Sumaila (2013) emphasized that more than 200 million people globally depend on fishing for their livelihoods, and therefore the FGF is critical not only in terms of ecological but also social impacts.

Parker et al. (2018) reported that annual greenhouse gas emissions from global fisheries reach approximately 200 million tons of CO_2 equivalent. Industrial bottom trawling and longline fishing, in particular, exhibit high carbon intensity per unit of product. Life Cycle Assessment (LCA) analyses show that the carbon footprint of small-scale fisheries is 40–60% lower than that of large industrial fleets (Thrane, 2006; Sala et al., 2021).

Amoroso et al. (2018) noted that bottom trawling activities have created habitat impacts on more than 20% of the world's continental shelves. This shows that the FGF seriously affects not only fishing pressure but also ecosystem health.

Sala et al. (2021) showed that the release of carbon stocks from the seabed due to trawling activities could accelerate climate change.

Amin et al. (2022) strengthened the socioeconomic context by revealing significant relationships between FGF and economic freedom indices.

Studies conducted specifically in the Mediterranean have shown that trawler fleets have a high carbon footprint per unit of product (FAO, 2023).

The analysis of the FGF areas has begun to attract the attention of researchers in recent years due to the pressure of human activity on the degradation of the marine environment (Aminizadeh et al., 2024).

The Importance of the Fishing Grounds Footprint

The FGF is a critical indicator not only for the protection of ecosystems, but also for economic sustainability and social justice. This concept:

- Enables policymakers to monitor fishing pressure,
- Provides scientists with an assessment tool for ecosystem-based fisheries management,

- Opens up discussions for communities on food security and the sustainability of livelihoods.

SDG 14: Life Below Water, one of the United Nations' 2030 Sustainable Development Goals, is directly related to the concept of the fishing footprint. This goal aims to promote the sustainable use of marine resources and prevent overfishing (UNEP, 2020).

The importance of the FGF can be explained under five main headings. These are:

1. Ecosystem-Based Management

The FGF contributes to ecosystem-based fisheries management by measuring the pressure that a fishing activity exerts on the ecosystem. This makes it possible to evaluate not only catch quantities, but also habitat destruction and impacts on biological diversity (Pauly and Christensen, 1995; Jennings and Kaiser, 1998).

2. Sustainability and Resource Use

The FGF is an important indicator for determining sustainable levels of resource use to prevent overfishing of target species. This approach is also consistent with the FAO's Principles of Responsible Fisheries (FAO, 1995).

3. Carbon and Energy Dimension

In recent years, the literature has shown that fishing has been examined not only from a biological perspective but also in terms of its carbon footprint. The fishing industry's footprint is also significant in terms of fuel consumption, emissions, and its contribution to climate change (Parker et al., 2018).

4. Socioeconomic Importance

The FGF is directly related to the sustainability of coastal communities' livelihoods, food security, and economic benefits. Therefore, it is used not only in ecological but also in socioeconomic decision-making mechanisms (Teh and Sumaila, 2013).

5. International Policies and Global Monitoring

The global FGF is directly linked to the United Nations Sustainable Development Goals (particularly SDG 14: Life Below Water). As such, it is increasingly being incorporated into international reporting and policy development processes (UNEP, 2020).

Conclusion

The Ecological Footprint, which represents the pressure of human activities on the environment, is used to identify terrestrial and marine areas that provide biological productivity worldwide and to show the proportion of these areas under human use (Wackernagel and Rees, 1998; McDonald and Patterson, 2004; Solarin and Bello, 2018).

The impacts of the Fishing Grounds Footprint can be summarized as follows:

- **Bottom Trawling and Habitat Destruction:** Bottom trawling activities leave traces in more than 20% of global continental shelves and accelerate climate change by exposing seabed carbon stores (Amoroso et al., 2018; Sala et al., 2021).
- **Carbon and Energy Dimension:** Total greenhouse gas emissions from global fisheries amount to approximately 200 million tons of CO_2 equivalent, with industrial fleets in particular having a high carbon footprint (Parker et al., 2018).
- **Regional Example – Mediterranean Sea:** In the Mediterranean Sea, small pelagic stocks have been found to be declining under fishing pressure, while bottom trawling activities have led to habitat loss (FAO, 2023; Ramirez et al., 2021).
- **Socioeconomic Impacts:** The FGF is linked not only to ecosystems but also to the livelihoods of coastal communities. Globally, more than 200 million people derive their income directly or indirectly from fishing (Teh and Sumaila, 2013).

The FGF is a powerful indicator that comprehensively addresses not only biological stocks but also ecosystem health, carbon emissions, and socioeconomic factors. Research shows that prioritizing fishing methods with a low footprint, ecosystem-based management, and reducing carbon emissions can ensure both ecological and socioeconomic sustainability (Sala et al., 2021; Parker et al., 2018; FAO, 2023).

FGF findings provide guidance for sustainable marine management and policy development processes. Some of the key recommendations highlighted in the literature are as follows:

Ecosystem-Based Management (EAFM): Management strategies that consider ecosystem health rather than stock quantity should be implemented (Steenbeek et al., 2018).

Promoting Low-Impact Fishing Methods: Small-scale pelagic fishing and filter-feeding shellfish harvesting, which have low ecological and carbon footprints, should be supported (Sala et al., 2021).

Monitoring Carbon Emissions: LCA and carbon footprint analyses should be used as a standard indicator in fisheries management (Parker et al., 2018).

Marine Protected Areas (MPAs) and No-Take Zones: Protected areas should be designated for ecosystems subject to intense fishing pressure, and their effectiveness should be monitored using tracking systems (AIS/VMS) (Kroodsma et al., 2018).

Support: The income and employment sustainability of small-scale fishers should be ensured, and policy integration should be implemented for local food security (Teh and Sumaila, 2013).

A study conducted by Clark and Longo (2019) revealed that FGF has increased over the past decade, supporting the possibility of food shortages. Therefore, it is crucial to adopt internationally accepted management decisions regarding fishing and aquaculture production to improve marine ecosystems and protect marine biodiversity.

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Chapter 4

Negative Effects of Climate Change On Pollen Germination in Fruit Trees

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In recent years, the biggest negative factor in agriculture and food security is climate change. The main issue caused by climate change is droughts, which result from irregular rainfall and water resource depletion. Intergovernmental Panel on Climate Change (IPCC) reports indicate that Mediterranean countries, including Turkey, will be among the most affected by climate change. The report states that as global temperatures rise, predicted to reach 1.5°C by 2100, an increase to 2.13 times the expected 3.2°C will lead to more natural disasters, decreased plant diversity, and a food crisis (Lee et al., 2023). Recent frequent disasters like floods, forest fires, and droughts are primarily caused by climate change in Turkey. The agriculture and food sectors will be the most impacted by climate change. Along with global warming, temperature fluctuations, pollution of clean water sources, erosion, high greenhouse gas emissions, and drought are key factors that harm agriculture. It has become essential to focus research on the sustainability of agriculture amid the adverse effects caused by climate change (Venkateswarlu and Shanker, 2012). Like other agricultural products, climate change and drought are expected to be the major factors limiting fruit cultivation. Climate change can increase winter air temperatures, and it has been reported that these projected temperature changes could impact regions that produce various fruits, grapevines, and nuts (Şahin et al., 2015).

Pollination is an ongoing process in fruit production that includes fertilization and fruit growth. The first necessary components for successful pollination and fertilization are the amount of pollen on the pistilthe ovarium's viability during this process, as well as its capacity to germinate and create a pollen tube (Tosun and Koyuncu, 2007; Güçlü et al., 2015). Optimal pollen germination levels can vary depending on the species and cultivars, environmental nutrients, temperature, pressure, pH, and ecology (Eti, 1991; Voyiatzis and Paraskevopoulou-Paroussi, 2002; Koyuncu, 2006). The healthy development, viability, and high germination capacity of pollen—the male sex cells of plants—

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are essential for successful fertilization (Engin and Ünal, 2002; Özcan, 2020). In addition to these qualities, which are considered pollen quality criteria, a high quantity of flower pollen is also desirable (Sütyemez and Eti, 1999). Pollination and fertilization are key factors influencing fruit set. Therefore, understanding the properties of pollen and other characteristics of species and cultivars is important for growers and breeders. Pollen properties (such as quantity and germination rate) significantly affect fertilization success and high fruit set in fruit species. Heat waves are predicted to become more common in a number of areas as a result of global warming, which poses a serious risk to agricultural security. Temperature rises, whether short-term or long-term, reduce crop production. These circumstances change the morphology, physiology, and biochemistry of plants, which has an adverse effect on their growth (Begcy and Dresselhaus, 2018).

It has been reported that the adverse effects of high temperatures on reproductive organs are greater than those on vegetative organs. Besides the harmful effects of high temperatures on bee activity—which is crucial for pollination and fertilization—they also adversely affect fruit growth by reducing pollen viability, morphological uniformity, pollen performances. Pollen and pollen tube growth serve as reliable indicators for how plant species, different cultivars within the same species, and even various genotypes respond to stress (Çetinbaş-Genç et al., 2019). Gametophyte development, the progamic phase, and embryo and seed development are the three phases of angiosperm reproduction. The male gametophyte (pollen), which produces male sperm cells and transfers them to the female gametophyte for double fertilization, is essential to plant reproduction and crop output (Carrizo Garcia et al., 2017). Because male organs are more susceptible to heat stress than female organs, temperature stress has distinct effects on male and female gametophytes (Zinn et al., 2010; Hedhly, 2011). Changes in temperature influence pollen quantity, shape, cell wall structure, and pollen metabolism (Hedhly, 2011). A thick callose membrane holds the four haploid microspores that are produced by meiosis in diploid pollen mother cells (microsporocytes) in anthers together in a tetrad. ndividual microspores are subsequently released when the callase enzyme, which is produced by the tapetum, breaks down the callose wall. These microspores grow, discharge their nuclei into the environment, and form vacuoles. A tiny generative cell and a big vegetative cell are produced as a result of the polarized microspores' following substantial asymmetric mitotic division. Consequently, the microspore generates cells with two distinct potentialities and serves as the male germline's pluripotent source (Berger and Twell, 2011). The two sperm cells required for double fertilization are created by another cycle of mitotic division of the germ cell. Whether the second mitotic division takes place during pollen germination or prior to pollen maturation determines whether mature pollen originates from the anthers in a two-celled or three-celled form. Successful and coordinated pollination and fertilization require the simultaneous development of microspores within an anther. Multiple checkpoints regulate this process, and when it malfunctions (for example, as a result of stress), developmental asynchrony causes physiological and metabolic variations in microspores (Giorno et al., 2013). Then, during development, competition for water increases for stigma rehydration and development of pollen tubes. Microspore formation at the onset of meiosis is the most susceptible to environmental stress in the majority of plants (De Storme and Geelen, 2014; Muller and Rieu, 2016; Rieu et al., 2017; Begcy et al., 2019). Climate change is expected to have a major influence on pollen formation and pollen tube enlargement, the most influenced by temperature stages of plant growth, which would lower agricultural crop (Scaven and Rafferty 2013; Bisbis et al., 2018; Rutley et al., 2021). Pollen metabolism, cell wall structure, and pollen quantity and quality are all impacted by abrupt temperature changes (Hedhly et al., 2005). Because it lowers pollen germination, reduces pollen viability, causes pollen to stay in anthers, and causes mononuclear microspore abortion, this has been connected to aberrant tapetum formation (Harsant et al., 2013). Temperature rises have been observed to cause faster pollen tube growth and quicker ovule degeneration (Hedhly, et al., 2005). Furthermore, heat stress causes premature deterioration of the tapetum, a layer of nutrient-rich cells that nourish developing pollen. The tapetum contains more mitochondria than vegetative tissues (Lee et al., 2023; Selinski and Scheibe, 2014). As a consequence of aerobic metabolism, ROS generation rises dramatically at high temperatures due to the large mitochondrial concentration (Mittler, 2017). ROS induce oxidative damage and necrosis when they build up in significant quantities as a result of stress (Sharma et al., 2012). Angiosperm sexual reproduction depends on pollen germination and tube development, two critical phases for fruit set and crop output. Pollen release and germination are necessary for successful fertilization after seed and fruitset (Mondal and Ghanta, 2012). In some cases, failure of plants to flower and pollen to germinate can greatly impact seed production and reduce crop yields. Pollen growth, viability, germination, and overall quality are all impacted by a variety of factors, including fertilization capacity and pollen tube development. These elements include high or extremely low temperatures, air pollution, chemical fertilizers, and pesticides (Padilla et al. 2017; Pers-Kamczyc et al. 2020). Environmental stress can also modify nutrient availability and phytohormone levels, which may impact plant reproduction (Cho et al. 2017; Pacini and Dolferus 2019; Dong et al. 2021). It has recently been observed that severe temperature swings are occurring in numerous areas (King and Harrington 2018; Kew et al. 2019; Perkins-Kirkpatrick and Lewis 2020). An increase or decrease of just a few degrees above or below the optimal growth limit can cause significant crop yield losses (Peng et al. 2004). Temperature changes can harm the pollination period, affecting the final fruit set and crop yield (Sanzol and Herrero, 2001). Additionally, research indicates that the environment during anther development influences bioactive pollen metabolites. Unfavorable ecological conditions during pollen development lead to notable changes in pollen performance and quality, potentially causing pollen sterility and resulting crop losses (Powell et al., 2012). All phases of pollen formation are impacted by abiotic stress, which commonly results in morphological, structural, and metabolic abnormalities that can cause male sterility, premature spore abortion, and dysfunctional pollen (Prasad and Djanaguiraman, 2011; Firon et al., 2012; Carrizo-Garcia et al., 2017). It can also prevent pollen from being released from the anthers and cause clustering on the stigma (Jagadish et al., 2010; Parish et al., 2013). Due to the tapetum's extreme susceptibility, any interference with its growth results in nonviable pollen, which drastically lowers dicotyledon grain yield. For example, early microspore stage temperature stress results in premature tapetum deterioration (Ku et al., 2003). Climate change could restrict the cultivation of certain crops in various regions. The effects of high temperatures on fruit tree pollen germination were investigated in a number of research. In a research on pollen from many Rosecea species, Beltrán et al. (2019) assessed pollen germination rates, average pollen tube length, and maximum pollen tube length at different temperatures. They found that pollen germination was highest between 15°C and 30°C across all species, with 30–52% germination occurring between 15°C and 20°C for quince, apple, cherry, plum, peach, pear. Germination declined in all species once the temperature reached 30°C. The ideal temperature for blackberry pollen germination is 20°C, according to a study on the effects of various temperatures and incubation periods on the process. High temperatures have a detrimental effect on pollen germination and tube development (Güçlü et al., 2021). According to a study on almond species, pollen germination and tube development are adversely affected by both high and low temperatures (Sorkheh et al., 2018). A study on mango found that the cardinal temperature ranges for pollen germination (Tmin, Topt, and Tmax) were 20.3-22.8 °C, 26.7-30.6 °C, and 30.4-34.3 °C, respectively. Similarly, the cardinal temperatures for pollen tube growth (Tmin, Topt, and Tmax) were 20.3-21.2 °C, 27.9-32.1 °C, and 30.2-34.4 °C, respectively (Liu et al., 2023). In a Korean research on apple varieties, the effects of temperature on pollen tube length and pollen germination percentage were investigated in a laboratory setting. Using a pollen germination medium, these parameters were assessed at intervals of 5 °C between 5 and 45 °C. Variations in temperature had a major impact on tube development and pollen germination in all cultivars. With an average of 85.2%, The highest percentage of pollen germination ranged from 99.9% ("Shinano Gold") to 61.5% ("Green Ball"). Averaging 855.1 µm, the maximum pollen tube length ranged from 716.5 µm ("Tsugaru") to 989.8 µm ("Arisoo") (Zebro et al., 2023). Plant reproductive organs are more sensitive to extreme temperatures than vegetative organs (Guo et al., 2019; Radović et al. 2019). According to earlier research on olive trees, excessive temperatures during flower development can drastically diminish fruit set (Benlloch-González et al. 2018). Reduced fruit set at severe temperatures is a result of reduced pollen viability and germinability in a number of crop species (Tolessa and Heuvelink 2018; Yang et al. 2019; Shenoda et al. 2021). Güclü et al. (2018) tested four temperature regimes in studies to determine pollen delivery in naturally growing blackberries. They found that 20 °C was the ideal temperature for both pollen germination and tube growth, and that as the temperature rose, the rate of pollen germination and tube length fell. Pollen germination investigations on the strawberry varietiesToyonoka' and Nyoho' revealed that both kinds' germination rates dramatically dropped at 30°C (Ladesma and .Sugiyama, 2005). The best temperatures for germination and tube development were found to be 20 and 25°C in a An investigation of the elongation of pollen tubes and pollen germination in sweet cherries at different temperatures (Koyuncu and Güçlü, 2009).

Since fruit farming is a perennial agricultural activity, it is greatly affected by climate change. Global warming could raise temperatures during winter, and these changes are expected to harm regions that grow various fruit species, grapevines, and nuts (Şahin et al., 2015). Besides the negative effects of high temperatures on bee activity—which is crucial for pollination and fertilization—they also harm fruit production by reducing pollen viability, affecting morphological consistency, and decreasing pollen germination and pollen tube length. Studies have shown that high temperatures negatively influence healthy pollen development, pollen germination, and fruit set, which are the initial stages of fruit formation. Additionally, it has been noted that high temperatures have a greater detrimental impact on tube development and pollen germination rates than low temperatures (Kakani et al., 2002). Temperatures in Turkey are increasing each year. Heat waves, caused by high temperatures and humidity during summer, have become more frequent, longer, and more intense. Rising nighttime temperatures will worsen urban heat islands, and the resulting thermal stress will pose existential threats. Rainfall has steadily decreased since the 1970s. Climate change and conditions across Turkey will create regional disparities. These changes will affect water sources, natural vegetation, agricultural potential, and human health and well-being. The risk of forest fires will rise. Insect populations will grow, negatively impacting the food chain and other organisms. Diseases and general health issues will increase, and some species will face extinction. Climate change and drought are ongoing and will continue to be serious issues in Turkey. The recent extreme temperatures and water shortage must be addressed urgently, and our water resources must be managed efficiently. Significant changes should be made in how we consume power, water, and other natural resources. Infrastructure improvements are needed to reduce losses in water and electricity distribution. Renewable energy sources (wind, solar, geothermal, etc.) should replace fossil fuels. Efforts to increase afforestation should be expanded, and measures to prevent forest fires should be put in place. Agricultural policies need reevaluation. Proper crop patterns should be established based on current developments. Illegal water use should be stopped, and groundwater consumption should be monitored. Major water projects such as dams and reservoirs should be planned. An integrated and sustainable approach to water management should be adopted. Stress management in fruit farming should be intensified, and breeding of varieties and rootstocks suited for extreme conditions should be promoted.

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Chapter 5

Aquarium Fish Production in the İskenderun Region: Current Status, Challenges, and Policy Recommendations

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1. INTRODUCTION

Aquarium fish are not only add aesthetic value but also play a significant role in enhancing psychological well-being and conributing to the ecological balance of aquatic environments. Scientific studies have shown that these species have beneficial effects in reducing stress, promoting mental relaxation, and producing therapeutic outcomes (Kazez and Nurqanatkyzy, 2025; Maia et al., 2025). Additionally, aquarium environments foster ecological awareness among individuals by encouraging water quality monitoring and the management of biological cycles. Today, aquariums have expanded from small glass tanks used in homes to large-scale public aquarium facilities (Göreci and Demirarslan, 2021). Aquarium fish attract human attention with their vibrant colors, diverse body forms, unique patterns, and behavioral characteristics, making the aquarium hobby one of the most popular recreational activities worldwide (Sharma, 2020; Shraborni et al., 2024). Today, aquarium keeping represents a global industry encompassing 125 countries, with an estimated annual trade volume of 15–30 billion USD (Evers et al., 2019; Biondo and Burki, 2020; Biondo et al., 2024). The sector contributes to national economies by generating foreign exchange earnings and providing employment opportunities for thousands of people (Anjur et al., 2021; Wijaya and Huda, 2021). It also offers social and ecological benefits, including rural development, job creation, enhanced environmental awareness, improved habits, and the conservation of biodiversity (Ziemann, 2001; Bruckner, 2005; Copp et al., 2005; Miller and Mitchell, 2009; Pailan et al., 2022). While the aquarium hobby in Türkiye dates back to the 1960s, the first commercial ornamental fish farming facility was established in the 1980s (Tolon and Emiroğlu, 2014). Import activities began in 1989, leading to an increase in traded

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species diversity and the emergence of market price competition (Tolon and Emiroğlu, 2014). According to the International Trade Centre (ITC, 2020), Türkiye ranks 42nd in the import of freshwater ornamental fish and 37th in marine ornamental species. However, the rapid growth of the sector has also brought various sustainability challenges. In particular, approximately 90–95% of marine ornamental fish are still collected from the wild due to their complex reproductive cycles (King, 2019), raising concerns about overexploitation (Raghavan et al., 2013; Evers et al., 2019). The aquarium fish trade also involves significant animal welfare concerns. Environmental stressors and physical risks encountered during collection, transport, wholesale and retail holding, and transfer to final aquarium environments are of critical importance in terms of welfare (Jones et al., 2021; Oralbek and Nurqanatkyzy, 2025).

Among these stages, transportation is considered one of the most stressful processes, primarily due to water quality deterioration, high stocking densities, oxygen depletion, and physical injuries (Millington et al., 2023). Post-transport recovery practices aimed at improving fish welfare have yet to been suficiently researched (Petty and Francis-Floyd, 2004).

In recent years, the sale of aquarium fish and other aquatic organisms through online platforms has become increasingly common (Walster et al., 2015). This trend has increased welfare concerns during transport and increased the risk of invasive species introductions. In Türkiye, the ornamental fish sector continues to expand both at the individual hobbyist level and on a commercial scale, with a growing number of small and medium-sized enterprises. Most of these businesses operate as family-run enterprises, contributing to local employment and rural development. This study is based on a survey conducted among aquarium fish producers operating in the İskenderun region of southern Türkiye. The primary objective of the research is to analyze the current status of the aquarium fish sector in the region, identify key challenges faced by producers, and develop practical policy recommendations to address these issues. Furthermore, the findings aim to contribute to national policy development processes concerning the ornamental fish industry in Türkiye.

2. MATERIALS AND METHODS

The data for this study were collected through structured surveys administered to five different aquarium fish enterprises operating in the İskenderun region. The survey was designed to designed to provide a comprehensive overview of the sector by including various thematic sections such as the type of enterprise activity, production capacity, workforce profile, sales channels, and major operational challenges. Additionally, specific questions were included to evaluate the impacts of the COVID-19 pandemic and the 6 February 2023 earthquakes on the aquarium fish sector in the region. The survey was conducted through direct interviews with enterprise owners and managers, ensuring the collection of reliable and context-specific information. Each questionnaire consisted of both closed-ended and open-ended questions. The closed-ended items allowed for quantitative assessment of production capacity, workforce, and market orientation, while the open-ended sections provided qualitative insights into participants' perceptions, experiences, and suggestions. The collected data were subsequently coded and prepared for analysis in order to facilitate a detailed examination of both structural characteristics and operational dynamics within the sector.

3. DATA ANALYSIS

The collected data were analyzed using both qualitative and quantitative analytical approaches to ensure a comprehensive interpretation of the findings. Within the qualitative analysis, open-ended responses provided by participants were systematically categorized through thematic classification, allowing for the identification of recurring concepts, challenges, and perceptions among producers. For the quantitative analysis, descriptive statistical methods such as frequency and percentage distributions were employed to summarize and compare key variables, including production capacity, workforce size, and marketing strategies. By combining these two analytical perspectives, the study aimed to present a multidimensional assessment of the operational structure, constraints, and developmental potential of aquarium fish enterprises in the İskenderun region.

4. RESULTS

4.1. Workforce and Production Profile

Most of the businesses examined in this study were identified as small-scale operations, typically run by one or two individuals. Annual production capacities range from 1,000 to 50,000 fish. The most commonly farmed species include guppies (*Poecilia reticulata*), goldfish (*Carassius auratus*), mollies (*Poecilia sphenops*), various tetra species, and other livebearers. The analysis results revealed strong and positive correlations among several operational variables, such as production capacity, number of employees, species diversity, and sales volume. This indicates that as enterprise scale increases, levels of institutionalization and digitalization also tend to improve (Table 1).

Table 1. Correlations among operational variables

Variables	Correlation (r)	Explanation
Production Capacity ↔ Sales	0.99	Very strong positive correlation
Number of Species ↔ Number of Employees	0.98	Greater species diversity is associated with more personnel
Online Sales ↔ Species Diversity	0.90	Enterprises selling online tend to produce a wider variety of species
Production Capacity ↔ Number of Employees	0.97	Employment increases as production capacity grows

These findings demonstrate that the expansion of enterprise size contributes not only to production volume but also to employment generation and diversification of cultured species.

4.2. SALES CHANNELS

Most enterprises conducted their sales primarily through physical stores. However, in recent years, there has been a noticeable shift toward online sales channels. The geographical distribution of sales activities showed that operations were mainly concentrated at local and regional levels. This suggests that the sector has not yet achieved a large-scale national market penetration. However, the increasing trend of digitalization points to the potential for broader customer reach in the near future, particularly through e-commerce platforms and social media-based marketing.

4.3. CHALLENGES FACED BY ENTERPRISES

The distribution of challenges reported by enterprises is presented in Figure 1. The findings indicate that the most frequently cited challenges were price volatility (27.3%), lack of government support (27.3%), and local competition (27.3%). These three factors emerged as the most prevalent issues across the sector. In contrast, legal proceedings (9.1%) and seasonal demand fluctuations (9.1%) were cited less frequently, indicating relatively limited impact compared to economic and structural factors. In summary, the most critical challenges identified by sector stakeholders were price instability, inadequate public support mechanisms, and intense local competition. In contrast, legal regulations and seasonal demand fluctuations appear to be of secondary importance. These results suggest that policymakers and support organizations should prioritize price stability, the development of government assistance programs, and the improvement of competitive market conditions for sustainable sector growth.

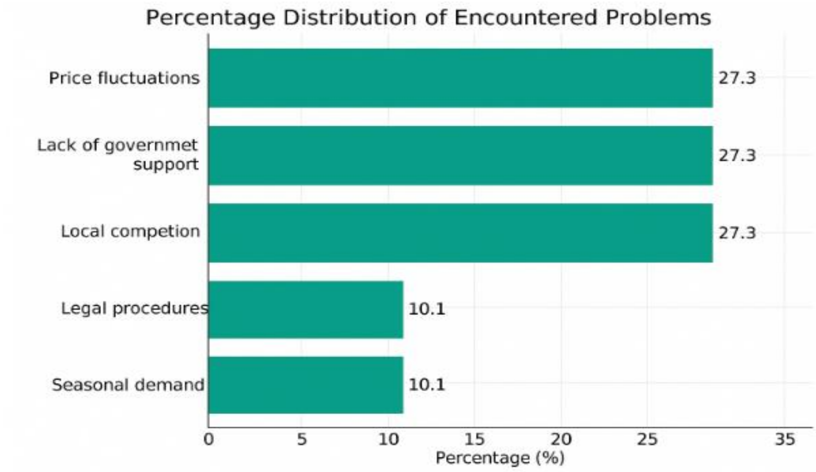

Figure 1. Distribution of key challenges faced by aquarium fish producers

4.4. IMPACTS OF THE COVID-19 PANDEMIC

The impacts of the COVID-19 pandemic on aquarium fish enterprises in the İskenderun region are illustrated in Figure 2. The most frequently reported problems during the pandemic were electricity and rent expenses (21.4%), indicating that businesses faced significant challenges covering fixed operating costs. Furthermore, 14.3% of respondents reported a shift toward online sales and a reduction in the number of employees. These findings highlight the need for businesses to accelerate digital transformation while simultaneously reducing labor costs during the pandemic. Other reported impacts, such as production disruptions, declines in domestic sales, export disruptions, production shutdowns, raw material shortages, lack of government support, and reduced export volume, accounted for 7.1% of the total. While less frequent, these findings illustrate the multifaceted nature of the pandemic's impact. Overall, the COVID-19 pandemic impacted businesses on both financial and operational levels, primarily through increased fixed costs, reduced workforce, and disruptions to production and marketing activities.

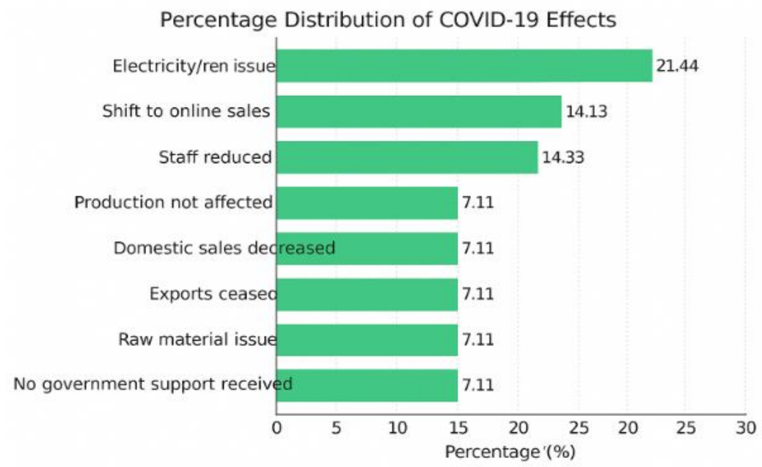

Figure 2. Percentage distribution of the impacts of COVID-19 on aquarium fish enterprises

4.5. IMPACTS OF THE 6 FEBRUARY 2023 EARTHQUAKES

Figure 3 shows the percentage distribution of the effects of the 6 February 2023 earthquake on aquarium fish enterprises.

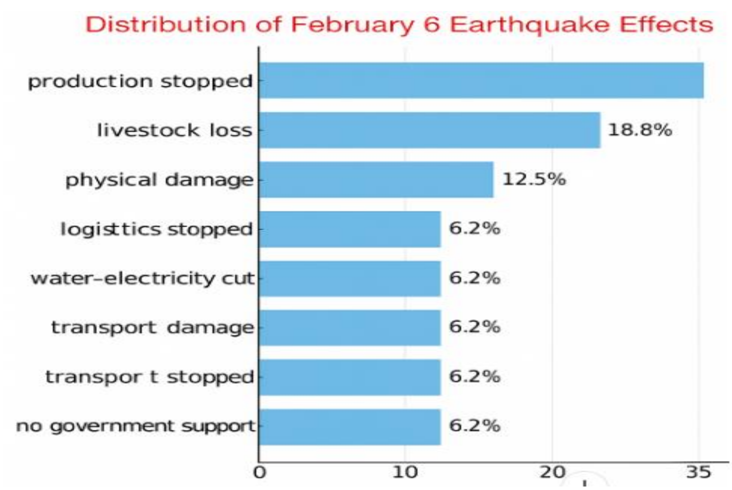

Figure 3 presents the percentage distribution of the impacts of the 6 February 2023 earthquakes on aquarium fish enterprises in the region.

The earthquakes had direct and devastating impacts on aquaculture-related businesses in İskenderun. The findings clearly illustrate the serious economic consequences of the disaster, with 25% of businesses having to completely shut down production. Furthermore, 18% of businesses reported significant fish losses, causing significant economic damage to the sector. Furthermore, 18.8% of businesses reported physical damage to their buildings, equipment, and infrastructure, resulting in significant financial losses. Other problems, each reported by 6.2%, included logistical disruptions, water and power outages, production interruptions, transportation disruptions, limited market access, and inadequate government support. These findings reflect the multifaceted operational challenges businesses faced in the aftermath of the disaster. Overall, earthquakes had the most severe impacts on production continuity and fish stock losses, while infrastructure failures, market access issues, and limited public assistance further disrupted business operations. Such results highlight the critical importance of identifying priority intervention areas for effective post-crisis recovery planning.

5. SWOT ANALYSİS OF THE AQUARIUM FISH PRODUCTION SECTOR IN ISKENDERUN

A SWOT analysis conducted for the aquarium fish production sector in the İskenderun region identifies the strengths, weaknesses, opportunities, and threats shaping the regional industry's performance. This analytical framework not only enhances understanding of the regional dynamics affecting the sector but also serves as a strategic planning tool for policymakers, investors, and stakeholders.

Strengths

From an internal perspective, the sector benefits from a rich diversity of products, providing flexibility in responding to changing market demands. The effective use of both physical retail outlets and digital marketing networks allows businesses to reach a broader consumer profile. Furthermore, low initial investment requirements and the prevalence of family businesses contribute to the sector's sustainability and resilience. This structure encourages entrepreneurs to enter the sector, supports local employment, and ensures continued production even with limited financial resources.

Weaknesses

Despite these strengths, several weaknesses pose challenges to the sector's long-term stability. Most notably, limited access to government support programs significantly constrains financial sustainability. Infrastructure deficiencies, such as electricity and water outages, and irregularities in feed supply chains threaten the continuity of production processes. Furthermore, complex licensing and permitting procedures make it difficult for businesses to formalize their operations, leading to a high rate of informal production and low levels of institutionalization within the sector. These weaknesses hinder the sector's capacity to modernize and compete effectively in national and international markets.

Opportunities

There are many significant opportunities for the further development of the aquarium fish sector in İskenderun. The proliferation of online sales platforms creates new marketing opportunities by facilitating access to larger markets and increasing product visibility. The establishment of cooperatives among small producers can enable them to benefit from economies of scale, reduce costs, and increase market competitiveness. Furthermore, collaborative R&D initiatives with universities and public institutions can support the development and implementation of innovative breeding and animal husbandry techniques. In addition to the domestic market, potential export opportunities exist to the Middle East, the Balkans, and the Turkic Republics, offering promising avenues for regional and international growth.

Threats

The sector faces many critical external threats. Natural disasters such as earthquakes, floods, and pandemics pose significant risks to production infrastructure and business continuity. Small-scale businesses struggle to compete with large retail chains, jeopardizing their long-term sustainability. Furthermore, price fluctuations in essential inputs such as feed and energy significantly increase production costs and reduce profitability. In the long term, climate change and the potential decline in water resources represent structural challenges that could undermine the sustainability of ornamental fish production. In summary, the SWOT analysis demonstrates that while the sector possesses significant biodiversity and entrepreneurial potential, it remains vulnerable to financial instability, infrastructure deficiencies, and environmental risks. Strengthening cooperative structures, investing in resilient infrastructure, and expanding R&D collaborations are strategic priorities for ensuring sustainable development in the Iskenderun aquarium fish production sector.

6. SECTORAL POLICY RECOMMENDATIONS

Based on the study's findings, several policy recommendations were developed to enhance the sustainability, competitiveness, and resilience of the aquarium fish production sector in the İskenderun region. These recommendations focus on strengthening cooperative structures, improving supply chains, advancing digitalization, and mitigating environmental and infrastructural risks.

6.1. Promotion of Regional Cooperatives

Priority should be given to establishing and supporting fish producer cooperatives to prevent local producers from being overexposed to intense market competition and to enable collective marketing power. Such cooperatives can increase cost efficiency and negotiating capacity in both local and national markets by facilitating joint action in logistics, feed supply, and marketing networks.

6.2. Supply Chain Improvements

To reduce price fluctuations and ensure production stability, regional subsidized procurement mechanisms for essential inputs such as feed and energy should be introduced. Furthermore, a prioritized producer support quota for critical raw materials could be established to ensure small-scale businesses maintain operational continuity in volatile market conditions.

6.3. Digitalization and Marketing Infrastructure

Despite a strong trend among producers to transition to online marketing, many businesses still lack sufficient technical and infrastructure support. To address this gap, regionally integrated e-commerce platforms (digital marketplaces) should be developed and made accessible to local producers. These efforts should be accompanied by training programs in digital marketing, product labeling, and online customer management to enhance producers' competitiveness in emerging digital markets.

6.4. Climate-Resilient and Disaster-Ready Infrastructure

Given the region's vulnerability to natural disasters such as earthquakes and floods, the establishment of "resilient production units" should be encouraged. Producers should be supported through grant-based financing for the installation of backup energy systems and emergency water supplies to ensure production continuity during times of crisis. These measures will enhance the adaptive capacity of smallholders and contribute to long-term sectoral resilience. Overall, these policy recommendations emphasize the importance of integrating cooperative organizations, financial incentives, digital infrastructure, and climate resilience into a unified regional development strategy for the aquarium fish production sector. Implementing such a framework will help strengthen the

İskenderun region's role as a major hub for sustainable ornamental fish farming in Türkiye.

7. STATE SUPPORT MODEL

A comprehensive three-stage state support model is proposed to enhance the financial sustainability, modernization, and innovation capacity of the aquarium fish production sector in the İskenderun region. This model integrates short-, medium-, and long-term instruments to ensure that state intervention effectively strengthens the sector's production base and competitiveness.

7.1. Three-Stage Support Package

- **Short-Term Measures:**

Provision of electricity and water bill subsidies and post-disaster microcredit schemes to support enterprises affected by crises such as earthquakes or pandemics. These measures are designed to stabilize operations and prevent immediate production losses.

- **Medium-Term Measures:**

Introduction of modernization credits, feed subsidies, and machinery grant programs to increase production efficiency and encourage technological upgrades within the sector.

- **Long-Term Measures:**

Transition toward an R&D-based production model supported by innovation-oriented state funds, along with export infrastructure incentives to expand access to international markets. This phase aims to institutionalize sustainable and knowledge-driven growth.

7.2. Local-Level Implementation Mechanism

To ensure effective coordination and accessibility, a dedicated liaison office should be established under the Provincial Directorates of Agriculture in İskenderun and its surrounding districts. This office would serve as a one-stop contact point for ornamental fish producers, facilitating access to incentives, monitoring fund utilization, and providing technical guidance. Additionally, existing incentive mechanisms should be simplified and made directly accessible to producers, minimizing bureaucratic delays and administrative complexity.

7.3. Fiscal Incentives

Fiscal measures should be designed to reduce the financial burden on small-scale producers and promote formalization: Tax exemptions and reduced VAT rates should be introduced for micro and small enterprises engaged in ornamental fish production. Producers operating under cooperative structures should be granted additional tax deductions to encourage collective marketing and shared investment. These fiscal incentives would improve profitability, enhance market competitiveness, and encourage small enterprises to formalize their operations within a transparent regulatory framework. In summary, this state support model proposes an integrated, multi-level policy architecture combining financial aid, infrastructural support, and institutional facilitation. Implemented effectively, such a model could significantly improve the resilience, innovation capacity, and international visibility of Türkiye’s ornamental aquaculture sector, particularly within the İskenderun region.

8. EDUCATION AND R&D COLLABORATION RECOMMENDATIONS

Enhancing human capital and promoting research-driven innovation are key prerequisites for ensuring the sustainable development of the aquarium fish production sector in the İskenderun region. Accordingly, the following education and research cooperation strategies are proposed to strengthen knowledge transfer, improve technical capacity, and increase production efficiency.

8.1. University–Enterprise Partnership Program

Each producer should be paired with a nearby university, particlarly those that have departments of aquaculture or fisheries sciences. Through this partnership, enterprises would gain access to knowledge exchange mechanisms, laboratory analyses, and technical consultancy services provided by academic experts. Such collaborations would contribute to the scientific monitoring **of production systems**, improved water quality management, and innovation in feed formulation and breeding techniques.

8.2. Mobile Training and Advisory Units

Specialized mobile training teams should be established for the İskenderun region to deliver on-site intensive training sessions to producers. These “accelerated production optimization programs” should cover essential operational topics such as feeding regimes, filtration systems, water quality control, and disease prevention. By providing hands-on capacity building, such initiatives would help standardize production practices and improve product quality across small enterprises.

8.3. National R&D Funding Mechanism

A dedicated national R&D fund should be established to support applied research on new species production, disease management, and feed technology innovations. Funding agencies such as TÜBİTAK should prioritize joint research projects conducted between universities and ornamental fish producers. This would not only foster innovation but also ensure that research outputs are directly translated into commercial and practical applications within the sector.

8.4. Online Training and Knowledge Portal

To enhance digital literacy and promote transparent business practices, an online training portal should be developed for producers. This platform should provide modular e-learning programs on topics such as e-commerce operations, licensing procedures, and record-keeping systems. Free and open access to such educational resources would help democratize knowledge, increase compliance with regulations, and support the digital transformation of small-scale enterprises. In conclusion, integrating education, digital training, and collaborative R&D mechanisms into sectoral policy design will strengthen the **innovation ecosystem** of the ornamental aquaculture industry. The proposed model emphasizes continuous learning, applied research, and institutional cooperation as key drivers of competitiveness and sustainability for the İskenderun region and beyond.

9. DISCUSSION

The research findings indicate that aquarium fish enterprises in the İskenderun region are predominantly small-scale and family-operated, reflecting a labor-intensive structure that requires low initial investment capital. The annual production capacity, ranging between 1,000 and 50,000 fish, and the employment of typically one to two workers per enterprise demonstrate the modest yet resilient nature of the sector. This observation aligns with international literature emphasizing the significant role of ornamental aquaculture in rural employment generation and livelihood diversification (Wijaya and Huda, 2021; Karunasagar, 2025). A strong positive correlation was observed between enterprise scale and variables such as production capacity, number of employees, and species diversity. This relationship suggests that as enterprises expand, they are better able to capitalize on economies of scale, achieve higher levels of institutionalization, and integrate digital technologies into their operations (Kılıçerkan and Çek, 2011). Similar patterns have been reported in India and Malaysia, where large-scale producers tend to diversify species portfolios and strengthen export orientation (Pailan et al., 2022). In Türkiye, Tolon and Emiroğlu (2014) also observed that the sector was initially dominated by small-scale enterprises, but growing market competition and species diversification have underscored the need for structural scaling and modernization. In terms of sales channels, the study found that physical retail stores remain dominant; however, a gradual shift toward online sales has become increasingly evident. Walster et al. (2015) highlighted that online distribution networks facilitate access to global consumers and generate new market opportunities for ornamental fish producers. Although enterprises in İskenderun show a similar digitalization trend, their operations largely remain localized at the regional level. In contrast, in Southeast Asian countries, even small-scale producers have leveraged e-commerce and export channels to access international markets (Evers et al., 2019). This comparison underscores the necessity for Türkiye to strengthen its logistics infrastructure, digital marketing platforms, and export facilitation mechanisms to remain competitive in global ornamental fish trade.

Among the most critical challenges reported by enterprises were price volatility, limited governmental support, and intense local competition (each 27.3%). These issues reveal the sector's financial fragility and vulnerability to market fluctuations. Consistent with findings from India, feed and energy price instability were among the most significant economic pressures for producers (Pailan et al., 2022). In contrast, producers in Europe and the United States primarily struggle with regulatory compliance, environmental certification, and sustainability standards (Evers et al., 2019; Biondo et al., 2024). Hence, while Türkiye's challenges are largely economic and structural, Western countries face regulatory and environmental constraints. However, as the Turkish sector expands, it is likely that issues related to environmental sustainability, licensing, and animal welfare will become more pronounced. The relatively low rate (9.1%) of participants citing regulatory complexity suggests that legal frameworks are still loosely enforced, but this situation may evolve as the industry matures. Like in many regions worldwide, the COVID-19 pandemic exerted a significant influence on enterprises in İskenderun. The most commonly cited difficulties were electricity and rent costs (21.4%), reflecting financial distress in meeting fixed operational expenses. In addition, shifts toward online marketing (14.3%) and reductions in workforce (14.3%) were major adaptive strategies adopted by producers. These results align with international literature documenting pandemic-related logistical disruptions, cost increases, and declines in sales (Akbulut and Yalnız, 2022; Oralbek and Nurqanatkyzy, 2025; Maia et al., 2025). Overall, the pandemic accelerated digital transformation but also caused job losses and supply chain vulnerabilities (Bulut and Özcan, 2022). The sector's exposure to natural disasters, particularly earthquakes, further highlights its structural fragility. The study revealed that 25% of enterprises halted production, 18% experienced fish mortality, and 18.8% reported infrastructure damage following the 6 February 2023 earthquakes. These findings confirm that small-scale enterprises are highly vulnerable to disaster-related shocks, consistent with prior research indicating that weak infrastructure exacerbates operational disruptions in aquaculture systems (Ziemann, 2001; Copp et al., 2005). The İskenderun case provides a clear regional manifestation of this phenomenon (Bulut and Özcan, 2022). Moreover, insufficient state support mechanisms during post-disaster recovery demonstrate a crucial gap in Türkiye's aquaculture resilience framework. Another critical issue emerging from this study is animal welfare in the ornamental fish trade. Inadequate handling during capture, transport, and maintenance, combined with poor water quality, overcrowding, and improper transportation practices, severely affects fish health and survival. The absence of effective monitoring systems and comprehensive welfare legislation remains a significant obstacle to improvement. Nonetheless, recent increases in public awareness and the publication of scientifically based welfare guidelines represent valuable opportunities for progress (Maia et al., 2025). In particular, data deficiencies and limited traceability in the marine ornamental fish trade continue to hinder sustainability and regulation (Kılıçerkan and Çek, 2011).

Overall, the findings from İskenderun reflect global trends and context-specific structural challenges in the Turkish ornamental fish farming sector. Key constraints include the dominance of small-scale, family-based businesses, price volatility, vulnerability to disasters, and insufficient government support. On the other hand, the sector also offers promising opportunities, such as digitalization, the expansion of e-commerce, the growth of university-industry partnerships, and potential access to international markets. Ensuring price stability, strengthening producer support mechanisms, improving logistics and digital infrastructure, implementing animal welfare regulations, and developing disaster-resilient production systems should be considered top policy priorities for achieving sustainable development. Furthermore, attracting large-scale investors to the sector and enhancing international competitiveness can simultaneously increase foreign exchange earnings and foster institutionalization in the sector. Given Türkiye's rich biodiversity and production potential in ornamental fish farming, it is clear that the sector's current status does not yet reflect its full potential. The growing body of academic research, combined with the empirical evidence from this study, highlights the urgent need for strategic, innovative, and sustainability-focused policy interventions in Türkiye aimed at overcoming existing barriers and fostering a modern, competitive, and resilient ornamental fish industry.

Based on the findings of this study, it is clear that the ornamental fish production sector in the İskenderun region has significant potential for regional economic growth, employment creation, and biodiversity conservation. However, its development is constrained by financial fragility, insufficient institutional support, and vulnerability to environmental and infrastructural risks. Several integrated strategies are recommended to ensure sustainable sectoral growth. First, priority should be given to the establishment of cooperative structures to strengthen collective marketing power, reduce production costs, and improve access to resources. Second, the implementation of regionally targeted subsidies and flexible financing mechanisms will help stabilize input prices and increase resilience to economic shocks. Third, promoting digital transformation through e-commerce platforms and online training programs can expand market access and enhance competitiveness. Additionally, collaboration between universities, public institutions, and producers should be institutionalized to foster innovation, research-based production, and technology transfer. Finally, policy frameworks should emphasize disaster-resilient infrastructure, animal welfare regulations, and sustainable water resource management to ensure the long-term ecological and economic sustainability of the sector. Combined, these measures, we suggest, could make Iskenderun a national model for sustainable ornamental fish farming in Türkiye.

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