THE SECRET WORLD OF WATERS

Editor:
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BİDGE

BİDGE Yayınları

The Secret World of Waters

Editor: Prof. Dr. Mevlüt Aktaş

ISBN: 978-625-5436-17-7

1st Edition
Page Layout By: Gözde YÜCEL
Publication Date: 2026-11-02
BİDGE Yayınları

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Krc Bilişim Ticaret ve Organizasyon Ltd. Şti.
Güzeltepe Mahallesi Abidin Daver Sokak Sefer Apartmanı No: 7/9 Çankaya / Ankara

CONTENTS

Zooplankton ın Well Waters: Ecology of Groundwater Ecosystem Fauna and Examples From Türkıye 1
AHMET BOZKURT

Effects of Metals on Zooplankton: A Toxicological, Physiological, and Ecological Assessment 40
AHMET BOZKURT, MEVLÜT AKTAŞ

Krustaselerin Gizli Kimyası: L-triptofan, Serotonin ve Melatonin 91
MEVLÜT AKTAŞ, AHMET BOZKURT, ESRA DEMİRKIRAN

CHAPTER 1

ZOOPLANKTON IN WELL WATERS: ECOLOGY OF GROUNDWATER ECOSYSTEM FAUNA AND EXAMPLES FROM TÜRKIYE

AHMET BOZKURT[1]

Introduction

Although groundwater ecosystems have long been considered biologically poor and static, recent studies have revealed that these environments harbour unique and highly adapted communities of organisms. Well waters, in particular, as windows into aquifer systems accessible to humans, offer an important research area for the study of groundwater biota. Zooplankton communities living in these systems have evolved under environmental constraints such as lack of light, limited food sources, low temperature variability, and specific oxygen regimes, and are distinctly different from their counterparts in surface aquatic environments (Gibert & Deharveng, 2002; Boulton et al., 2008).

The zooplankton fauna in groundwater ecosystems mostly consists of basic groups such as Copepoda, Cladocera, and Rotifera. However, species defined as stygobionts, which are completely

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adapted to underground environments, exhibit characteristics such as morphological reduction (loss of eyes, decreased pigmentation) and metabolic slowing. These organisms play a critical role in the organic matter cycle, occupying the lower levels of trophic networks. They also form an important link between microbial production in aquifer systems and higher trophic levels (Galassi, 2001; Hahn, 2006).

The distribution and diversity of zooplankton in well waters vary depending on factors such as aquifer type, hydrogeological structure, water renewal time, and degree of connection to the surface. Karstic aquifers, in particular, can host richer and more dynamic zooplankton communities due to their fractured structures and high hydraulic conductivity. In contrast, species diversity is generally more limited in porous aquifers. Furthermore, while the presence of epigean (surface-derived) species is noteworthy in wells connected to surface waters, more unique subsurface species become dominant in isolated systems (Malard et al., 2002; Hahn & Fuchs, 2009).

Turkey has significant potential in terms of subsurface aquatic ecosystems due to its geological and hydrological diversity. Particularly in the Mediterranean and Aegean regions, karst systems and the semi-arid aquifers in Central Anatolia provide opportunities for the development of different zooplankton communities. Although studies on zooplankton in well waters in Turkey are limited, existing research has revealed the prevalence of groups such as Cyclopoida and Harpacticoida. The distribution of these species is influenced by anthropogenic effects as well as local environmental conditions (Ustaoğlu, 2015; Özkan et al., 2020).

In recent years, the importance of zooplankton communities in the context of the protection and sustainable management of groundwater ecosystems has been increasingly emphasised. Zooplankton found in well waters can be used as biological

indicators in the assessment of water quality and reflect the effects of pollutants on the ecosystem. Pressures such as agricultural activities, excessive groundwater extraction, and urban pollutants can lead to a decrease in biodiversity in these sensitive ecosystems (Danielopol et al., 2003; Humphreys, 2006). Therefore, more comprehensive research into the subsurface aquatic biota is critically important for both increasing scientific knowledge and ensuring the effective management of water resources.

Groundwater and Well Ecosystems: A General Assessment

Definition, Formation and Classification of Groundwater

Groundwater forms when atmospheric water reaches the surface through precipitation, then passes through the porous structures of soil and rocks via infiltration and percolation, and is deposited underground. During this process, water movement occurs under the influence of physical forces such as gravity, capillarity, and hydraulic gradient. Groundwater accumulates particularly in permeable geological units known as aquifers, which serve as important components of the hydrological cycle (Freeze & Cherry, 1979; Todd & Mays, 2005).

Groundwater classification is generally based on aquifer type, hydraulic properties, and geological structure. In this context, unconfined, confined, and semi-confined aquifers are primarily distinguished. In unconfined aquifers, the water table is in direct contact with the atmosphere, while in confined aquifers, the water is trapped between impermeable layers. Additionally, porous (granular), karst, and fractured aquifer systems are important classification categories from a hydrogeological perspective. These different aquifer types play a decisive role in the chemical composition, flow rate, and biodiversity of the water (Fetter, 2001; Custodio & Llamas, 2013).

The processes that form groundwater also shape its physicochemical properties. During long-term groundwater flow, the water interacts with surrounding rocks, becoming enriched in dissolved minerals. This directly affects the physiological adaptations of organisms living in groundwater ecosystems. In particular, low light conditions, limited nutrient input, and a stable temperature regime lead to the evolutionary specialisation of groundwater aquatic biota (Gibert et al., 1994; Humphreys, 2009).

Hydrogeological and Ecological Characteristics of Well Types

Wells are artificial structures drilled to access groundwater reserves and are classified into different types according to their hydrogeological characteristics. The main types include boreholes, drilled wells, and artesian wells. Boreholes are typically drilled at shallow depths and obtain water from undisturbed aquifers. Drilled wells reach deeper aquifers and generally provide more stable physicochemical conditions. In artesian wells, water is brought to the surface from pressurised aquifers by natural pressure (Driscoll, 1986; Todd & Mays, 2005).

These well types also show significant differences in the biological communities they support. Shallow wells, being more exposed to surface waters, are open to the entry of epigean organisms and generally have higher zooplankton diversity. In contrast, deep and isolated wells, due to more limited energy input and low environmental variability, have lower species diversity but can support highly specialised stygobiont species. These species are typically characterised by low metabolic rates and long life cycles (Hahn, 2006; Boulton et al., 2008).

Wells are also important research tools for sampling groundwater ecosystems. However, as these structures are external interventions in natural systems, they can partially alter the structure

of biological communities. In particular, physical disturbances during drilling and hydraulic changes caused by water extraction can affect sensitive groundwater fauna. Therefore, when evaluating well ecosystems, both anthropogenic effects and hydrogeological characteristics should be considered (Malard et al., 2002; Hahn & Matzke, 2005).

Importance and Conservation of Groundwater Ecosystems Worldwide

Groundwater ecosystems are among the important, yet often overlooked, components of global biodiversity. These systems support high diversity, particularly of microfauna and meiofauna, and provide habitat for many endemic species. The isolation of groundwater habitats results in geographically restricted species distributions and high levels of endemism. This highlights groundwater aquatic biota as one of the sensitive ecosystems that require protection on a global scale (Gibert & Culver, 2009; Culver & Pipan, 2019).

Groundwater ecosystems are significant not only for biodiversity but also for ecosystem services. They perform critical functions such as natural water filtration, pollutant retention, and regulation of biogeochemical cycles. In particular, a substantial portion of drinking water for human consumption is sourced from groundwater. Therefore, the quality and sustainability of groundwater are directly linked to human health and economic activities (Danielopol et al., 2003; Griebler & Avramov, 2015).

However, groundwater ecosystems face serious threats from increasing anthropogenic pressures. Excessive water extraction, agricultural chemicals, industrial waste, and urban pollutants can cause irreversible damage to these sensitive systems. Additionally, climate change threatens habitat continuity by causing fluctuations in groundwater levels. In this context, integrated management

approaches for the conservation of groundwater ecosystems must be developed. Conservation strategies should address both biological components and hydrogeological processes, and the use of biological indicators, particularly for monitoring groundwater fauna, should be encouraged (Boulton et al., 2008; Hancock et al., 2005).

Underground Water Fauna: The Concept and Classification of Stygofauna

Distinction Between Stygofauna, Troglofauna and Subsurface Fauna

Subsurface biota is studied under different conceptual categories depending on the physical characteristics of the habitat and the life strategies of the organisms. In this context, the term "stygofauna" refers to organisms living in subsurface aquatic environments (aquifers, subsurface rivers, wells). In contrast, "troglofauna" encompasses species that inhabit cave systems and are generally found in cavities in contact with air. Although both groups are important components of subsurface ecosystems, they are exposed to different environmental conditions in terms of hydraulic connections and energy inputs (Gibert & Deharveng, 2002; Culver & Pipan, 2019).

The main difference between stygofauna and troglofauna is the degree of water saturation in their environment and the resource dynamics present. Stygofauna live in cavities completely filled with water and are generally adapted to environments with low oxygen concentration and limited organic matter input. In contrast, troglofauna can access more heterogeneous microhabitats in cave cavities and have greater connections with the surface. Additionally, the concept of "subsurface fauna" includes organisms living in habitats that form a transition between surface and subsurface systems, such as the hyporheic zone, and can include both epigean and subsurface species (Boulton et al., 2008; Hancock et al., 2005).

These classifications provide an important framework for understanding the ecological and evolutionary processes of subsurface biota. In particular, the isolation of subsurface habitats leads to organisms in these environments following independent evolutionary paths, resulting in high levels of endemism. Therefore, the distinction between stygofauna and troglofauna is critically important not only terminologically but also biogeographically and ecologically (Gibert & Culver, 2009).

Ecological Categorization: Stygobiont, Stygophile, and Stygoxene Organisms

Another commonly used approach to classifying subsurface aquatic fauna is based on the degree of dependence of organisms on subsurface environments. In this context, species are generally divided into three main groups: stygobionts, stygophiles, and stygoxenes. Stygobiont organisms are species that spend their entire life cycle in subsurface aquatic environments and are obligately dependent on these habitats. These organisms cannot survive in surface environments and exhibit a high degree of morphological and physiological adaptation (Sket, 2008; Galassi, 2001).

Stygophile organisms, by contrast, include species that can live in both subsurface and surface environments but have adapted to subsurface habitats to some extent. This group can switch between habitats depending on environmental conditions and demonstrates ecological flexibility. In contrast, stygoxenes are species that are only incidentally present in subsurface systems and cannot establish permanent populations in these environments. These organisms, generally of surface origin, are transported to aquifers by water flow or infiltration processes (Hahn, 2006; Boulton et al., 2008).

This ecological categorisation is an important tool in the biological assessment of groundwater ecosystems. In particular, the presence of stygobiont species is considered an important biological

indicator reflecting the natural and isolated nature of the habitat. Conversely, systems dominated by stygoxene species generally indicate environments where surface influence is strong or where anthropogenic interventions are present (Malard et al., 2002).

Adaptation Mechanisms to the Underground Environment

Organisms inhabiting subsurface aquatic ecosystems have developed various morphological, physiological, and behavioural adaptations to cope with extreme environmental conditions. Among the most notable of these adaptations are the reduction or complete loss of eyes, decreased pigmentation, and elongation of body structure. These features provide advantages, particularly in lightless environments, by enabling energy conservation and increased mobility in confined spaces (Culver & Pipan, 2019; Sket, 2008).

Physiological adaptations also play a crucial role in the survival of subsurface organisms. Species living in environments with low nutrient input are generally characterised by low metabolic rates and long lifespans. Additionally, some species have been reported to develop tolerance to low oxygen conditions and can efficiently utilise anaerobic metabolic processes. These features enable survival in resource-scarce conditions by optimising energy use (Hervant et al., 1999; Hüppop, 2000).

Behavioural adaptations are related to habitat use and feeding strategies. Subsurface aquatic organisms are generally integrated into food webs based on detritus and microbial biomass. Consequently, their foraging behaviours are highly selective and have evolved to maximise energy efficiency. Furthermore, low population densities and limited dispersal capacity directly affect the genetic diversity and population dynamics of these organisms. In this context, the adaptation mechanisms of subsurface fauna are considered the result of long-term interactions between evolutionary

processes and environmental pressures (Gibert et al., 1994; Humphreys, 2006).

Zooplankton: Definition, Biology and Role in Freshwater Ecosystems

The Concept of Zooplankton and its Basic Biological Characteristics

Zooplankton is the general term for heterotrophic organisms that live freely in aquatic environments, are transported by currents, or have limited mobility. Unlike phytoplankton, these organisms lack photosynthetic capabilities and meet their energy needs by consuming organic matter. Zooplankton displays wide biological diversity, including both microscopic (protozoa, rotifers) and macroscopic (larvae of some crustaceans) organisms (Wetzel, 2001; Lampert & Sommer, 2007).

The biological characteristics of zooplankton vary greatly depending on the species. However, they are generally characterised by short life cycles, high reproductive potential, and a rapid response to environmental changes. Many zooplankton species can alternate between asexual reproduction (parthenogenesis) and sexual reproduction depending on environmental conditions. This ability provides a significant advantage in ensuring population continuity, especially in freshwater ecosystems with variable environmental conditions (Allan, 1976; Lampert, 2011).

Furthermore, zooplankton can efficiently utilise resources within their habitat by exhibiting vertical and horizontal movement behaviours. In particular, daily vertical migration (diel vertical migration) ensures a balance between avoiding predator pressure and accessing food sources. These behavioural characteristics directly affect the role of zooplankton in energy flow and matter cycling in aquatic ecosystems (Hays, 2003; Lampert & Sommer, 2007).

Major Groups of Freshwater Zooplankton: Rotifera, Cladocera, Copepoda

Freshwater zooplankton comprises organisms from systematically distinct phylogenetic groups, but is studied under three main groups: Rotifera, Cladocera, and Copepoda. These groups are fundamental components of freshwater ecosystems in terms of both species diversity and functional roles within the ecosystem (Wetzel, 2001; Thorp & Covich, 2010).

Rotifers are generally microscopic and are notable for their rapid reproduction and short generation times. This group can experience rapid population increases, especially under eutrophic conditions, and plays an important role in controlling primary production through phytoplankton consumption. The Cladocera group, commonly known as "water fleas," is effective in regulating particulate matter in the water column due to its filter-feeding strategies. Some species within this group can significantly reduce phytoplankton biomass (Lampert, 2011).

The Copepoda group, by contrast, has more complex life cycles and can exhibit both herbivorous and carnivorous feeding strategies. Orders within this group, such as Cyclopoida and Calanoida, occupy different levels of the trophic network and play an important role in energy transfer. Furthermore, copepods are characterised by longer lifespans and lower reproductive rates compared to other zooplankton groups (Thorp & Covich, 2010; Mauchline, 1998).

Ecosystem Functions of Zooplankton: Food Web and Bioindicator Characteristics

Zooplankton functions as a central component of the food web in freshwater ecosystems. These organisms facilitate energy transfer between phytoplankton, the primary producers, and fish and other aquatic organisms at higher trophic levels. In this context,

zooplankton is described as an "energy bridge" and plays a critical role in determining ecosystem productivity (Wetzel, 2001; Lampert & Sommer, 2007).

The role of zooplankton within the food web is also important for regulating water quality. Filtering species, in particular, can limit eutrophication by controlling algal density in the water column. This indirectly affects light penetration and oxygen balance in aquatic ecosystems. However, population dynamics shaped by predation pressure on zooplankton increase the complexity of trophic interactions (Carpenter et al., 1985; Lampert, 2011).

Zooplankton are also considered an important bioindicator group due to their sensitivity to environmental changes. Parameters such as species composition, abundance, and diversity can reflect changes in water quality and pollution levels. For example, some rotifer species tolerate high organic pollution, while certain copepod species are commonly found in cleaner and more stable environments. Therefore, zooplankton community structure is widely used in assessing the ecological status of aquatic ecosystems (Jeppesen et al., 2011; Ejsmont-Karabin, 2012).

Zooplankton Ecology in Groundwater and Well Waters

General Structure and Characteristics of Zooplankton Communities in Well Waters

Zooplankton communities in well waters are characterised by lower species diversity and abundance compared to surface water ecosystems, but the dominance of certain taxonomic groups is notable. Among the most frequently encountered organisms in these environments are Copepoda (especially Cyclopoida and Harpacticoida), Rotifera, and, to a lesser extent, Cladocera. These groups possess physiological and ecological traits that enable them to survive with low energy input, limited nutrient resources, and stable environmental conditions (Galassi, 2001; Hahn, 2006).

Zooplankton communities in well waters generally exhibit simplified structures with low densities but dominance by specific species. This is particularly associated with the advantage gained by stygobiont and stygophilic organisms in a limited competitive environment. In addition, epigean (surface-derived) species are also observed in these communities, depending on the hydrogeological characteristics of the wells. Therefore, well zooplankton communities can display a complex structure reflecting the interaction between subsurface and surface systems (Malard et al., 2002; Hahn & Fuchs, 2009).

Differences in Zooplankton Diversity Between Surface and Groundwater

There are significant differences in zooplankton diversity between surface and groundwater. In surface aquatic environments, factors such as light, temperature, and nutrient input are more variable and generally higher, allowing for increased species diversity. In contrast, groundwater systems, due to low energy input and limited environmental variability, support fewer but highly specialised species (Gibert et al., 1994; Boulton et al., 2008).

Zooplankton communities in groundwater generally consist of smaller, slower-growing species with low metabolic rates. The Copepoda group, in particular, is the dominant taxon in these environments and includes species adapted to different aquifer types. In contrast, Cladocera species are less represented in groundwater systems, mostly observed in wells connected to the surface. Rotifera, however, are widely found in both surface and groundwater systems due to their small size and high reproductive capacity (Galassi, 2001; Hahn, 2006).

These differences arise not only from biological characteristics but also from ecological factors such as habitat continuity and dispersal capacity. While surface waters exhibit more

intense competition and predation between species, these interactions are more limited in subsurface systems. This directly affects the ecological niches and population dynamics of the species (Culver & Pipan, 2019).

Passive Transport and Active Colonization: Zooplankton Entry Routes into Wells

The presence of zooplankton in well water is directly related to the mechanisms by which these organisms enter such environments. The arrival of zooplankton in wells is primarily explained by two main processes: passive transport and active colonisation. Passive transport occurs through infiltration from surface waters into groundwater systems, specifically involving the movement of zooplankton to aquifers during precipitation and surface runoff (Malard et al., 2002).

In passive transport, small and hardy species are more likely to reach groundwater systems. In this context, Rotifera and some Copepoda species can be easily transported by water movements. Additionally, dormant eggs and resistant life stages also play an important role in this process. These structures withstand adverse conditions and can resume development when suitable environmental conditions are present (Brendonck & De Meester, 2003).

Active colonisation, although more limited, refers to the movement of some organisms through fracture systems and porous media to reach new habitats. Stygobiont species, in particular, have limited dispersal capacity within subsurface systems but can spread to different aquifer regions over long timescales. In this context, wells can function both as part of natural dispersal processes and as accumulation points for organisms (Hahn & Matzke, 2005).

Physicochemical Parameters Affecting Zooplankton Diversity in Well Waters

Zooplankton diversity in well waters is largely determined by physicochemical environmental conditions. Parameters such as temperature, dissolved oxygen, pH, conductivity, and nutrient salts directly affect the distribution and population dynamics of organisms. In groundwater systems, temperature is generally stable throughout the year, causing species to adapt to narrow tolerance ranges (Hahn, 2006; Griebler & Avramov, 2015).

Dissolved oxygen concentration is a limiting factor, especially for aerobic organisms. Low oxygen conditions allow only species with certain tolerance levels to exist, which reduces species diversity. In addition, high conductivity and mineral content can create stress, particularly for organisms with low ion tolerance (Malard et al., 2002).

The limited availability of nutrient salts (nitrogen and phosphorus compounds) results in low levels of primary production in groundwater ecosystems. This causes zooplankton communities to depend largely on detritus and microbial production. However, increased nutrient input in surface-connected wells may cause temporary increases in species diversity. Therefore, there is a strong and multidimensional relationship between physicochemical parameters and zooplankton community structure (Griebler & Avramov, 2015; Boulton et al., 2008).

Overview of Zooplankton Research in Türkiye's Inland Waters

Historical Development of Limnological Research in Türkiye

Although limnological research in Turkey was institutionalised later than the classical limnological tradition in Europe, it gained significant momentum, particularly after the

second half of the 20th century, with the development of freshwater biology and aquaculture research. Assessments of the development of limnology in Türkiye emphasise that the field initially focused more on physical and chemical water properties, while detailed studies on biological components became widespread later. Studies summarising the historical development of limnology in Turkey show that research capacity has been strengthened by universities, aquaculture faculties, and regional freshwater research projects (Kazancı, 2008).

From the perspective of zooplankton research, it has been noted that inventory studies in Turkish freshwater are relatively new, with pioneering records dating back to the early 1900s, but regular and cumulative species list studies accelerated mainly after 1940. Ustaoğlu (2004) states in his checklist for Turkish inland waters that the pioneering records are based on the early works of researchers such as Daday, Vavra, Zederbauer, and Brehm; however, the majority of the literature has been produced in the last 65 years. This shows that zooplankton knowledge in Turkey has progressed from fragmented regional studies to national-scale syntheses (Ustaoğlu, 2004).

From the 1990s onwards, there has been a significant increase in the number of studies conducted on lakes, reservoir lakes, rivers, deltaic wetlands, and transitional waters. A 2015 update revealed that zooplankton research published on Turkish inland waters has gradually become more than just species records; it has also begun to include ecological dimensions such as distribution, seasonal variation, trophic status, and relationships with environmental variables. Thus, limnological research in Turkey has evolved from descriptive fauna studies to an ecological interpretation and biological monitoring approach (Ustaoğlu, 2015).

Rotifera, Cladocera and Copepoda Fauna Lists in Turkish Inland Waters

One of the most fundamental syntheses concerning the zooplankton of Turkish inland waters is the checklist published by Ustaoğlu. This study compiled publications from 1940 to 2004 and reported a total of 427 taxa, including 229 Rotifera, 92 Cladocera, and 106 Copepoda. It is a key reference because it is the first to comprehensively present the diversity of zooplankton in Turkish inland waters on a national scale (Ustaoğlu, 2004).

Subsequent updates have shown a rapid increase in knowledge, particularly regarding the rotifer fauna. A separate checklist for Turkish rotifers reported that the number of taxa, given as 167 in 1999 and 229 in 2004, had reached 341 by 2012. In the updated review published in 2015, the zooplankton biodiversity of Turkish inland waters increased to a total of 650 taxa, including 417 Rotifera, 98 Cladocera, and 135 Copepoda. This increase reflects not only a higher level of actual biodiversity but also greater sampling density, wider geographical coverage, and more taxonomic studies (Ustaoğlu et al., 2012).

Regional checklists have also contributed significantly to understanding the spatial patterns of Türkiye's zooplankton fauna. For example, the review of the inland waters of European Türkiye reported 256 species: 138 Rotifera, 65 Cladocera, and 53 Copepoda. Such regional syntheses show that faunal information is not homogeneously distributed across the country, and that data accumulation is stronger for some basins and regions. It is also clear that regional catalogues play a complementary role in updating national checklists (Güher, 2014).

Zooplankton Studies Conducted in Different Water System Types: Lake, Dam and Stream Patterns

Zooplankton research in Türkiye has been most intensively conducted in lake and reservoir ecosystems. Studies in lakes have mainly focused on seasonal variation, species composition, and relationships with trophic status. For example, the İznik Lake study evaluated the zooplankton fauna through monthly sampling and demonstrated that species richness and seasonal dynamics in lakes can be examined in detail. Such studies show that natural lakes are effective model systems for monitoring both long-term limnological processes and regional fauna patterns (Yağcı and Ustaoğlu, 2012).

Research on reservoir lakes constitutes a significant part of the zooplankton literature in Türkiye. The main reason for this is that dams are widespread throughout the country, and their hydromorphological and trophic characteristics are suitable for comparative ecological analyses. In a comparative study conducted in seven different reservoirs, the relationships between zooplankton community structure and limnological variables were evaluated, and it was shown that rotifers are particularly important in interpreting trophic status. Similarly, studies conducted in individual dam reservoirs have revealed that in most systems, Rotifera is the dominant group in terms of species number, while Cladocera and Copepoda constitute more limited but ecologically complementary components (Dorak et al., 2019).

Although zooplankton studies on rivers are more limited compared to those on lakes and reservoirs, these systems have become an important research area in recent years. Studies conducted in systems such as the Sakarya River Basin and the Kars River have shown that the zooplankton structure in rivers is shaped by the flow regime, pollution load, suspended solids, and associated lentic habitats. It is understood that river zooplankton is generally less dense and more variable than in lakes and dams, but provides

important information as a biological component sensitive to environmental pressures. Therefore, evaluating lake, dam, and river studies together in Türkiye is necessary for a holistic interpretation of zooplankton patterns in the country's inland waters (Dorak, 2013).

Zooplankton Fauna of Well Waters in Türkiye: Current Research

Zooplankton in Adana (Kuyubeli Village) Well Waters: First Record of the Genus Speocyclops

Zooplankton research focusing on well waters in Türkiye is quite limited, and the study conducted in Kuyubeli Village, Kozan district, Adana province, represents a significant milestone in the development of this field. In this study, a total of 24 zooplankton taxa were identified from well waters: 13 belonged to the Rotifera group, 9 to the Copepoda group, and 2 to the Cladocera group. The most remarkable finding is the first recorded occurrence of the genus *Speocyclops* from inland waters in Türkiye. This record demonstrates that well waters can harbour not only transient taxa of surface origin but also unique microcrustacean communities associated with subsurface aquatic environments (Bozkurt, 2019) (Table 1).

The importance of the Kuyubeli study does not stem solely from the recording of a new genus. It also shows that subsurface aquatic zooplankton research in Türkiye can move beyond the classical lake- and river-centred limnological approach. The limited number of species, but relatively strong copepod component, can be considered an example supporting the ecological significance of taxa adapted to the subsurface environment in well waters. Therefore, the Kuyubeli data have established a preliminary reference area for zooplankton in Turkish well waters (Bozkurt, 2019).

Relationship Between Zooplankton Fauna and Water Quality in Well Waters of Hatay (Yayladağı)

Studies conducted in the Yayladağı district of Hatay province are among the most detailed in Turkey regarding the relationship between well zooplankton and the physicochemical environment. In a 2019 study, seasonal sampling was carried out in 14 different wells, and a total of 51 species were identified: 30 Rotifera, 9 Cladocera, and 12 Copepoda (Table 1). *Keratella cochlearis*, *Bosmina longirostris*, and *Tropocyclops prasinus* were among the most common taxa. These results suggest that surface-derived freshwater zooplankton constitute a significant component of the wells in Yayladağı (Bozkurt and Bozça, 2019).

In a subsequent study conducted in 2022 on 10 wells in the same region, a total of 30 species were identified: 14 Rotifera, 10 Copepoda, and 6 Cladocera. The study indicated a significant positive correlation between parameters such as water temperature, pH, and conductivity, and zooplankton diversity and abundance (Table 1). Furthermore, the widespread distribution of species such as *Rotaria neptunia*, *Daphnia curvirostris*, *Megacyclops viridis*, and *Tropocyclops prasinus* across multiple wells suggests that some taxa are tolerant of or adapted to well environments. The researcher specifically emphasised that some rotifers and cladocerans, and a significant proportion of copepods, are species adapted to groundwater (Bozkurt, 2022).

The Yayladağı example demonstrates that well zooplankton can be evaluated not only as a taxonomic list but also as a community of biological indicators linked to water quality. In particular, the variation in species richness among different wells and the concentration of the highest diversity in certain wells reveal that local hydrogeological conditions and water chemistry strongly influence community structure. In this respect, the Yayladağı studies

provide one of the strongest ecological data sets for well water zooplankton in Türkiye (Bozkurt and Bozça, 2019; Bozkurt, 2022).

Studies on Zooplankton Fauna in Well Waters of Kilis Province

The study conducted in Kilis province is among the most detailed investigations of subsurface zooplankton in well waters in Türkiye, both in terms of scope and sampling density. In this study, a total of 27 taxa were identified in 29 wells; 12 taxa were classified as Rotifera, one as Cladocera, and 14 as Copepoda (Table 1). The dominance of Copepoda in both taxon number and distribution suggests that the wells in Kilis have a more pronounced subsurface aquatic character. Furthermore, the fact that Cladocera was represented only by *Pleuroxus aduncus* indicates that this group remains quite limited in the well environment (Bozkurt, 2023).

A notable aspect of the Kilis study is the wide distribution of certain copepod and harpacticoid taxa in well systems. *Kinnecaris xanthi* was recorded in 27 wells, *Diacyclops languidoides* and *Monchenkocyclops mehmetadami* in 25 wells, and *Nitocrella stammeri* in 23 wells. In contrast, many rotifer species were observed in only a single or very few wells. Additionally, the study reports that the genus *Ectinosoma* was recorded for the first time from inland waters in Türkiye. These findings indicate that a community structure closer to subsurface life, particularly copepod-dominated, has developed in the wells of Kilis (Bozkurt, 2023).

The Kilis data also showed that species distribution at the well level was quite heterogeneous. While 14 species were found in the richest wells, only two copepod species were detected in some wells. This suggests that even within the same provincial boundaries, aquifer characteristics, well depth, associated microhabitats, and water chemistry can strongly influence community structure. Therefore, the Kilis study clearly demonstrates that zooplankton

research in well waters in Türkiye should be conducted not only with presence/absence records but also within ecological frameworks that explain spatial differentiation (Bozkurt, 2023).

Regional Differences and Common Patterns of Zooplankton in Turkish Well Waters

When the studies conducted in Adana, Hatay, and Kilis are considered together, two main patterns emerge in Turkish well water zooplankton. First, Copepoda is a strong and often ecologically decisive component in all regions. Second, the presence of Rotifera and Cladocera varies more with surface contact, well type, and local water quality conditions. The record of *Speocyclops* in Adana and the predominance of harpacticoid-cyclopoid forms in Kilis indicate more unique subsurface fauna components, while the higher total species count and the abundance of taxa also common in surface waters in Yayladağı suggest a stronger surface-subsurface interaction. This conclusion is an interpretation based on findings in publications (Bozkurt, 2019).

Another common finding is that well zooplankton communities are not uniform. Even within the same region, species richness and dominant taxa can vary significantly between wells. This variability is influenced by well depth, aquifer connection, physicochemical properties, and the degree of surface transport, all acting together. The current literature also shows that research on zooplankton in well waters in Türkiye still has a limited geographical scope. Therefore, the most reliable conclusion for now is that well zooplankton in Türkiye exhibits a regionally heterogeneous structure, is predominantly copepod-like, and occasionally contains special subsurface-cooperative taxa.

Table 1. *Zooplankton content of well waters in different localities (1: Kuyubeli 2019, 2; Yayladağ 2019, 2022, 3; Kilis 2023)*

Study areas	1	2	3	Total	%F
Rotifera	x/y (x: Number of wells where this species was found, y: total number of wells)				
Ascomorpha ovalis (Bergendahl, 1892)	-	4/24	-	4/61	**6.6**
Brachionus urceolaris Müller, 1773	-	2/24	-	2/61	**3.3**
Cephalodella catellina (Müller, 1786)	-	1/24	-	1/61	**1.6**
Cephalodella forficula (Ehrenberg, 1838)	-	-	9/29	9/61	**14.8**
Cephalodella gibba (Ehrenberg, 1830)	-	6/24	1/29	7/61	**11.5**
Cephalodella tenuiseta (Burn, 1890)	-	1/24	-	1/61	**1.6**
Cephalodella ventripes (Dixon-Nuttall, 1901)	-	1/24	-	1/61	**1.6**
Colurella adriatica Ehrenberg, 1831	2/8	3/24	-	5/61	**8.2**
Colurella colurus (Ehrenberg, 1830)	2/8	-	-	2/61	**3.3**
Colurella uncinata (Müller, 1773)	2/8	4/24	1/29	7/61	**11.5**
Dicranophorus epicharis Harring & Myers, 1928	-	2/24	-	2/61	**3.3**
Euchlanis dilatata Ehrenberg, 1832	-	2/24	-	2/61	**3.3**
Filinia longiseta (Ehrenberg, 1834)	-	1/24	-	1/61	**1.6**
Heterolepadella ehrenbergi (Perty, 1850)	1/8	-	-	1/61	**1.6**
Hexarthra fennica (Levander, 1892)	-	1/24	-	1/61	**1.6**
Kellicottia longispina (Kellicott, 1879)	-	1/24	-	1/61	**1.6**
Keratella cochlearis (Gosse, 1851)	1/8	13/24	-	14/61	**23**
Keratella quadrata (Müller, 1786)	1/8	8/24	-	9/61	**14.8**
Keratella tecta (Gosse, 1851)	1/8	-	-	1/61	**1.6**
Keratella tropica (Apstein, 1907)	-	2/24	-	2/61	**3.3**
Lecane bulla (Gosse, 1886)	1/8	3/24	1/29	5/61	**8.2**
Lecane closterocerca (Schmarda, 1859)	1/8	6/24	15/29	22/61	**36.1**
Lecane flexilis (Gosse, 1886)	-	5/24	2/29	7/61	**11.5**
Lecane hamata (Stokes, 1896)	-	5/24	12/29	17/61	**27.9**
Lecane inermis (Bryce,1892)	-	-	8/29	8/61	**13.1**
Lecane ludwigi (Eckstein,1893)	-	-	1/29	1/61	**1.6**
Lecane lunaris (Ehrenberg, 1832)	3/8	1/24	-	4/61	**6.6**
Lecane pumila (Rousselet, 1906)	-	1/24	-	1/61	**1.6**
Lecane pyriformis (Daday,1905)	-	3/24	13/29	16/61	**26.2**
Lecane tenuiseta Harring, 1914	-	5/24	2/29	7/61	**11.5**
Lepadella acuminata (Ehrenberg, 1834)	-	2/24	-	2/61	**3.3**
Lepadella patella (Müller, 1773)	1/8	2/24	5/29	8/61	**13.1**
Lophocharis salpina (Ehrenberg, 1834)	-	1/24	-	1/61	**1.6**
Mytilina bisulcata (Lucks, 1912)	-	3/24	-	3/61	**4.9**
Mytilina unguipes (Lucks, 1912)	-	1/24	-	1/61	**1.6**
Platyias quadricornis (Ehrenberg, 1832)	-	1/24	-	1/61	**1.6**
Rotaria neptunia (Ehrenberg, 1830)	-	7/24	-	7/61	**11.5**
Synchaeta stylata Wierzejski, 1893	-	9/24	-	9/61	**14.5**
Testudinella elliptica (Ehrenberg, 1834)	-	2/24	-	2/61	**3.3**
Testudinella patina (Hermann, 1783)	8/8	7/24	-	18/61	**29.5**

Trichocerca similis (Wierzejski, 1893)	-	11/24	-	11/61	**18**
Trichocerca tigris (Müller, 1786)	-	1/24	-	1/61	**1.6**
Trichotria tetractis (Ehrenberg, 1830)	1/8	3/24	-	4/61	**6.6**
Cladocera					
Bosmina longirostris (Müller, 1785)	-	16/24	-	16/61	**26.2**
Ceriodaphnia pulchella Sars, 1862	-	1/24	-	1/61	**1.6**
Ceriodaphnia reticulata (Jurine, 1820)	-	6/24	-	6/61	**9.8**
Daphnia curvirostris (Eylmann, 1887)	-	8/24	-	8/61	**13.1**
Diaphanosoma birgei Korinek, 1981	-	1/24	-	1/61	**1.6**
Simocephalus vetulus (Müller, 1776)	-	1/24	-	1/61	**1.6**
Alona guttata Sars, 1862	-	1/24	-	1/61	**1.6**
Chydorus sphaericus (Müller, 1776)	1/8	10/24	-	11/61	**18**
Coronatella rectangula (Sars, 1862)	-	6/24	-	6/61	**9.8**
Leydigia acanthocercoides (Fischer, 1854)	-	3/24	-	3/61	**4.9**
Pleuroxus aduncus (Jurine, 1820)	5/8	11/24	21/29	37/61	**60.7**
Copepoda					
Acanthocyclops robustus (Sars, 1863)	-	5/24	-	5/61	**8.2**
Cyclops vicinus Uljanin, 1875	-	1/24	-	1/61	**1.6**
Diacyclops bisetosus (Rehberg, 1880)	2/8	-	-	2/61	**3.3**
Diacyclops bicuspidatus (Claus, 1857)	6/8	1/24	-	7/61	**11.5**
Diacyclops languidoides (Lilljeborg, 1901)	-	-	25/29	25/61	**41**
Diacyclops languidus (Sars, 1863)	-	8/24	-	8/61	**13.1**
Eucyclops serrulatus (Fischer, 1851)	-	3/24	14/29	17/61	**27.9**
Macrocyclops albidus (Jurine, 1820)	-	8/24	-	8/61	**13.1**
Megacyclops viridis (Jurine, 1820)	-	11/24	21/29	32/61	**52.5**
Microcyclops sp.	-	-	1/29	1/61	**1.6**
Monchenkocyclops mehmetadami Karaytuğ, Bozkurt &Sönmez, 2018	-	-	25/29	25/61	**41**
Paracyclops chiltoni (Thomson, 1883)	-	1/24	11/29	12/61	**19.7**
Paracyclops fimbriatus (Fischer, 1853)	4/8	-	-	4/61	**6.6**
Speocyclops sp.	1/8	-	-	1/61	**1.6**
Thermocyclops dybowski (Lande, 1890)	-	-	11/29	11/61	**18**
Tropocyclops prasinus (Fischer, 1860)	6/8	18/25	11/29	35/61	**57.4**
Eudiaptomus drieschi (Poppe and Mrazek, 1895)	-	9/25	-	9/61	**14.8**
Attheyella crassa (Sars, 1863)	4/8	2/25	5/29	11/61	**18**
Bryocamptus minutus (Claus, 1863)	1/8	-	-	1/61	**1.6**
Bryocamptus zschokkei (Schmeil, 1893)	-	1/25	-	1/61	**1.6**
Canthocamptus microstaphylinus Wolf, 1905	1/8	1/25	-	2/61	**3.3**
Ectinosoma sp.	-	-	4/29	4/61	**6.6**
Elaphoidella sp.	-	-	9/29	9/61	**14.8**
Kinnecaris xanthi Bruno & Cottarelli, 2015	-	1/25	27/29	28/61	**46**
Nitocra hibernica (Brady, 1880)	-	1/25	-	1/61	**1.6**
Nitocrella kosswigi Noodt, 1954	1/8	-	-	1/61	**1.6**
Nitocrella stammeri Chappuis, 1938	-	1/25	23/29	24/61	**39.3**
Phyllognathopus viguieri (Maupas, 1892)	-	1/25	1/29	2/61	**3.3**

References: Bozkurt, 2019; Bozkurt and Bozça, 2019; Bozkurt, 2022; Bozkurt, 2023

According to Table 1, the frequency of some zooplankton species in well waters is quite variable. One species was detected in 37 out of 61 wells. Most of these species are also commonly found in lentic (stagnant water) and lotic (stream) ecosystems. Among the Rotifera group, *Lecane closterocerca* was detected in 22 out of 61 wells (36.1%). This was followed by *Testudinella patina* in 18 wells (29.5%), *Lecane hamata* in 17 wells (27.9%), *Lecane pyriformis* in 16 wells (26.2%), and *Keratella cochlearis* in 14 wells (23%) (Bozkurt, 2019; Bozkurt and Bozça, 2019; Bozkurt, 2022; Bozkurt, 2023).

In the Cladocera group, *Pleuroxus aduncus* was one of the most frequently encountered species, found in 37 out of 61 wells (60.7%). This was followed by *Bosmina longirostris* in 16 wells (26.2%), *Chydorus sphaericus* in 11 wells (18%), *Daphnia curvirostris* in 8 wells (13.1%), and *Ceriodaphnia reticulata* and *Coronatella rectangula* in 6 wells (9.8%) (Bozkurt, 2019; Bozkurt and Bozça, 2019; Bozkurt, 2022; Bozkurt, 2023).

In the Copepoda group, *Tropocyclops prasinus* was recorded as one of the most common species, found in 35 out of 61 wells (57.4%). This species was followed by *Megacyclops viridis* in 32 wells (52.5%), *Kinnecaris xanthi* in 28 wells (46%), and *Diacyclops languidoides* and *Monchenkocyclops mehmetadami* in 25 wells (41%) (Bozkurt, 2019; Bozkurt and Bozça, 2019; Bozkurt, 2022; Bozkurt, 2023).

Apart from species exhibiting hyporheic characteristics, such as *Monchenkocyclops mehmetadami* (Karaytuğ et al., 2018) and *Kinnecaris xanthi* (Bruno & Cottarelli, 2015), the other species are considered hardy, with broad environmental tolerance. This is because the characteristics of well waters differ from those of other surface water ecosystems.

Relationships Between Zooplankton and Physicochemical Parameters in Well Waters

Effects of Water Temperature, Dissolved Oxygen and pH on Zooplankton Diversity

The structure of zooplankton communities in well waters is largely determined by fundamental physicochemical parameters. In groundwater systems, temperature generally shows little variation throughout the year, resulting in organisms having narrow tolerance ranges. Stable temperature conditions are advantageous, especially for stygobiont organisms with low metabolic rates, while abrupt temperature changes can limit the persistence of some epigean species in the system (Hahn, 2006; Griebler & Avramov, 2015).

Dissolved oxygen concentration is considered one of the most important factors limiting zooplankton distribution in well waters. Oxygen levels are generally low in underground environments due to organic matter mineralisation and limited aeration. Under these conditions, only organisms with low oxygen requirements can survive, leading to a decrease in species diversity. Some Copepoda species are reported to be better adapted to low oxygen conditions, while many Cladocera species are limited in these environments (Malard et al., 2002; Galassi, 2001).

The pH value is another important parameter that directly affects the physiological tolerance ranges of zooplankton species. In groundwater, pH generally ranges from neutral to slightly alkaline, providing a suitable environment for many zooplankton species. However, excessively acidic or alkaline conditions reduce species diversity and allow only the presence of species with a specific tolerance range. Studies show that Rotifera species, in particular, are found widely in different well systems due to their broad pH tolerance (Ejsmont-Karabin, 2012; Griebler & Avramov, 2015).

Relationship Between Conductivity and Salinity and Zooplankton Composition

Conductivity and salinity indicate the amount of dissolved ions in groundwater systems and are important environmental variables influencing zooplankton composition. High conductivity values, in particular, reflect a high mineral content in the water, which can create stress for many zooplankton species. Increased ionic composition impairs osmoregulation, leading to the loss of sensitive species (Williams, 2001; Griebler & Avramov, 2015).

Although groundwater salinity is generally low, it can increase in some areas due to geological structure and anthropogenic effects, resulting in significant changes in zooplankton community structure. Greater species diversity is observed under low salinity conditions, while only highly tolerant species dominate under high salinity. Some Rotifera species and certain Copepoda taxa are reported to be more resistant to high conductivity and salinity (Hammer, 1986; Williams, 2001). The relationship between conductivity and zooplankton composition is also influenced by aquifer type and water renewal time. Waters that remain underground for extended periods generally have higher mineral content, resulting in a more limited but specialised subsurface fauna. In contrast, wells with closer connections to the surface have lower conductivity and greater species diversity (Malard et al., 2002).

Effects of Nutrient Salt Concentrations on Zooplankton Abundance and Diversity

Nutrient salts, particularly nitrogen and phosphorus compounds, are among the main factors that indirectly influence the abundance and diversity of zooplankton communities. Nutrient salt concentrations in groundwater systems are generally low, resulting in limited primary production. Consequently, zooplankton largely

depend on a food web based on detritus and microbial production (Boulton et al., 2008; Griebler & Avramov, 2015).

Rising nutrient salt concentrations can increase zooplankton abundance, especially in wells connected to surface waters. This is associated with increased phytoplankton production and the subsequent proliferation of herbivorous zooplankton species. However, excessive nutrient input can cause hypoxic conditions by increasing oxygen consumption, leading to the loss of sensitive species (Jeppesen et al., 2011).

Responses to nutrient changes also differ among zooplankton groups. For example, Cladocera species can increase their populations by directly benefiting from increased phytoplankton biomass. In contrast, Copepoda species may respond indirectly and more slowly to increased nutrient salts due to their more complex feeding strategies. Rotifera, however, stand out as a group that can respond rapidly to short-term increases in nutrients due to their high reproductive capacity (Lampert & Sommer, 2007).

In summary, the relationships between zooplankton and physicochemical parameters in well waters involve multidimensional and interconnected processes. Evaluating these parameters together is crucial for accurately understanding the biological structure of groundwater ecosystems and monitoring water quality.

Use of Well Water Zooplankton as a Bioindicator

Zooplankton Diversity Indices and Water Quality Assessment

Zooplankton communities are widely used indicator groups in the biological assessment of water quality because they respond rapidly to environmental changes. The use of zooplankton as bioindicators in well water involves calculating quantitative indices

based on species diversity, abundance, and community structure. The most commonly used metrics in this context include the Shannon-Wiener diversity index (H′), the Simpson index, and the Pielou evenness index. These indices enable a more comprehensive assessment of ecosystem status by considering not only the number of species but also the evenness of species distribution (Magurran, 2004; Washington, 1984).

As low species diversity is generally observed in groundwater systems, caution is required when interpreting diversity indices. Low diversity does not always indicate poor water quality; it can be a characteristic feature of naturally resource-limited groundwater environments. Therefore, when conducting biological assessments of well water, it is recommended that index values be considered alongside reference conditions and hydrogeological characteristics (Hahn, 2006; Griebler & Avramov, 2015).

However, sudden changes in diversity indices or significant shifts in species composition may indicate the presence of environmental stress or pollutants. Fluctuations in diversity indices can be observed, especially in surface-connected wells, with increasing anthropogenic impacts. Therefore, zooplankton diversity-based indices are considered a complementary tool for monitoring water quality in well water (Jeppesen et al., 2011).

Determining Pollution Indicator Species and Trophic Status

Zooplankton communities can serve as biological indicators in water quality assessment, as certain species exhibit tolerance to specific environmental conditions. In this context, some rotifer species are particularly associated with high organic matter loads and eutrophic conditions. For example, species belonging to the genera *Brachionus* and *Keratella* have been reported to increase in

abundance with high nutrient salt concentrations and can therefore be considered indicators of pollution (Ejsmont-Karabin, 2012).

Similarly, some species within the Cladocera group are sensitive to changes in trophic status and show a direct relationship with phytoplankton biomass. For instance, small-sized cladocerans tend to become more dominant under eutrophic conditions, while large-sized species are generally more prevalent in systems with lower nutrient levels (Jeppesen et al., 2011).

The Copepoda group, by contrast, may respond more slowly but more comprehensively to environmental changes due to their longer life cycles and complex trophic relationships. Some copepod species are more common in clean and stable water conditions, whereas species composition shifts in polluted environments. Therefore, more reliable conclusions about trophic status and pollution levels can be drawn by evaluating the entire zooplankton community structure, rather than focusing on individual species (Galassi, 2001; Griebler & Avramov, 2015).

Potential for Integration into Well Water Monitoring Programs

The use of zooplankton as bioindicators in well waters holds significant potential for the sustainable management of water resources. Traditional water quality monitoring programmes are mostly based on physicochemical parameters, with biological components often neglected. However, biological monitoring is gaining importance as it allows a holistic assessment of cumulative impacts on the ecosystem (Boulton et al., 2008).

Integrating zooplankton-based monitoring approaches into well waters offers advantages, particularly for early warning systems. As zooplankton communities can respond rapidly to pollutants, deteriorations in water quality can be detected before significant changes in physicochemical parameters occur. This

provides a critical advantage, especially for protecting drinking water sources (Hahn, 2006).

However, there are challenges in developing biological monitoring programmes for groundwater ecosystems. The lack of standardised sampling methods, the need for taxonomic expertise, and insufficient reference data are among the main challenges. In Türkiye, the limited database on well water zooplankton restricts the widespread adoption of such applications. Therefore, future studies should focus on developing standard methodologies and creating long-term data sets (Malard et al., 2002; Griebler & Avramov, 2015).

In conclusion, well water zooplankton can be used as a powerful biological tool in water quality assessment when supported by appropriate methodological approaches. When integrated with physicochemical analyses, it is possible to obtain more effective and reliable results in the conservation and management of groundwater resources.

Discussion and Evaluation

Positioning Türkiye's Well Water Research in a Global Context

Zooplankton research in well waters in Türkiye has only recently begun and remains quite limited. Studies on subsurface aquatic ecosystems in Europe, Australia, and North America are characterised by long-term datasets, broad geographical coverage, and a strong taxonomic infrastructure (Danielopol et al., 2003; Gibert & Culver, 2009). In contrast, studies in Türkiye have mostly developed as regional, short-term research with limited sampling intensity. Nevertheless, Türkiye has high potential for research into subsurface water biota due to its geological diversity and, in particular, the prevalence of karst aquifer systems. Its location in the Mediterranean belt forms a transition zone connecting both European and Middle Eastern biogeographic regions, which is

considered a factor that can increase the diversity of subsurface fauna. Indeed, the discovery of new taxonomic records even in a limited number of studies shows that Turkish well waters are a biodiversity reserve that has not yet been sufficiently explored (Galassi, 2001; Sket, 2008). In this context, while current research in Türkiye is still relatively unrepresentative in the global literature, it can be considered to have high potential for generating original data. Increasing regional studies and integrating them into international databases will contribute to Türkiye becoming more visible in groundwater ecology research.

Research Gaps and Methodological Discussions

One of the most significant gaps in zooplankton research in well waters in Türkiye is the lack of long-term monitoring studies. As existing studies are generally based on single-period or short-term sampling, the presentation of seasonal and interannual variations remains limited. Although groundwater systems are considered relatively stable, temporal variability can be significant, especially in surface-connected wells. Therefore, the creation of long-term datasets is critical for understanding ecosystem dynamics (Hahn, 2006; Griebler & Avramov, 2015).

In recent years, the use of molecular methods (DNA barcoding, environmental DNA analysis) in zooplankton research has offered a significant opportunity to address these problems. However, the application of these methods to zooplankton in well waters in Türkiye remains very limited. In the future, developing integrated methodologies that combine classical morphological approaches with molecular techniques will increase the accuracy of species identification and help reveal cryptic species diversity (Boulton et al., 2008; Culver & Pipan, 2019).

In conclusion, zooplankton research in well waters in Türkiye is a developing field with significant research gaps and

methodological limitations. Nevertheless, current findings clearly demonstrate that these ecosystems have high scientific value and can make significant contributions through more comprehensive studies.

Conclusion

The findings evaluated in this section clearly demonstrate that well waters are important systems not only from a hydrogeological perspective but also in terms of biodiversity and ecosystem functions. Zooplankton communities, as components of subsurface aquatic ecosystems, are noteworthy for their unique adaptations developed under low energy input, limited nutrient resources, and stable environmental conditions. Even the limited number of studies conducted in Türkiye show that these systems have considerable biological potential, particularly due to their predominantly Copepoda community structure and, in some cases, unique taxonomic records. Although the current body of knowledge regarding zooplankton in Turkish well waters is still in its infancy, the data obtained reveal that these ecosystems are heterogeneous and possess region-specific characteristics. Studies conducted especially in the Mediterranean Region show that Rotifera and Cladocera groups become more prominent in systems with strong surface-subsurface interactions, whereas Copepoda species are dominant in more isolated systems. This supports the idea that hydrogeological structure, water chemistry, and surface connectivity are fundamental factors determining zooplankton community structure (Griebler & Avramov, 2015; Galassi, 2001).

However, the geographical distribution of studies on well water zooplankton across Türkiye is uneven, and many regions remain unexplored. In particular, the Central Anatolia, Eastern Anatolia, and Black Sea regions are underrepresented due to a lack of data, despite potentially harbouring diverse groundwater ecosystems. This situation limits the accurate assessment of species

diversity and ecological patterns on a national scale. Therefore, it is crucial that future research focuses on understudied regions and that a more balanced dataset is created across the country.

From a methodological perspective, the lack of standardised sampling protocols and difficulties in taxonomic identification are significant factors limiting the comparability of existing studies. In this context, supplementing classical morphological approaches with molecular methods (DNA barcoding, environmental DNA analysis) will both increase the accuracy of species identification and contribute to uncovering cryptic species diversity. Furthermore, initiating long-term monitoring studies is a critical requirement for understanding the temporal dynamics of groundwater ecosystems (Boulton et al., 2008; Culver & Pipan, 2019).

The use of well water zooplankton as bioindicators offers an important application for the sustainable management of water resources. The sensitivity of zooplankton communities to physicochemical changes enables these organisms to detect deteriorations in water quality at an early stage. Therefore, integrating biological monitoring approaches with traditional physicochemical analyses will yield more effective results in the conservation and management of groundwater resources (Hahn, 2006; Jeppesen et al., 2011).

In conclusion, well water zooplankton research in Türkiye is a developing but high-potential scientific field. In the future, adopting interdisciplinary approaches and considering hydrogeology, ecology, and molecular biology together will contribute to a more comprehensive understanding of these systems. Scientific studies developed in this direction will make significant contributions not only to the protection of groundwater ecosystems but also to global biodiversity and water management policies.

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CHAPTER 2

EFFECTS OF METALS ON ZOOPLANKTON: A TOXİCOLOGİCAL, PHYSİOLOGİCAL, AND ECOLOGİCAL ASSESSMENT

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Introduction

The presence of metals in aquatic ecosystems results from natural geochemical processes, but has increased significantly, particularly in the last century, due to rising anthropogenic activities. Heavy metals entering the environment through mining, industrial discharges, agricultural inputs, and urban waste can have lasting and often irreversible effects on aquatic organisms. In this context, zooplankton are among the critical biological model groups for studying the effects of metal pollution because of their short life cycles, high reproductive rates, and sensitivity to environmental changes (Rainbow, 2002; Wang & Rainbow, 2008).

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Zooplankton communities are a fundamental component of the trophic network in the water column, facilitating the transfer of energy and matter between phytoplankton and higher trophic levels. Therefore, the effects of metals on zooplankton are not limited to the physiology of individual organisms but also have cascading consequences that can affect the entire ecosystem. Studies, particularly on model species such as *Daphnia magna*, have shown that metals directly suppress nutrient uptake, growth, and reproduction (Lampert, 2011; OECD, 2004).

The toxic effects of metals on zooplankton vary significantly depending on the chemical form of the metal, its bioavailability, and the duration of exposure. Metals in dissolved form can be readily absorbed, particularly through gill-like structures and integuments, leading to cellular accumulation. This accumulation causes physiological disturbances such as enzymatic inhibition, oxidative stress, and cellular damage. For example, oxidative stress is a common response in zooplankton exposed to metals and is associated with increased reactive oxygen species (Livingstone, 2001; Barata et al., 2005).

These physiological effects observed at the individual level can lead to changes at the population and community levels over time. The decline of metal-sensitive species and the dominance of tolerant species cause significant shifts in zooplankton community structure. This results in reduced biodiversity and altered trophic interactions, threatening ecosystem stability. Furthermore, the transfer of metals to higher trophic levels through bioaccumulation and biogrowth processes along the food chain further increases the ecotoxicological significance of zooplankton (DeForest et al., 2007; Luoma & Rainbow, 2008).

In recent years, studies integrating molecular, biochemical, and ecological approaches have come to the forefront to more comprehensively assess the effects of metal pollution. Omics

technologies, biomarker analyses, and experimental ecotoxicology applications provide a more detailed understanding of zooplankton responses to metal stress. In this context, zooplankton is considered not only an indicator group revealing the effects of metal pollution, but also an indispensable tool for assessing the health status of aquatic ecosystems (Hook & Fisher, 2002; Baird et al., 2007).

Aquatic Ecosystems and Pollutant Pressure

Aquatic ecosystems are dynamic systems where natural processes intersect with human influences. Industrialisation, rapid population growth, and intensive land use have been reported to exert significant polluting pressure on these systems. Heavy metals, in particular, are pollutants that are not biodegradable and can persist in the environment for extended periods, causing chronic toxicity in aquatic environments (Förstner & Wittmann, 2012). Rivers, lakes, and coastal areas are especially sensitive to the accumulation of these pollutants, leading to increased metal concentrations in sediment and the water column over time (Chapman et al., 2013). This situation threatens not only the health of individual organisms but also ecosystem functionality and biodiversity.

Global Distribution and Significance of Metals in Aquatic Environments

The distribution of metals in aquatic environments is determined by geological structure, hydrodynamic processes, and human activities. While metals entering the environment through natural processes exist in a certain equilibrium, anthropogenic inputs disrupt this balance, significantly increasing the metal load. Metals such as mercury, cadmium, lead, and copper are considered priority pollutants globally due to their toxic effects and potential for bioaccumulation (Tchounwou et al., 2012). The presence of these metals in the water column, whether dissolved or in particulate form, directly affects their bioavailability and determines the severity of

their effects on organisms. Global studies have shown that metal pollution is particularly pronounced in developing countries and places significant pressure on aquatic ecosystems (UNEP, 2019).

The Critical Position of Zooplankton in Aquatic Food Webs

Zooplankton is an essential biotic component that enables the flow of energy and substances between phytoplankton and higher trophic levels. Species such as *Daphnia magna* are used both as model organisms in ecological studies and as bioindicators due to their sensitivity to environmental stress factors. The short life cycle and rapid population dynamics of zooplankton allow them to respond quickly to environmental changes (Lampert, 2011). These characteristics make it possible to detect the effects of stress factors such as metal pollution at an early stage. In addition, zooplankton plays a critical role in bioaccumulation and biomagnification processes by serving as an important intermediate step in the trophic transfer of metals (Wang & Rainbow, 2008).

This section evaluates the effects of metals on zooplankton from a holistic perspective, considering toxicological, physiological, and ecological aspects. Drawing on existing literature, the study investigates the cellular and biochemical changes induced by metals in zooplankton, as well as their effects at the population and community levels. Additionally, it addresses the indirect effects of metal exposure on food web dynamics and the implications for ecosystem health. In this context, the integration of classical ecotoxicological approaches with current molecular and biomarker-based methods is considered, aiming to present the subject from a multidimensional perspective (Luoma & Rainbow, 2008; Rainbow, 2002).

General Framework of Metal Pollution in Aquatic Environments

Definition, Classification and Basic Properties of Metals

Metals are defined as elements with characteristic properties such as high density, electrical conductivity, and chemical reactivity. In aquatic environments, metals may exist in dissolved ionic form, complex-bonded form, or particulate phase, and these forms play a critical role in determining their effects on organisms. From an ecotoxicological perspective, metals differ from other pollutants because they are non-biodegradable and persist in the environment over the long term (Förstner & Wittmann, 2012). Therefore, the environmental fate and biological effects of metals are assessed not only by their total concentration but also by their chemical forms and environmental conditions (Luoma & Rainbow, 2008).

Essential and Non-Essential Metals

Metals are classified as essential or non-essential based on their biological functions. Essential metals, such as iron (Fe), zinc (Zn) and copper (Cu), act as cofactors in enzymatic reactions and are required by organisms within specific concentration ranges. However, high concentrations of these metals can be toxic. In contrast, non-essential metals, such as cadmium (Cd), lead (Pb) and mercury (Hg), have no significant physiological function in biological systems and can be toxic even at low concentrations (Tchounwou et al., 2012). In this context, metal toxicity is assessed along a spectrum that depends on dose and exposure time.

The Toxic Trio: Cadmium, Lead, and Mercury

Cadmium, lead, and mercury are among the metals of greatest toxicological significance in aquatic ecosystems. Cadmium, in particular, inhibits kidney and cellular enzyme systems, suppressing growth and reproductive processes in zooplankton. Lead affects the nervous system and disrupts ion regulation mechanisms, resulting in physiological imbalances in organisms. Mercury, especially in its methylmercury form, has high bioaccumulation and

biomagnification potential and can be transported across trophic levels, causing severe toxic effects in higher organisms (Boening, 2000; Wang & Rainbow, 2008). The combined assessment of these three metals is now a common approach in aquatic ecotoxicology studies.

Sources of Metal Entry into the Aquatic Environment

Natural Sources: Geological Processes and Volcanic Activity

Metals are continuously transported into aquatic environments through natural processes. Rock weathering, soil erosion, and hydrothermal activity are primary mechanisms for the entry of metal ions into water systems. Volcanic eruptions and atmospheric deposition also contribute to large-scale metal transport. While these natural inputs are generally tolerable for ecosystems, they can lead to local accumulations depending on environmental conditions (Förstner & Wittmann, 2012).

Anthropogenic Resources: Industry, Mining, Agriculture

Anthropogenic activities are considered the primary source of metal pollution in aquatic environments. Mining, ore processing, and metallurgical industries release large amounts of metal-containing waste into the environment. Fertilisers and pesticides used in agriculture also contribute to the transport of metals, particularly cadmium and arsenic, into water systems. Furthermore, urban wastewater and industrial discharges can increase heavy metal loads, creating chronic stress on aquatic organisms (UNEP, 2019). These inputs often result in metal concentrations far exceeding natural background levels.

Metal Specimen and Bioavailability in Aquatic Environments

The effects of metals in aquatic environments vary greatly depending on their chemical forms, that is, their specificities. Metals

in their free ionic form generally have the highest bioavailability and are readily taken up by organisms. In contrast, metals that form complexes with organic matter or are bound to the particulate phase may have lower bioavailability. Therefore, accurate assessment of metal toxicity requires consideration of not only total metal concentrations but also their chemical species (Luoma & Rainbow, 2008).

Effects of pH, Salinity, and Temperature on Bioavailability

Environmental parameters directly affect metal speciation and, consequently, bioavailability. Low pH conditions increase bioavailability by enhancing the solubility of metal ions. Salinity modulates metal uptake, particularly in marine environments, through ion competition and complex formation. Increased temperature can affect the rate of metal uptake by organisms by increasing metabolic rates. Considering these factors together is important for understanding the spatial and temporal variability of metal toxicity (Rainbow, 2002).

Metal Dynamics in Sediment and Water Column

Metals are present in the water column either dissolved or bound to suspended particles, but over time they can accumulate by precipitating into sediment. Sediments act as both a reservoir and a potential source of re-release for metals. Processes such as physical mixing, redox conditions, and bioturbation can cause metals bound in sediment to return to the water column. These dynamic processes result in constantly changing metal concentrations to which pelagic organisms such as zooplankton are exposed (Chapman et al., 2013).

International Environmental Quality Standards and Regulatory Framework

To control metal pollution in aquatic environments, various environmental quality standards and regulatory frameworks have been developed internationally. The European Union Water

Framework Directive sets limit values for heavy metals in assessing the chemical and ecological status of water bodies. Similarly, water quality criteria established by the United States Environmental Protection Agency provide reference values for protecting human health and aquatic life. These regulations serve as fundamental tools for monitoring and managing metal pollution. Additionally, studies conducted globally by the United Nations Environment Programme contribute to developing strategies to reduce metal pollution (UNEP, 2019).

Biology and Ecological Function of Zooplankton

Zooplankton: Systematic Diversity and Ecological Groups

Zooplankton comprises a large and taxonomically diverse group of free-living heterotrophic organisms in aquatic ecosystems. These organisms occupy different ecological niches according to their morphological and physiological characteristics and play a fundamental role in the functioning of pelagic systems. Zooplankton communities are generally studied within major groups such as Copepoda, Cladocera, and Rotifera. These groups differ in their feeding strategies, reproductive methods, and environmental tolerances, which diversifies their functional roles within the ecosystem (Barnes et al., 2001; Lampert & Sommer, 2007).

Copepods

Copepods are crustaceans found in both freshwater and marine environments and make up a significant portion of zooplankton biomass. Their varied feeding strategies, including filter feeding, predation, and detritivory, make them a versatile component of trophic networks. In particular, calanoid and cyclopoid copepods play a crucial role in regulating primary production by exerting intense grazing pressure on phytoplankton and microzooplankton (Mauchline, 1998).

Cladoceran (Daphnia* spp. *and others)

Cladocerans are dominant organisms, particularly in freshwater ecosystems, and consume phytoplankton through filter feeding. Species in this group, such as *Daphnia magna*, are widely used as model organisms in ecotoxicological studies. The high filtration capacity of cladocernae helps regulate water clarity by controlling algal density in the water column (Lampert, 2011). Additionally, their rapid parthenogenetic reproduction enables quick changes in population dynamics.

Rotifers

Rotifers, due to their microscopic size and rapid reproductive strategies, form an important zooplankton group, especially in eutrophic systems. These organisms link the microbial cycle and the classical food web by feeding on bacteria, algae, and organic particles. The rapid response of rotifers to environmental stressors makes them an important biological indicator in water quality assessments (Wallace et al., 2006).

Other Holoplanktonic and Meroplanktonic Groups

Zooplankton includes holoplanktonic species, which spend their entire life cycle as plankton, as well as meroplanktonic organisms, which are planktonic during certain stages of their life cycle. In particular, the larvae of benthic invertebrates contribute to dispersal and colonisation processes by being present in planktonic environments during their early developmental stages. These groups result in more complex interspecies interactions and energy flows (Barnes et al., 2001).

Role in Food Webs: Bridging from Primary Producers to Higher Trophic Levels

Zooplankton serve as a critical link between primary producers and higher trophic levels in aquatic food webs. Organic matter produced by phytoplankton is consumed by zooplankton and

transferred to higher trophic levels. This process is a fundamental mechanism for the continuity of energy flow. Zooplankton also constitute a primary food source for fish larvae and small invertebrates; therefore, changes in their population dynamics can directly affect fisheries productivity (Lampert & Sommer, 2007). In this context, zooplankton play a central role not only in energy transfer but also in matter cycling.

Use of Zooplankton as a Bioindicator

Zooplankton communities are widely used in water quality assessment because of their sensitivity to environmental changes. Parameters such as species composition, abundance, and diversity provide important information about the trophic status and pollution levels of aquatic ecosystems (Jeppesen et al., 2011). Their short generation times enable rapid responses to environmental stressors and allow them to serve as an early warning system.

Zooplankton Communities in Water Quality Assessment

Changes in zooplankton community structure are considered a biological indicator of water quality deterioration. Factors such as eutrophication, metal pollution, and increased organic load cause a decrease in sensitive species and the dominance of tolerant species. These changes are an important data source in biological monitoring programmes (Jeppesen et al., 2011).

Ecotoxicological Test Organisms

Zooplankton species are widely used as model organisms, particularly in standard ecotoxicological tests. In this context, *Daphnia magna* is accepted as a reference species in acute and chronic toxicity tests. These tests are conducted according to international standards to determine the effects of chemical substances on aquatic organisms (OECD, 2004).

Metal Uptake and Accumulation Mechanisms

Zooplankton interact with metals both directly and indirectly. Metal uptake varies according to the physiological characteristics of the organism, environmental conditions, and the chemical form of the metal. These processes underlie the mechanisms of bioaccumulation and biomagnification (Wang & Rainbow, 2008).

Passive Diffusion and Active Transport

Metals can be absorbed by zooplankton through passive diffusion or active transport mechanisms. Metals in their free ionic form can cross cell membranes by passive diffusion, depending on the concentration gradient. Additionally, some metals, due to their similarity to essential ions, can be actively transported into the cell via carrier proteins. This process facilitates the entry of toxic metals into biological systems (Rainbow, 2002).

Routes of Nutritional Intake

One of the main pathways for metal exposure in zooplankton is dietary intake. Metals enter the organism through the consumption of metal-containing phytoplankton, bacteria, and detritus particles. This pathway is particularly important in the bioaccumulation of particle-bound metals. Dietary uptake underpins trophic transfer processes, contributing to the movement of metals to higher trophic levels (Wang & Rainbow, 2008).

Toxicological Effects of Metals on Zooplankton

Acute Toxicity: LC50 Values and Lethal Threshold Concentrations

The acute toxicity of metals to zooplankton is assessed primarily through mortality or immobilisation endpoints determined after 24–96 hours of exposure. One of the most commonly used criteria for this purpose is the LC50 or, in some test setups, the EC50 value. In standard tests, particularly those based on *Daphnia*,

immobilisation is considered the primary endpoint for defining the acute effect. The OECD Test No. 202 guideline states that *Daphnia magna* is commonly used in the assessment of acute toxicity and that this test provides an international standard framework for determining the acute effects of chemicals on daphnids (OECD, 2004).

Acute toxicity data show considerable variability depending on the metal species, the organism's taxonomic position, life stage, and environmental conditions. However, metals such as copper, cadmium, and mercury have been reported to cause significant acute effects in many zooplankton taxa even at low concentrations. A meta-analysis of copepods found that copper sensitivity varies significantly between species, and LC50-based comparisons remain a key tool in environmental risk assessments. This underscores the limitations of generalisations based on a single species or a single metal (Heuschele et al., 2022).

Comparative Toxicity Data by Species and Metal

Comparisons by species and metal indicate that zooplankton groups do not display equal sensitivity to metals. For instance, studies on various species show that copper frequently causes rapid acute effects, whereas cadmium can induce sublethal and chronic deterioration at lower thresholds in some species. In a study of *Notodiaptomus iheringi*, a copepod species from Brazil, the LC50 value for Cu was reported as 27.7 µg/L and for Cd as 8.0 µg/L in 48-hour acute tests; this suggests that cadmium may have greater toxic potential at acute levels, at least for this species (Rocha et al., 2025).

Similarly, interspecies sensitivity is not limited to mortality. In some studies, metal accumulation and metallothionein response have proved to be more sensitive early warning indicators than mortality. In a study on *Daphnia magna* exposed to cadmium-contaminated sediment, cadmium accumulation and metallothionein

response were reported to be more sensitive outcomes than mortality. This finding suggests that focusing solely on LC50 data in comparative toxicity assessments may not reflect the full biological effect (Li et al., 2017).

Methodologies Used in Toxicity Testing

Methodologies used in the assessment of acute metal toxicity are crucial for experimental standardisation. According to OECD TG 202, acute *Daphnia* tests are generally conducted using a short-term exposure regimen and evaluated based on the immobilisation endpoint. For the investigation of chronic effects, OECD TG 211 is used; in this test, young female *Daphnia magna* are monitored for 21 days at a minimum of five different concentrations, and reproductive output is evaluated as the primary endpoint. The OECD recommends using at least 10 individuals for each concentration in semi-static tests and a total of 40 individuals in four groups in flow-through systems (OECD, 2004).

Although these standard tests were developed to ensure comparability between different laboratories, variables such as water chemistry, dissolved organic matter, hardness, and temperature must also be considered when interpreting metal toxicity. Therefore, standard test results are more meaningful when evaluated alongside mechanistic and ecological interpretations (Heuschele et al., 2022).

Sublethal Effects and Chronic Exposure

Metal exposure in zooplankton often begins with sublethal effects that do not cause death but reduce life performance. Growth retardation, prolonged development time, decreased energy reserves, reduced reproductive success, and behavioural changes are frequently observed in long-term or low-dose exposures. A recent review summarising multi-generational and chronic *Daphnia* studies highlights that long-term tests reveal impairments in growth,

reproduction, and physiological responses more effectively than acute tests (Padilla Suarez et al., 2023).

Effects on Growth and Development

The effects of metals on growth and development are linked to a shift in the energy budget towards maintenance and detoxification processes. Cadmium, even at environmentally realistic levels, was clearly shown in a 2025 *Daphnia magna* study to delay somatic growth. Similarly, zinc oxide exposure has been reported to reduce stem length and deplete energy reserves, with rising temperatures potentially worsening this toxicity. These findings suggest that growth suppression is a key chronic indicator of metal stress (Padilla Suarez et al., 2023).

Changes in Reproduction and Fecundity

Reproduction is one of the most sensitive endpoints for metal exposure. The main objective of OECD TG 211 is to assess the impact of chemicals on the reproductive output of *Daphnia magna*. Cadmium, copper, and metal mixtures have been reported to reduce egg production and the number of live pups; in some cases, they can also reduce egg viability. In a study on *Notodiaptomus iheringi*, mortality was observed during the acute phase, while subchronic exposure resulted in significant impairments, particularly in egg production and egg viability. A review of multi-generational *Daphnia* also emphasises that reproductive endpoints are central to long-term environmental risk assessment (OECD, 2012).

Behavioral Changes: Nutrition and Swimming Activity

Behavioural endpoints are among the sensitive indicators that can change before mortality. Recent studies have shown that feeding inhibition can be a rapid and sensitive sublethal endpoint for *Daphnia*. A 2024 review indicates that feeding rate is an endpoint that deteriorates as early as, or in some cases even earlier than, mortality for many chemicals, and that cadmium, in particular, can

affect feeding even at very low levels. Changes in swimming activity due to metal exposure can also have indirect consequences for predation avoidance, energy use, and foraging success (Aruoja et al., 2024).

Biochemical and Physiological Mechanisms of Action

The toxic effects of metals extend beyond external performance changes. At the cellular level, disruption of oxidative balance, alteration of ion regulation, enzyme inhibition, activation of detoxification systems, and reorganisation of energy metabolism are key mechanistic responses in zooplankton. Recent studies on *Daphnia magna* indicate that cadmium and other metals increase reactive oxygen species, alter antioxidant defences, and cause metabolic disruptions (Mahmut et al., 2022).

Oxidative Stress and Reactive Oxygen Species (ROS)

Oxidative stress is one of the most frequently reported mechanisms of metal toxicity. Cadmium exposure has been shown to increase reactive oxygen species in *Daphnia magna*, which is linked to growth and accumulation processes. Similarly, experiments involving metal and pesticide mixtures have reported increased oxidative damage and lipid peroxidation. Therefore, increased ROS production is considered one of the biochemical starting points of sublethal effects (Hu et al., 2022).

Responses in Antioxidant Enzyme Systems (SOD, CAT, GPx)

Under metal exposure, antioxidant defence systems often constitute the first physiological response. It has been reported that antioxidant enzyme activities change in response to Cd and Pb exposure in *Daphnia magna*, with significant regulation, especially in enzymes such as SOD and CAT. Some studies have shown that GPx-related defence responses also vary depending on the level of oxidative stress. However, these enzymatic responses are not always

linear; while a compensatory increase may be observed at low doses, suppression of the defence system may occur at higher doses (Kim et al., 2018).

Metallothionein Synthesis and Detoxification

Metallothioneins are low molecular weight proteins involved in binding and detoxification, particularly of soft metals such as cadmium. Studies on *Daphnia magna* have shown that cadmium exposure induces metallothionein synthesis, and this response may serve as a more sensitive biomarker than mortality. Additionally, the metallothionein response in pulsed cadmium exposures has been linked to recovery processes and intergenerational effects. Thus, the metallothionein response may indicate not only the zooplankton's defence mechanism against the metal but also its history of chronic stress (Li et al., 2017).

Enzyme Inhibition and Metabolic Disorders

Metals can directly or indirectly disrupt many enzymatic processes. ATPase systems involved in ion transport, energy production under oxidative stress, and key metabolic pathways are particularly affected. Studies on *Daphnia magna* have shown that exposure to Cd alters ATPase activity, lipid peroxidation, and energy utilisation patterns. Moreover, the association between growth retardation and reduced energy reserves suggests that toxicity is not limited to target organ damage but reshapes the organism's entire metabolism (Mahmut et al., 2022).

Genotoxic Effects: DNA Damage and Repair Mechanisms

The effects of metals on zooplankton can extend to the genetic material. DNA damage associated with metal pollution in marine zooplankton communities has been monitored using the comet assay. A 2024 review also emphasises that various pollutants can disrupt genomic integrity in coastal mesozooplankton, and DNA damage is a significant endpoint for ecotoxicological monitoring. It

has been shown that micronucleus and comet assay applications can be used to measure genotoxic responses in *Daphnia magna* and some copepods. Studies of *Daphnia* exposed to metals such as arsenic and cadmium have reported that DNA damage, epigenetic changes, and physiological disturbances occur together (Goswami et al., 2014).

The balance between DNA damage and repair capacity is a critical factor in determining an individual's ability to adapt to metal stress. However, detailed characterisation of repair pathways in zooplankton is more limited in the current literature compared to mortality and reproductive data. Therefore, genotoxicity represents a significant research gap, particularly concerning metal mixtures and multiple stress interactions. This assessment is based on the limited but consistent findings reported in current reviews and experimental studies (Thirunavukkarasu and Hwang, 2024).

Morphological and Histopathological Changes

Morphological changes in zooplankton due to metal exposure are reported as reductions in body length, developmental abnormalities, embryonic disruptions, and, in some cases, structural changes related to osmoregulation. Studies on *Daphnia magna* show that metal exposure can alter morphometric characteristics, and these changes may be linked to oxidative stress. Zinc oxide exposure has been associated with decreased body length and impaired energy reserves. Although histopathological changes are more limited and technically more difficult to study in zooplankton than in fish or larger invertebrates, disruptions in cellular tissue integrity are becoming increasingly apparent, particularly through experimental microscopy and biomarker approaches (Mahmut et al., 2022).

Generally, the toxic effects of metals on zooplankton extend far beyond acute responses resulting in death; they cause multi-layered disruptions in growth, reproduction, behaviour, oxidative balance, genetic integrity, and morphological structure. Therefore,

interpreting LC50 data alongside chronic and mechanistic biomarkers in zooplankton-based metal toxicity assessments provides a more realistic ecological risk framework (OECD, 2004).

Metal-Specific Effects: Focusing on Selected Metals

Effects of Mercury (Hg) on Zooplankton

Mercury is important in aquatic ecosystems in both its inorganic Hg(II) and organic methylmercury (MeHg) forms. This distinction is critical for zooplankton, as MeHg is more strongly transported through the food web and tends to bioaccumulate. Studies on *Daphnia magna* have shown that MeHg can accumulate effectively and that dietary composition can significantly alter bioaccumulation. Furthermore, the acute toxicities of Hg(II) and MeHg differ; in 48-hour tests, Hg(II) has been reported to be more toxic than MeHg for *D. magna* (He et al., 2022).

The effects of mercury on zooplankton are not limited to acute mortality. Previous exposure history and maternal influence can alter the tolerance and physiological performance of subsequent generations. Experiments with *Daphnia magna* have shown that maternal mercury exposure affects the offspring's response to mercury. This suggests that mercury toxicity can have intergenerational consequences at the population level (Tsui and Wang, 2005).

From an ecological perspective, mercury, particularly as methylmercury, makes zooplankton both a target group and an intermediary for transfer to higher trophic levels. Therefore, Hg exposure is important not only for its effects on zooplankton growth and reproduction, but also for its role in trophic transfer and biogrowth processes (He et al., 2022).

Effects of Cadmium (Cd) on Zooplankton

Cadmium is one of the most frequently studied non-essential metals in zooplankton and has a strong evidence base for both acute and chronic toxicity. Studies of *Daphnia magna* have shown that sublethal effects can occur even at environmentally realistic levels, particularly impaired growth, disrupted energy balance, and altered physiological stress responses. Recent studies reveal that cadmium exerts significant pressure not only on mortality but also on long-term life performance (Pinto et al., 2025).

The importance of the exposure route in cadmium toxicity is particularly emphasised. Although regulatory considerations often focus on dissolved metal, experiments on *Daphnia magna* have shown that dietary cadmium intake also has significant negative effects on reproduction. Dietary cadmium has been reported to increase the body burden of daphnids and reduce reproductive performance. This finding indicates that particulate-bound metal should not be ignored ecotoxicologically (Geffard et al., 2008).

At the mechanistic level, cadmium is associated with oxidative stress, metallothionein synthesis, and multigenerational physiological adaptation processes. Multigenerational experiments have shown that *Daphnia magna* evolves an energy-tolerance balance between metallothionein and Hsp70 in response to cadmium, although this may impose life history costs. It has also been reported that variables such as calcium concentration and pH in water chemistry alter cadmium toxicity (Haap et al., 2016).

Effects of Lead (Pb) on Zooplankton

Although lead has been studied less extensively than cadmium and copper in zooplankton, current data suggest that Pb has significant effects, particularly on oxidative balance, motility, and reproduction. Experimental studies on *Daphnia magna* have reported altered antioxidant enzyme responses to Pb exposure,

which are associated with oxidative stress. Lead exposure can activate cellular defence systems, but suppression of these defences can also occur as exposure levels increase (Kim et al., 2017).

The effects of lead may also vary depending on the life stage. Classic studies on early life stages have shown that *Daphnia magna* eggs can be more tolerant to some metals than adults, but as development progresses, the effect of Pb becomes more apparent. It has also been reported that Pb may show a different sensitivity pattern compared to other metals during embryo development (Bodar et al., 1989). An important point for lead is that, in most cases, behavioural and physiological outcomes may be impaired before acute mortality occurs. Therefore, it seems more appropriate to evaluate Pb effects not only with immobilisation or LC50 data, but also with antioxidant responses and developmental parameters. This interpretation is directly based on the common pattern of experimental results (Kim et al., 2017).

Effects of Copper (Cu) on Zooplankton

Although copper is an essential metal, it becomes highly toxic to zooplankton when its threshold is exceeded. A meta-analysis, particularly on copepods, has shown that copper sensitivity varies widely among species; nauplius stages are more sensitive than adults, and pelagic calanoids are more sensitive than some benthic harpacticoids. The same study reports that copper toxicity increases at temperatures above 25 °C (Heuschele et al., 2022).

Copper toxicity is not limited to exposure in the aquatic phase. A study on *Acartia tonsa* showed that copper taken up through the phytoplankton diet can significantly reduce reproduction. This result indicates that copper can reach zooplankton through both dissolved and dietary pathways, and that both exposure routes should be considered in risk assessment (Wang and Wang, 2022).

More recent studies on freshwater copepods also show that copper exerts strong pressures on both survival and reproduction. In the study of *Notodiaptomus iheringi*, copper, alone and in combination with cadmium, caused significant toxicity, especially in the early stages. This confirms that copper is not only an essential microelement but also a powerful stressor when environmental conditions are favourable (Rocha et al., 2025).

Threshold Effects of Zinc (Zn) and Other Essential Metals

Zinc, like copper, is an essential metal with biological functions; however, above certain thresholds, it has negative effects on development, reproduction, and gene expression. Studies on *Daphnia magna* have shown that water hardness significantly alters zinc toxicity, with the toxic effect decreasing in harder water. This finding suggests that bioavailability-focused interpretations are more meaningful for zinc than total concentration (Paylar et al., 2022).

Zinc is notable in early life stages. Classical experiments have shown that *Daphnia magna* eggs can be more tolerant to Cd, Cu, Zn, and Pb than adults; however, sensitivity to Zn has been reported to increase in later stages of embryonic development. This pattern indicates that the toxicity of essential metals varies according to life stage and developmental window (Bodar et al., 1989).

Similar threshold effects occur for other essential metals as well. A study of *Acartia tonsa* showed that zinc, copper, and nickel, when ingested through the phytoplankton diet, can reduce reproductive output. This suggests that although essential metals are physiologically necessary at low levels, once the threshold is exceeded, they are among the first stressors to affect energy-costly processes, especially reproduction (Bielmyer et al., 2006).

Mixed Exposure Scenarios of Metals: Synergistic and Antagonistic Effects

In natural environments, zooplankton are often exposed to mixtures of metals rather than a single metal. Therefore, mixture toxicity is considered more realistic than single-metal assessments. In 21 studies of binary metal mixtures on the marine/euryhaline rotifer *Proales similis*, synergistic effects were detected in 20 mixtures. This result shows that the additive assumption is often insufficient for metal mixtures (Arreguin-Rebolledo et al., 2024).

However, not all mixtures are synergistic. Species, life stage, exposure route, and metal combination influence the outcome. A study on the freshwater copepod *Notodiaptomus iheringi* using a mixture of Cu and Cd showed that Cd contributed to Cu toxicity at some stages, but this pattern did not occur in the same way in adults. This finding suggests that mixture effects are context-dependent rather than constant (Rocha et al., 2025).

Overall, the effects of metal mixtures on zooplankton are often more complex than can be predicted from single-metal data. Synergism, antagonism, and additive behaviour can occur within the same system under different conditions. Therefore, approaches based solely on single-metal LC50 or NOEC data are insufficient for environmental risk assessments, and mixture toxicity should be given greater prominence in future research and regulatory agendas (Espericueta et al., 2014).

Metals and Zooplankton Community Structure

Effects on Species Richness, Diversity and Abundance

Metal pollution is often primarily associated with decreases in species richness, diversity, and abundance in zooplankton communities. Field studies have reported that stations with lower metal loads have higher total density, biomass, Shannon diversity,

and species richness, while these measures decline in areas with increased metal pressure. A study in the Salado River basin showed that main stream sections with lower metal pollution were characterised by higher density, biomass, diversity, and species richness. Similarly, it has been noted that zooplankton diversity decreases in wetlands with high urbanisation pressure, and metals such as chromium can suppress functional group densities (Gagneten and Paggi, 2009).

This pattern does not completely disappear in marine systems, but the direction of the response appears to depend more on the type of metal and accompanying environmental conditions. A 2025 study conducted on the Yellow Sea coast showed that Zn and As are the main metals affecting zooplankton abundance and community composition; it was reported that total abundance and diversity increase under higher Zn and lower As conditions. This finding suggests that the metal-community relationship is not always linear in the form of "more metal = lower diversity," and that bioavailability, threshold levels, and co-varying environmental factors determine the outcome (Weng et al., 2025).

Changes in Community Structure: Sensitive and Tolerant Species

Metal exposure not only causes an overall numerical decline in zooplankton communities but also results in a significant compositional shift between sensitive and tolerant species. The Salado River study showed that as Pb and Cu concentrations increased, copepod biomass was negatively affected, while some tolerant species proliferated in polluted tributaries. The same study reported that a small number of tolerant species, such as *Eucyclops neumani*, became dominant in polluted environments. This suggests that under metal stress, communities tend towards a simpler but more tolerant structure (Gagneten and Paggi, 2009).

Data on the relative sensitivity of freshwater planktonic crustaceans also support this interpretation. Studies of species sensitivity distribution have shown that cladocerans can be particularly more sensitive to Cd and Cu than copepods. More recent marine data, however, report that copepods in coastal zooplankton communities can exhibit relatively higher tolerance to heavy metals. Therefore, a significant part of the community-level restructuring can be explained by the decline of large and sensitive filter feeders and the relative advantage gained by more tolerant taxa (Wong et al., 2009).

Communal Shift in Favor of Rotifers: The Dominance of r-Strategists

It is frequently reported that in systems under metal stress, taxa with small bodies, rapid reproduction rates, and quick responses to environmental fluctuations gain an advantage. In particular, rotifers have been shown to remain relatively resistant under metal and general pollution pressure, and therefore can become dominant in community structures. A study of the Salado River reported that rotifers were the most tolerant group to heavy metal pollution, followed by copepods and then cladocerans. The same study clearly indicated the proliferation of r-selected species in polluted tributaries (Gagneten and Paggi, 2009).

This shift does not appear to be unique to a single field study. A study examining urbanisation pressure also reported that rotifers exhibited strong resistance to pollution. A 2024 field study argues that rotifer dominance in tropical waters may be related to their small size, wide temperature tolerance, and rapid reproductive capacity under deteriorating environmental conditions. Therefore, metal pollution, often in conjunction with eutrophication and other stressors, may facilitate a community shift in favour of rotifers, even if not acting alone. This last sentence is not a direct experimental

cause-and-effect statement, but a cautious ecological interpretation derived from current field findings (Shen et al., 2021).

Adaptation and Resistance Mechanisms to Metal Pollution

The response of zooplankton communities to metal stress is not limited to species elimination; true tolerance development has also been observed in some populations. Natural populations of *Daphnia longispina* historically exposed to metal stress have shown higher metal tolerance than reference populations. Furthermore, experimental selection studies on *Daphnia magna* have shown that populations under Cu and Zn stress can develop greater tolerance, which may be related to changes in genetic structure. These findings suggest that changes in community composition can be explained not only by the loss of susceptible species but also by intra-population evolutionary responses (Lopes et al., 2006).

Resistance mechanisms include changes in metal uptake and removal kinetics, metallothionein-based binding, reorganisation of energy allocation, and intergenerational plasticity. A review of *Daphnia magna* highlights that uptake, assimilation, and elimination pathways for toxic metals are metal-specific, and that tolerance development is closely related to these biokinetic processes. Therefore, the resistance observed in communities under metal exposure involves not only survival but also physiological restructuring in the way the metal enters and is processed by the organism (Tsui and Wang, 2007).

Comparative Assessment of Freshwater and Marine Ecosystems

While the overall direction of metal impacts is similar between freshwater and marine systems, there are significant differences in dominant taxa and exposure dynamics. In freshwater, metal pollution is often associated with the decline of large-bodied

cladocerans, loss of diversity, and a community shift favouring rotifers, whereas in marine systems, copepods appear to play a more dominant and relatively more tolerant role. A study on the Yellow Sea coast reported that copepods were the most frequently encountered group and exhibited higher tolerance to heavy metals. In contrast, freshwater sensitivity comparisons show that cladocerans may be more sensitive to metals such as Cd and Cu (Weng et al., 2025).

Furthermore, in marine systems, it is more evident that zooplankton are not only affected by metals but also act as mediators of transport and trophic transfer. A study in the Gulf of Guinea showed significant bioaccumulation of several metals, including As, Cd, and Pb, in different size fractions of plankton, with accumulation increasing in larger plankton fractions. Therefore, in marine community assessments, plankton size structure and trophic transport appear to be as critical as species composition. In general, "community simplification" is a slightly more dominant theme in freshwater systems, while "transport and bioaccumulation function" is more prominent in marine systems; this latter comparison is a synthesis based on the combined interpretation of existing studies (Chevrollier et al., 2022).

Bioaccumulation, Biomagnetism and Trophic Transfer

Metal Accumulation Levels in Zooplankton Tissues

Zooplankton is considered one of the most critical biotic steps through which metals enter aquatic food webs. Metals can enter zooplankton both directly through uptake from the dissolved phase and via the nutritional route through the consumption of metal-containing phytoplankton, bacterioplankton, and detrital particles. Therefore, metal levels in zooplankton tissues reflect not only the concentration in the ambient water but also species composition, feeding path, and plankton size class. A study conducted in the Gulf

of Guinea showed that 12 metals were bioaccumulated at significant levels in different size fractions of plankton, with toxic metals such as As, Cd, and Pb accumulating particularly in zooplankton fractions. The same study reported that bioaccumulation increased from smaller to larger plankton fractions (Chevrollier et al., 2022).

The levels of accumulation in zooplankton tissues show significant variability depending on the metal species and regional conditions. In a study conducted in the Edremit Gulf, the average metal accumulation order in zooplankton was Fe > Zn > Pb > Mn > Cu > Cr > Cd > Hg; wide concentration ranges were reported for Hg, Cd, Pb, Cr, Cu, Zn, Mn, and Fe based on dry weight. This finding shows that zooplankton can accumulate both essential and toxic metals in their tissues at measurable levels and can be used as a biomarker (Uluturhan et al., 2023).

Bioconcentration Factors (BCF) and Calculation Methods

The bioconcentration factor (BCF) is defined as the ratio of the chemical concentration in an organism to the chemical concentration in water. Review sources indicate that, under laboratory conditions, BCF is primarily based on the concentration measured in the organism resulting from exposure to water, whereas BAF reflects the combined effect of water and nutrient sources in the natural environment. Therefore, in metal studies, BCF is generally expressed as the basic ratio: concentration in organism divided by concentration in water. However, interpreting this ratio for metals is more complex than for organic pollutants because the chemical form of the metal, its modifiability, and whether it is essential can significantly alter the result (DeForest et al., 2007).

For zooplankton, BCF calculations are often performed by dividing dry weight or wet weight tissue concentrations by dissolved water concentrations. Biokinetic studies on *Daphnia magna* have

shown that BCF can be monitored over time and evaluated alongside uptake and elimination rates. A study on coastal mesozooplankton reported that BCF values were consistently above 1 and could exceed 100 for some metals. This indicates that zooplankton are not passive carriers but active, metal-accumulating biotic pools (Tsui and Wang, 2007).

Metal Transfer to Higher Trophic Levels via Zooplankton

As zooplankton occupy an intermediate trophic level between primary producers and fish, metals accumulating in their tissues can be transferred to higher trophic levels. It is emphasised that, in marine systems, plankton act as a "gateway for metals," facilitating trophic transfer to fish and other consumers. The Gulf of Guinea study indicates that high bioaccumulation in plankton can pose risks to coastal communities dependent on seafood. Similarly, the Kalpakkam coast study states that bioconcentration plays a significant role in the transport of metals from lower to higher trophic levels (Chevrollier et al., 2022).

Indirect Effects on Fish

Fish can acquire metal loads either by directly consuming zooplankton or through intermediate organisms that feed on zooplankton. This process is particularly well established for mercury. A 2025 study conducted off the Changshan Archipelago reported that total mercury and methylmercury concentrations increased with trophic level in both invertebrates and fish, with trophic amplification factors above 1, indicating biomagnification. This suggests that metal accumulation beginning in zooplankton and other invertebrates is transferred to fish (Huo et al., 2025).

The indirect effect on fish is not limited to tissue accumulation. When metal loads in zooplankton increase, the nutritional quality of fish during early life stages may decrease, and exposure can place pressure on growth and physiological

performance. The current marine trophic transfer literature consistently shows that zooplankton-based input, especially for Hg, has ecotoxicological significance at higher trophic levels. This result is inferred from the combined interpretation of trophic level and tissue accumulation data, rather than from direct experimental fish–zooplankton manipulation (Huo et al., 2025).

Implications for Human Health

Metal accumulation in zooplankton is significant not directly for human health, but primarily through the consumption of fish and other aquatic products. Recent studies, particularly on mercury, highlight that biomagnification across trophic levels can lead to higher accumulation in seafood, which should be monitored for seafood safety. The Changshan study clearly states that mercury biomagnification is important for assessing local seafood safety. The Gulf of Guinea study also indicates that metal accumulation originating from plankton can pose a food safety risk for coastal communities (Huo et al., 2025).

Therefore, metal load in zooplankton should be considered not only an ecological indicator but also one of the lowest links in the human exposure chain. High bioaccumulation at the zooplankton level, especially for toxic metals such as mercury, cadmium, and lead, can be considered an early predictor of potential accumulation at higher trophic levels. This statement is based on a literature synthesis combining trophic transfer data with an emphasis on food safety (Chevrollier et al., 2022).

Seasonal and Spatial Variability

Metal accumulation in zooplankton is not constant; it varies with season, hydrodynamic conditions, station characteristics, and community structure. The Edremit Bay study showed that the metal pollution index in zooplankton was higher in spring, and point stations had higher average metal levels than transect lines. The

Kalpakkam coast study reported that metal levels were higher in both water and zooplankton tissue during the summer. When these two studies are considered together, it is clear that the seasonal pattern can vary by region, but seasonality remains a strong determinant that cannot be ignored (Uluturhan et al., 2023).

Similar seasonal and spatial patterns have been reported in freshwater systems. A 2024 study in the Rosetta branch of the Nile Delta found that the highest average values of heavy metals were concentrated at certain stations, zooplankton reached its highest density in spring, and there was a significant negative correlation between dominant zooplankton groups and heavy metals. These results indicate that metal accumulation and community structure should be evaluated together with spatial heterogeneity and seasonal cycles (Younis et al., 2024).

Modulator Role of Environmental Factors

Effect of Temperature and Climate Change on Metal Toxicity

Temperature is one of the strongest modulators influencing the effect of metals on zooplankton. Generally, an increase in temperature can alter metal uptake and the rate at which toxic effects appear, as it raises metabolic rate, ventilation, and feeding activity. Recent experimental and field studies show that this effect is not always unidirectional; in some cases, warming increases metal toxicity, while in others it can mask certain low-dose effects. For example, a 2025 study on *Brachionus plicatilis* reported that cadmium at environmental levels delayed the suppression of algal populations by rotifers, but this delay disappeared under warming. The same study highlighted a significant interaction between temperature and cadmium (Sun et al., 2025).

However, it is also clear that warming does not alleviate metal stress in every case. The same study showed that at higher but

non-lethal cadmium levels, rotifers could not completely eliminate algal populations; that is, while warming can mask some low-dose effects, it cannot compensate for stronger metal stress. This suggests that under climate change, the grazing pressure of zooplankton on primary producers may become unpredictable. In the literature, this pattern is interpreted as temperature altering not only organism physiology but also predator-prey interactions and, consequently, energy flow (Sun et al., 2025).

Similar complexity is observed at the community level. An observational study conducted along the Yellow Sea coast between 2021 and 2023 reported that warming and acidification trends in seawater occurred simultaneously, with zooplankton abundance and biomass negatively correlated with temperature, while diversity and evenness were positively correlated. The same study indicated that temperature and pH together were among the main drivers of nonlinear community fluctuations. These results demonstrate that the effects of climate change can reshape metal toxicity not only at the individual level but also at the level of community dynamics and ecological stability (Li and Ding, 2026).

The Role of pH and Acidification

pH is a key environmental variable in interpreting metal toxicity because it directly affects metal speciation and bioavailability. For divalent metals such as Cd, pH and calcium levels together strongly determine toxicity. A study on *Daphnia magna* showed that higher calcium concentrations and lower pH reduced Cd uptake and acute toxicity, indicating that Cd toxicity was closely related to the rate of metal entry into the organism. This finding clearly demonstrates that metal effects should be evaluated not only in terms of ambient concentration but also in conjunction with water chemistry (Tan et al., 2011).

In marine systems, a decrease in pH, i.e., acidification, can also act as an independent stressor on zooplankton communities. A Yellow Sea coastal study reported that pH showed an inverse pattern with zooplankton abundance and biomass compared to temperature, with warming and acidification together driving community fluctuations. Furthermore, shallow hydrothermal fields where low pH and high metal load coexist offer specific systems for observing this interaction in the natural environment. A 2024 marine study showed increased mesozooplankton mortality rates in acidified environments, with effects varying with distance from hydrothermal vents. This result suggests that acidification can increase lethal pressure on zooplankton in metal-rich environments (Li and Ding, 2026).

Therefore, pH is not merely a technical water quality parameter in metal toxicity. Acidification can simultaneously affect metal solubility, ion competition, the organism's ionoregulatory capacity, and community composition. For this reason, future ecotoxicological assessments should consider pH not as a fixed background variable, but as an active regulatory axis; this conclusion is based on a synthesis of current experimental and field data (Tan et al., 2011).

Multiple Stressor Interactions: Metals and Other Pollutants

Zooplankton in natural environments are often exposed to more than one pollutant. Metals, pesticides, microplastics, nutrient stress, temperature increases, and salinity changes frequently act simultaneously. Recent studies on multiple stressors show that these combined effects are often not additive, but can lead to synergistic or, more rarely, antagonistic outcomes. A 2025 study found that pesticide exposure in *Daphnia magna*, combined with rising temperature and nutrient restriction, more strongly disrupted life history characteristics. Similarly, a 2024 study showed that the co-

occurrence of toxicant and nutrient stress can create synergistic effects on *Daphnia* (Shahid et al., 2025).

Metal-pollutant interactions are also important. Previous experimental studies and recent reviews indicate that microplastics can affect the bioavailability, uptake, and trophic transfer of heavy metals. A 2026 review highlights that microplastics can increase the uptake of xenobiotics, such as heavy metals, and facilitate their transport across food webs. At the zooplankton level, this suggests that metals should be considered not only for their direct toxicity but also as stressors that are "transported" or "modified" by other pollutants (Banaee et al., 2026).

In this context, multi-stressor ecotoxicology has moved beyond being a secondary subfield of metal studies. Especially in the age of climate change, the combined assessment of temperature, acidification, nutrient stress, and chemical pollution yields more realistic results than risk assessments based on individual metal tests. This statement is not the direct result of a single study, but a synthesis based on the common thread in recent multi-stressor literature (Double trouble: Shahid et al., 2025).

Special Ecosystems: Polar Regions, Coastal Areas and River Delta Environments

Specific ecosystems highlight the intersection of metal toxicity and environmental modulators. Coastal areas are particularly complex systems for zooplankton due to terrestrial inputs, eutrophication, salinity fluctuations, and multiple pollutant pressures. A study of the Yellow Sea coast has shown that temperature and pH changes strongly influence coastal zooplankton dynamics. Research based on 2018–2023 data from the Doce River estuary and adjacent coastal area indicated that metal(loid) levels and bioavailability varied with river flow, the ratio of particulate organic to inorganic matter, and seasonal hydrodynamic conditions.

This suggests that the impact of metals in coastal and deltaic systems should always be considered alongside physical transport processes (Li and Ding, 2026).

River deltas are also important transitional environments where metal loads are transported into the marine system. The Doce River study demonstrates that a zooplankton-based monitoring index can be useful in assessing metal(loid) pollution and that bioavailability can differ with metal load during periods of high river flow. Because suspended particulate matter, organic matter content, and flow regime vary in delta systems, the same total metal level can lead to different biological outcomes. Therefore, delta environments exemplify how metal toxicity is determined by the "contextual environment" as much as by "concentration" (Rocha et al., 2025).

While direct zooplankton–metal interaction data are more limited for polar and other extreme environments, it is thought that low temperature, seasonality, acidification, and local pollutant inputs may differentiate metal bioavailability in these systems. In contrast, metal-rich natural systems such as shallow hydrothermal vents present an "extreme habitat" model due to the combined effect of low pH, high metal levels, and thermal variability. A systematic review from 2025 reports that mesozooplankton communities in shallow hydrothermal vents are significantly affected by low pH, high metal levels, and thermal variability. Therefore, such specific habitats, along with polar regions, coastal areas, and deltas, should be considered priority monitoring areas in future metal ecotoxicology studies (Thirunavukkarasu et al., 2025).

Monitoring Methods and Ecotoxicological Testing Approaches

Field Studies and Biomonitoring Protocols

Field studies are the primary method for evaluating the effects of metal pollution on zooplankton. These studies typically involve spatial comparisons between stations, seasonal sampling,

and simultaneous physicochemical measurements. Zooplankton samples are usually collected with plankton nets, followed by species identification, abundance, biomass, and diversity assessments; metal analyses are conducted on water and sometimes sediment samples. The Edremit Bay study showed that zooplankton can serve as a biomarker for metal pollution by combining trace metal measurements in zooplankton tissues with spatial and temporal sampling. Similarly, the Yellow Sea coastal study linked community responses to environmental metal pressure by evaluating zooplankton community composition alongside heavy metal concentrations (Uluturhan et al., 2023).

Biomonitoring protocols increasingly include both community composition and biomarker responses. A 2021 study found that metallothioneins and lipid peroxidation in zooplankton can distinguish between polluted and less polluted areas, and that these indicators are useful for predicting ecological impact. This approach complements classical taxonomic monitoring with biochemical responses, allowing detection of metal pressure at an earlier stage (Zebral et al., 2021).

Laboratory-Based Toxicity Tests: Standards and Methodology

Laboratory-based tests enable the quantitative determination of metal toxicity under controlled conditions. One of the most widely used international standards in this field is OECD Test No. 202. This guideline describes the use of a 48-hour acute immobilisation test, typically employing *Daphnia magna*. In the test, young daphnids are exposed to the chemical, and immobilisation is assessed at 24 and 48 hours. The guideline also specifies the pH, light, temperature, and technical requirements for preparing the test solution (OECD, 2004).

For the evaluation of chronic and sublethal effects, OECD Test No. 211 serves as the primary reference. In this test, young

female *Daphnia magna* are monitored for 21 days at a minimum of five concentrations, with reproductive output measured as the main endpoint. According to the OECD, at least 10 individuals are used at each concentration in semi-static tests, and 40 individuals in flow-through tests. This standardisation enhances interlaboratory comparability (OECD, 2012).

However, methodological interpretation in metal testing is insufficient if based solely on nominal concentrations. As variables such as pH, calcium, hardness, temperature, and dissolved organic matter can affect the bioavailability of metals, results must be interpreted in the context of water chemistry. Therefore, standardised tests are evaluated alongside bioavailability and multiple stressor approaches to increase environmental realism (OECD, 2004).

Model Organisms: *Daphnia magna*, *Acartia tonsa*, *Tigriopus* spp.

Daphnia magna is the most widely used model organism in freshwater ecotoxicology. The fact that OECD 202 and OECD 211 are directly based on this species demonstrates its central role in studies of acute and chronic metal toxicity. Furthermore, with the development of genomic tools, *Daphnia* has become a powerful model not only in standard mortality and reproduction tests but also in mechanistic toxicity studies. A study on *Daphnia magna* ecotoxicogenomics showed that different metals produce distinct gene expression profiles even within the same chemical class (OECD, 2004).

Acartia tonsa is a prominent copepod model for marine and brackish water systems. A 2021 study of eight years of control data reported that this species could be stably maintained in multi-generational cultures and exhibited consistent susceptibility to the reference toxicant in acute tests. A 2024 study also demonstrated that *A. tonsa* strains from different geographical origins could be used in

standardised acute and chronic assays. This species has also been used in experiments investigating the dietary intake of metals (Rotolo et al., 2021).

Tigriopus species have gained importance, particularly in coastal and tidal zone ecotoxicology. Studies on *Tigriopus japonicus* have detailed the effects of heavy metals on ROS production, antioxidant enzyme activities, and heat shock proteins. Furthermore, multi-generational proteomic and transcriptomic studies demonstrate that this group is a suitable model for investigating the molecular mechanisms of metal toxicity (Kim et al., 2014; Rhee et al., 2013).

Omics Approaches: Transcriptomics, Proteomics, and Metabolomics

Omics approaches are increasingly used to detect early and subtle effects of metal toxicity that are not apparent with classical endpoints. Ecotoxicogenomics studies on *Daphnia magna* have shown that metal exposures generate specific gene expression profiles and enhance the mechanistic interpretive power of genomic data. This approach has enabled the definition of responses to metal stress not only through mortality or reproductive loss, but also through molecular pathways (Santos et al., 2009).

Proteomic approaches have made significant progress, particularly in marine copepods such as *Tigriopus japonicus*. Proteomic studies under multi-generational mercury exposure have revealed possible protein-level mechanisms associated with Hg toxicity and tolerance. Similarly, studies using global proteome profiling have shown that ocean acidification can alter Hg toxicity and metabolic responses. These findings suggest that proteomic data are valuable for understanding the physiological cost of metal stress (Xu et al., 2016).

Transcriptomics and metabolomics, when used together, provide a more holistic picture. A 2025 study reported that warming and nickel pollution elicit multigenerational transcriptome responses in *Tigriopus japonicus*, and that warming increases Ni toxicity. Metabolomics is considered a complementary tool in metal ecotoxicology because it can capture disruptions in energy metabolism, amino acid cycling, and redox balance. Therefore, omics technologies will make zooplankton-based metal monitoring studies more precise and mechanistic in the future (Zhang et al., 2025).

Use and Evaluation of Biomarkers

Biomarkers are used to detect early and measurable biological responses to metal exposure. The most commonly used biomarkers in zooplankton studies include reactive oxygen species (ROS), lipid peroxidation, antioxidant enzymes, metallothioneins, and heat shock proteins. A study on *Tigriopus japonicus* found that exposure to As, Cd, Cu, Ag, and Zn increased ROS levels and altered heat shock protein responses, as well as antioxidant enzyme activity. These results indicate that metal-induced oxidative stress is a key factor in biomarker-based assessments (Kim et al., 2014; Rhee et al., 2013).

The value of biomarkers in field applications is further increased when combined with community data. A 2021 zooplankton biomonitoring study showed that metallothionein and lipid peroxidation are effective indicators for predicting environmental impact. Additionally, the development of metallothionein-based transgenic *Daphnia* highlights the potential for next-generation biosensor approaches for rapid and sensitive metal detection. Thus, biomarkers are regarded not only as classical biochemical endpoints but also as the foundation for future biosensor-based monitoring tools (Zebral et al., 2021).

Overall, monitoring and testing approaches no longer depend on a single scale. When field biomonitoring, standard laboratory tests, model organisms, omics platforms, and biomarkers are used together, the effects of metal pollution on zooplankton can be assessed much more reliably. This integrated approach provides a stronger scientific basis for both environmental risk assessment and aquatic ecosystem management (Uluturhan et al., 2023).

Conclusion

Synthesis of Existing Knowledge and Key Findings

The literature discussed in this section demonstrates that the effects of metals on zooplankton have a multifaceted and cross-scale structure. At the individual level, mechanisms such as acute toxicity, sublethal effects, oxidative stress, and genotoxicity have been identified; these effects result in species loss, reduced diversity, and the dominance of tolerant species at the population and community levels. Experimental findings, particularly from model organisms such as *Daphnia magna*, reveal the suppressive effects of metals on growth, reproduction, and energy budget (Lampert, 2011; Wang & Rainbow, 2008).

At the ecosystem scale, zooplankton are not only affected by metal stress but also play a central role in bioaccumulation, biomagnification, and trophic transfer processes of metals. In this context, zooplankton are considered both "target organisms" and "carrier vectors" in aquatic food webs. Furthermore, environmental factors such as temperature, pH, salinity, and multiple stressor interactions significantly modulate metal toxicity; therefore, metal effects should be considered contextually (Luoma & Rainbow, 2008; DeForest et al., 2007).

Research Gaps and Limitations

While the current literature has made significant progress, several critical research gaps remain. Firstly, a large proportion of studies focus on a limited number of model species and do not fully represent the species diversity of natural zooplankton communities. Data deficiencies are particularly evident in understudied regions such as tropical and semi-arid areas. Secondly, although laboratory-based studies of single metals are common, scenarios involving multiple stressors and mixture toxicity, which are more frequently encountered in natural environments, are still inadequately represented (Rainbow, 2002).

Furthermore, studies on long-term biological consequences such as genotoxicity, epigenetic changes, and multigenerational effects remain limited. Although omics technologies are rapidly developing, integration of these data with ecological scales is still in its infancy. In addition, methodological differences and lack of standardisation in integrating field data and laboratory results constitute a significant limitation. This can restrict the generalisability of the results obtained (Baird et al., 2007).

Management and Protection Policy Recommendations

Zooplankton-based approaches offer significant opportunities for managing metal pollution. It is recommended that zooplankton community structure and biomarker responses be integrated into water quality monitoring programmes. Within regulatory frameworks such as the European Union Water Framework Directive, it is necessary to assess not only chemical parameters but also biological quality elements. In this context, integrated monitoring approaches proposed by organisations such as the United Nations Environment Programme can contribute to more effective management of metal pollution (UNEP, 2019).

Controlling metal pollution at its source is also crucial. Limiting industrial discharges, reducing the environmental impact of mining activities, and regulating agricultural inputs are fundamental strategies for protecting zooplankton and, consequently, all aquatic ecosystems. Additionally, considering processes such as bioavailability and trophic transfer in risk assessment will enable the development of more realistic environmental quality standards (Luoma & Rainbow, 2008).

Recommendations for Future Research

Future research should adopt multidisciplinary and multi-scale approaches. First, integrating omics technologies with classical ecotoxicological methods will enable monitoring of responses to metal stress from the molecular to the ecosystem level. In addition, increasing long-term and multi-generational experimental studies will improve understanding of the effects of metal pollution on evolutionary and population dynamics.

Second, metal toxicity should be re-evaluated in the context of climate change. The effects of temperature increase, acidification, and hydrodynamic changes on metal bioavailability are not yet fully understood. Therefore, future studies should address multiple stressor scenarios more comprehensively.

Finally, modelling studies that integrate field and laboratory data will play a critical role in predicting ecosystem-level effects of metal pollution. In this context, developing integrated models that use zooplankton as both a bioindicator and a component of trophic transfer will help bridge the gap between scientific knowledge and environmental management. This approach is considered one of the most important future research directions for the sustainable management of aquatic ecosystems (DeForest et al., 2007).

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CHAPTER 3

KRUSTASELERİN GİZLİ KİMYASI: L-TRİPTOFAN, SEROTONİN VE MELATONİN

MEVLÜT AKTAŞ[1]
AHMET BOZKURT[2]
ESRA DEMIRKIRAN[3]

Giriş

Krustase yetiştiriciliği, diğer su ürünleri yetiştiriciliğinde olduğu gibi kaliteli hayvansal proteine olan talebi karşılamayı amaçlayan; su ürünleri yetiştiriciliği sektörü içerisinde oldukça hızlı büyüyen; sürdürülebilir yeni teknolojilerin kullanılabildiği; yüksek fiyatlı ürünlerin yetiştirildiği bir gıda üretim sektörüdür. Global krustase üretiminde yaygın olarak yetiştiriciliği yapılan grupları sırasıyla deniz karidesleri, kerevitler, tatlı su karidesleri, ıstakozlar

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ve yengeçler şeklinde sıralamak mümkündür. Bu gruplar içerisinde ise yetiştiriciliği en baskın şekilde yapılan türleri *Litopenaeus vannamei* (Pasifik beyaz karidesi), *Procambarus clarkii* (Kırmızı bataklık kereviti), *Eriocheir sinensis* (Çin eldiven yengeci), *Penaeus monodon* (Siyah kaplan karidesi), *Macrobrachium rosenbergii* (Tatlısu karidesi) şeklinde sıralamak mümkündür. Bu türlerin toplam krustase üretiminde %93 civarında bir paya sahip olduğu belirtilmektedir (Tacon, 2020). Üretimi yapılan krustaselerin %90'a yakın bir kısmının Asya ülkeleri tarafından yetiştirildiği; yetiştirilen bu ürünün %50'sinin ise tek başına Çin tarafından karşılandığı bildirilmektedir.

Yüksek kaliteli hayvansal proteine yönelik artan küresel talep, krustase yetiştiriciliğinin ivme kazanmasına ve buna bağlı olarak sürdürülebilir üretim sistemleri ile yenilikçi teknolojilerin geliştirilmesine zemin hazırlamıştır. Bu sektörel büyüme, yetiştiriciliği yapılan türlerin büyüme, üreme, morfogenez, osmoregülasyon ve metabolik faaliyetleri üzerindeki endokrin kontrol mekanizmalarının süreçlerini inceleyen bilimsel araştırmaların stratejik bir yoğunluk kazanmasını zorunlu kılmıştır. Küresel sucul ekosistemlerin kilit taksonomik gruplarından biri olan krustase alt şubesi, karmaşık fizyolojik süreçlerini sinir sistemiyle entegre çalışan sofistike bir endokrin mekanizma aracılığıyla yönetmektedir.

Krustaselerde kabuk değişimi (ecdysis), büyüme, üreme, osmoregülasyon, morfogenez ve metabolik faaliyetlerin endokrin kontrolü dikkate değer bir regülasyonel ağ sergilemektedir. Sıcaklık, besin bulunabilirliği, salinite ve pH gibi çevresel faktörler; hormon ve nörotransmitter sentezi ile salınımını modüle ederek organizmanın fizyolojik adaptasyonunda belirleyici rol oynamaktadır. Özellikle son otuz yılda ivme kazanan genomik, transkriptomik ve metabolomik tabanlı araştırmalar, bu karmaşık nöroendokrin yapıların deşifre edilmesine olanak tanımış; yeni

hormonal dizilerin ve enzim yolaklarının tanımlanmasıyla krustase fizyolojisine dair literatüre derinlik kazandırmıştır.

Bu kitap bölümünde, krustaselerde önemli biyolojik işlevlere sahip olan L-triptofan, serotonin ve melatoninin tanımları, işlevleri ve metabolizmaları; son yıllarda yayımlanan araştırma makaleleri ve uluslararası derlemeler ışığında ele alınarak hazırlanmıştır.

L-TRİPTOFAN

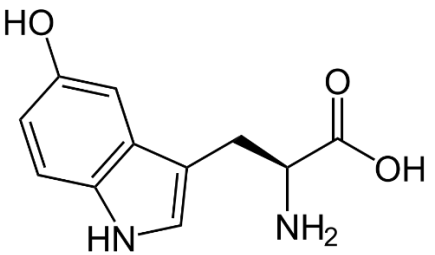
L-Triptofan İşlevi, Yapısı ve Metabolizması

L-triptofan, krustaseler dahil birçok canlı için dışarıdan sağlanması gereken esansiyel bir aromatik amino asittir; organizma tarafından sentezlenemediği için diyetle alınması zorunludur. Kimyasal olarak, bir indol halkası içeren aromatik bir amino asittir ve bu yapı onun karakteristik özelliklerini ve metabolik kaderini belirler (Şekil1). Esansiyel amino asit triptofan, protein biyosentezinin yapısal bileşeni olmasının ötesinde, 5-hidroksitriptofan aracılığıyla nörotransmitter serotonin gibi biyoaktif bileşiklerin ve melatonin gibi nöroendokrin düzenleyicilerin öncüsü olarak da görev yapar. Bu metabolik dönüşümler, triptofandan türetilen aktif bileşiklerin hem nöroendokrin hem de immün fonksiyonlarda sinyalleşme rolünü üstlendiğini göstermektedir. Dolayısıyla, L-triptofan ve metabolitleri bağışıklık da dahil olmak üzere birçok metabolik, fizyolojik ve patofizyolojik fonksiyonda önemli roller oynamaktadır (McCoole vd., 2012, Zhang vd., 2018, Huang vd., 2020).

Krustaselerde L-triptofan besinlere eklendiğinde hem büyüme performansı hem de fizyolojik dayanıklılık üzerinde olumlu etkiler rapor edilmiştir. Örneğin, Çin yengeci *(Eriocheir sinensis)* üzerinde yapılan çalışmalarda diyetle L-triptofan takviyesinin hem

ölüm oranını azalttığı hem de hemolymph ve dokulardaki bağışıklık ve antioksidan enzim aktivitelerini artırdığı gösterilmiştir. Ayrıca triptofanın nörotransmitter öncüsü olarak davranması, agresiflik gibi davranışsal tepkilerin modülasyonu ve stres yanıtının düzenlenmesinde önemli bir role sahip olduğunu düşündürmektedir (Zhang vd., 2018).

Yapısal olarak, L-triptofan bir alfa-amino grubu, bir karboksil grubu ve bir indol halkasına sahip bir yan zincir içerir. Bu indol halkası triptofanın ultraviyole ışığı absorbe etme yeteneğini açıklar ve proteinlerin floresans özelliklerine katkıda bulunur. Kiral bir merkez içerdiğinden, L-izomeri biyolojik olarak aktif formudur.



Şekil 1. L-Triptofan'ın kimyasal yapısı (5-Hydroxy-L-Tryptophan)

Triptofan metabolizması, başlıca iki ana yol üzerinden gerçekleşir: serotonin yolu (5-hidroksitriptofan yolu olarak da bilinir) ve kinürenin yolu. Serotonin yolu, triptofanın triptofan hidroksilaz (TPH) enzimi tarafından 5-hidroksitriptofana (5-HTP) dönüştürülmesiyle başlar. Daha sonra 5-HTP, aromatik L-amino asit dekarboksilaz (AADC) tarafından serotonin (5-HT) adı verilen önemli bir nörotransmiter ve hormona dönüştürülür. Serotonin, ruh hali, uyku, iştah ve sindirim üzerinde düzenleyici etkilere sahiptir. Serotonin ayrıca, uyku-uyanıklık döngüsünü düzenleyen bir hormon

olan melatoninin öncüsüdür. Hipofiz bezinde ve gastrointestinal sistemde bu dönüşümler gerçekleşebilir.

Kinürenin yolu ise, triptofanın metabolizmasındaki ana yoldur ve alınan triptofanın %95'inden fazlasını oluşturur. Bu yol, indolamin 2,3-dioksijenaz (IDO) veya triptofan 2,3-dioksijenaz (TDO) enzimleri tarafından N-formilkinürenine dönüşümü ile başlar. Kinürenin yolu boyunca, kinürenin, kinüregenin asit, antranilik asit, pikolinik asit ve nikotinamid adenin dinükleotid (NAD+) gibi çeşitli biyoaktif metabolitler üretilir. Bu metabolitlerin bazıları nöroaktif, immünomodülatör ve antioksidan özelliklere sahiptir. Örneğin, kinüregenin asit bir NMDA reseptör antagonisti olarak işlev görürken, NAD+ hücresel enerji metabolizmasında kritik bir koenzimdir. Kinürenin yolunun disregülasyonu, nörodejeneratif hastalıklar, inflamatuar bozukluklar ve bazı kanser türleri dahil olmak üzere çeşitli patolojilerle ilişkilendirilmiştir.

Karasal omurgalılarla karşılaştırıldığında, denizel organizmaların triptofan metabolizması konusu oldukça yenidir ve detaylı bilgi bulunmamaktadır. Bununla birlikte, bazı denizel türlerde bağışıklık, büyüme ve gelişme ile çeşitli fizyolojik süreçlerde birtakım işlevlere sahip olduğu yapılan çalışmalarla bildirilmektedir (Tablo 1). *Eriocheir sinensis*'te L-triptofanın yaşama oranı, immün yanıt ve bağırsak mikrobiyotası üzerine etkisi araştırılmış, araştırma sonunda %0,73 düzeyinde L-triptofan katkılı yemlerle beslenen yengeçlerin yaşama oranının arttığı, bağırsak mikrobiyotasının zenginlik ve çeşitliliğinin arttığı bildirilmiştir (Yang vd., 2019). Benzer şekilde, triptofan ve ara ürünlerinin Tatlısu karidesi *Macrobrachium* rosenbergii'nin embriyonik gelişimini iyileştirdiği (Tinukul, Poljaroen, Tinukul ve Sobhon, 2016), *S. serrata*'nın agresyon (saldırganlık ve kavga) düzeyini azalttığı (Leopoldo, Lacivita, Berardi, Perrone ve Hedlund, 2011), Tatlısu

yengeci *Barytelphusa guerini'*nin de embriyonik gelişimine olumlu katkılar yaptığı bildirilmiştir.

Krustaselerde kabuk değişimi ve yenisinin oluşumu sürecinde ve uzuv kaybı olması durumunda bağışıklık ve yaşama oranı düşerken, besinlerine triptofan ilave edilen bireylerde toplam hemosit sayısı, antioksidan kapasitesi gibi immün sistem belirteçlerinin iyileştiği ifade edilmektedir (Zhang vd., 2018).

Serotonin'in bir aminoasit öncüsü olan triptofan Türk kereviti *Astacus leptodactylus* dietlerine farklı oranlarda ilave edilerek büyüme, gelişme parametrelerine bakılmıştır (Harlıoğlu, Harlıoğlu, Yonar ve Duran, 2014) . Araştırmada %1 oranında yemlere ilave edilen triptofanın hemolenfteki serotonin düzeyini yükselttiği, dolayısıyla yaşama ve büyüme oranının yükselmesine önemli ölçekte etki ettiği bildirilmiştir. L-Triptofanın Büyüme performansını artırdığına yönelik olarak benzer sonuçlar çamur yengeci, *Scylla serrata* (Laranja, Quinitio, Catacutan ve Coloso, 2010) ve *Litopenaeus vannamei* (Sun, Guan, Xiong, Xi ve Zhang, 2015) için de bildirilmiştir.

Yemlerine %0.73 düzeyinde Triptofan ilave edilen Çin yengeci (*Eriocheir sinensis)'*nde uzuv regenerasyonunun arttığı bildirilmiştir (Zhang vd., 2019). Araştırılar bu düzeyde triptofan destekli yemlerle beslenen yengeçlerin MIH genlerinin aşağı yönlü, ecdystreoidlerin yukarı yönlü düzenlendiğini ifade etmişlerdir. Tüm bunlara ilaveten triptofan destekli yemlerle beslenen yengeçlerin sindirim enzim aktivitelerinin iyileştiğini, dolayısıyla sindirim ve absorbsiyon işlevinin arttığını belirtmişlerdir. Kısaca Triptofan destekli yemlerle beslenen yengeçlerde regenereasyon kapasitesinin arttığını, bu durumun diğer krustaseler için de geçerli olduğunu ifade etmek mümkündür.

Tablo.1 L-Triptofan uygulamasının krustaselerde temel etkileri

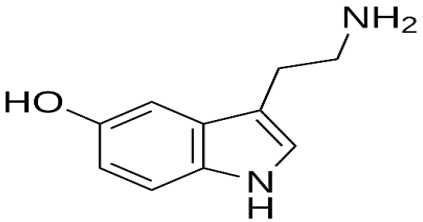
Tür	L-triptofan,	Uygulama dozu/ uygulama yolu	Temel etki ve bulgular	Kaynak
Macrobrachium nipponense Nehir karidesi	L-triptofan	%0,35 - %0,39 yem	Büyüme performansının artması ve saldırgan davranışın azaltılması	Cao vd., (2025)
Eriocheir sinensis Çin eldiven yengeci	L-triptofan	7,3-10,5 g/kg yem	Kabuk değişimini indükleme, besin alımını ve sindirim enzimi aktivitelerini önemli ölçüde iyileştirme	Zhang vd, (2021)
Scylla paramamosain	L-triptofan	3.94–3.98 g/kg yem	Büyümeyi uyarma,sindirim enzim aktivitesini artırma, bağışıklığı güçlendirme ve agresifliği azaltma	Feng vd., (2024)
Scylla serrata Çamur Yengeci	L-triptofan	0.5%–1% yem	Büyüme performansı ve yaşama oranında artma, Agonistik davranışda baskılanma.	Laranja vd., (2010)
Astacus leptodactylus Türk Kereviti	L-triptofan	%1 Yem	Hemolenfte serotonin düzeyi artmış; yaşama ve büyüme oranı yükselmiştir.	Harlıoğlu vd., (2014)
Eriocheir sinensis Çin eldivenYengeci	L-triptofan	%0.7 Yem	Uzuv rejenerasyonu artmış; MIH genleri baskılanmış, ecdysteroidler ve sindirim enzim aktivitesi artmıştır.	Zhang vd., (2018)

Eriocheir sinensis Çin eldiven yengeci	L-triptofan	0.53 -0.70% Yem	Diyetle alınan triptofan, THC, hemosiyanin, ACP ve ALP aktivitesini ve hemosit fagositoz aktivitesini artırır.	Zhang vd., (2019)
Eriocheir sinensis Çin eldiven yengeci	L-triptofan	0.36 -0,73 %Yem	Hayatta kalma oranını artırır, bağırsak mikrobiyotasının zenginliğini ve çeşitliliğini önemli ölçüde artırır.	Yang vd., (2019)
L. vannamei Pasifik beyaz karidesi	L-triptofan	0.36 % yem - 1.72 % yem proteini	5-HT ve GABA yollarını düzenleyerek büyüme performansını artırır.	Sun vd., (2015)
Eriocheir sinensis Çin eldiven yengeci	L-triptofan	0.75% yem	Erken dönemlerde diyetle alınması durumunda bağışıklığın ve antioksidan yeteneğin geliştiği, uzun süreli L-triptofan desteğinin ise bağışıklık ve antioksidan yeteneği üzerine olumlu bir etkisinin olmadığı	Hou vd., (2022)
Procambarus clarkii Kırmızı bataklık kereviti	L-triptofan	0.39% yem	Büyüme, kas kalitesi ve bağırsak mikrobiyotasını iyileştirir.	Chen vd., (2026)

SEROTONİN

Serotonin 5-HT, (5-Hidroksitriptamin) İşlevi, Yapısı ve Metabolizması

İlk kez 1948 yılında tanımlanan ve etimolojik kökeni Latince serum ile Yunanca tonus kelimelerine dayanan serotonin (5-Hidroksitriptamin, 5-HT), biyojenik bir monoamin olup; epinefrin, norepinefrin, dopamin ve histamin ile aynı grupta yer almaktadır (Şekil 2). 5-HT; davranış, uyku döngüsü, stres yanıtı, agresyon, glikoz homeostazı ve reprodüksiyon gibi geniş bir fizyolojik spektrumda modülatör rol oynamaktadır (Lam ve Heisler, 2007; Donovan ve Tecott, 2013; Jones, Sun, Martin ve Keating, 2020). Hem omurgalılarda hem de krustaselerde temel sentez bölgeleri gastrointestinal mukozadaki enterokromafin hücreleri ve periferik sinir sistemidir. Krustaselerde serotonin biyosentezi, esansiyel bir amino asit olan L-triptofanın, triptofan hidroksilaz (TPH) enzimi aracılığıyla 5-hidroksitriptofana (5-HTP) hidroksillenmesi ve ardından aromatik L-amino asit dekarboksilaz ile dekarboksilasyona uğramasıyla gerçekleşir. B3, B6 vitaminleri ve magnezyumun kofaktör olarak katıldığı bu enzimatik süreçte, diyetle alınan triptofanın yalnızca yaklaşık %5'i serotonerjik yolağa aktarılmaktadır. Geri kalan triptofan fraksiyonu, hepatopankreasta triptofan pirrolaz aktivitesiyle kinurenin yoluna girmektedir (Boadle-Biber, 1993). Bu doğrultuda, su ürünleri yetiştiriciliğinde rasyonun triptofan içeriği, serotonerjik sinyal iletimi ve buna bağlı olarak bağışıklık ile ekdaysis (kabuk değişimi) gibi kritik süreçlerin regülasyonu için belirleyici bir parametredir (Meunpol, Hall ve Kapoor, 1998).



Şekil 2. Serotonin'in kimyasal yapısı (5-Hidroksitriptamin; 5-HT)

Serotonin (5-Hidroksitriptamin; 5-HT), hem omurgalı hem de omurgasız taksonlarında çift yönlü işlevselliğe sahip kritik bir biyojenik amindir. Gastrointestinal sistemde sindirim süreçlerini ve motiliteyi regüle eden bir periferik hormon olarak işlev görmesinin yanı sıra; merkezi sinir sisteminde (MSS) nöronlar arası sinyal iletimini sağlayan temel bir nöromodülatördür. Bu nörokimyasal, presinaptik terminallerden salınarak hedef hücrelerdeki spesifik reseptörler aracılığıyla mesaj iletimini gerçekleştirmekte ve böylece organizmanın kompleks fizyolojik yanıtlarını koordineli bir şekilde yönetmektedir.

5-HT bazı omurgalılarda kan-beyin bariyerini (kan ile beynin interstisyumu arasında seçici yarı geçirgen bir zar nedeniyle) geçemediğinden (yani bağırsakta üretilen serotoninin beyne ulaşması mümkün değildir) merkezi ve periferik havuzları anatomik olarak ayrılabilir. Bu nedenle kan-beyin bariyerini geçen TRP miktarı beyinde ne kadar 5-HT üretilebileceğini düzenler (Gupta, Dhanda ve Sandhir, 2019). Memelilerde vücuttaki 5-HT'nin yaklaşık %90-95'i periferde, çoğunlukla hücre içi gastrointestinal hücrelerde bulunur. Monoamin oksidaz beyindeki 5-HT gibi monoaminlerin oksidasyonunu katalize eden birincil katobolik enzimdir. Kabuklularda 5-HT, fizyolojik ve davranışla ilgili tepkilere neden olan serotoninerjik sinyal iletiminde rol oynar. 5-HT'nin üretimi ve salınımı mekanizması, nöromusküler preparat

üzerindeki etki mekanizması ve krustaselerdeki reseptörleri Boadle-Biber tarafından 1993'te açıklanmıştır. 5-HT'nin çok çeşitli etkilerinin en az 14 reseptörden kaynaklandığı ileri sürülmektedir. 5-HT_3 tıpkı gama-aminobütirik asit gibi ligand kapılı Na+/K+ iyon kapılarıyla benzersiz bir şekilde çalışır. 5-HT_3 hariç tüm 5-HT reseptörleri G proteinine bağlıdır. Kabuklularda şimdiye kadar üç reseptör alt tipi belirlenmiş olmasına rağmen, yapılan çalışmalarda çok sayıda reseptörün tanımlanabileceği belirtilmektedir. Postsinaptik 5-HT reseptöründeki sinyallemenin gücü ve süresi hem omurgalılarda hem de omurgasızlarda sinaptik aralıktaki (bir nöronun akson terminali ile hedef hücre, doku veya bir sonraki nöron arasındaki boşluk*)* 5-HT'nin bolluğuna göre belirlenir. Sinaptik aralıktaki 5-HT'nin mevcudiyeti, 5-HT'nin otoreseptörü olan 5-HT_{1A} reseptörüne bağlanması ve 5-HT taşıyıcılarının aktivitesi tarafından kontrol edilir. 5-HT otoreseptörünün uyarılması negatif geri bildirim oluşturur ve 5-HT salınımını azaltır. 5-HT taşıyıcıları ise sinaptik aralıktan 5-HT'yi uzaklaştırır (Nichols ve Nichols, 2008). Serotonerjik düzenleme altında olan nöropeptitler arasında oreksin, madde P, vasküler büyüme faktörü ve nöropeptit Y bulunur (Muller ve Cunningham, 2020).

Enterik sinir sistemi bağırsak-beyin ekseninden sorumludur ve omurgalılarda besin sindirimi ve emiliminin tüm sürecini kontrol eder. 5-HT'nin enterokromafin (EC) hücrelerinden salınımı, omurgalı ve omurgasızlarda bağırsak kaslarının kasılıp gevşemesini, hareketliliğini ve salgılarını kontrol eder. Bazı sindirim sistemi bozukluklarının 5-HT yetersizliği veya enterositlerdeki taşıyıcıların yetersizliği ile ilgili olduğu ifade edilmektedir. Diğer yandan 5-HT'nin bağlanması, taşınması veya metabolizmasının manipüle edilmesi, vücutta aşırı 5-HT seviyesinin artmasına neden olur ki bu durum 5-HT sendromu olarak tanımlanmaktadır (Nichols ve Nichols, 2008). Sınırlı olmakla birlikte, balık ve krustaselerde 5-HT sendromunun gözlendiğini ve hem periferik fizyolojiyi hem de

merkezi sinir sistemini bozduğunu bildiren çalışmalar bulunmaktadır.

Serotoninin oldukça uzun bir evrimsel geçmişi vardır. Kabuklular gibi tüm hayvansal organizmaların gelişiminde, büyümesinde, kabuk değişiminde, üremesinde, stres tepkimesinde, immün sisteminde ve davranışlarında geniş bir etki yelpazesine sahiptir. Karides, yengeç, kerevit, istakoz gibi krustase türlerinde günümüze kadar agresif davranış sonucu ortaya çıkan kanibalizm, çevresel etkenler ve diğer bazı etkenlere bağlı oluşan stres, günümüzde dahi çok yaygın bir teknik olarak kullanılan gözsapı kesimiyle yumurta ve yavru üretimine alternatif olarak seretonin kullanarak gonad gelişiminin indüklenmesi gibi farklı konularda çok sayıda çalışma ile bu maddenin etkinliği test edilerek ispatlanmıştır. Sonuç olarak, 5-HT'nin krustaselerdeki varlığı, metabolizması ve etkileri ilgi literatür kullanılarak değerlendirilmiştir.

Bilindiği gibi, besin metabolizması; besinlerin vücudun kullanabileceği bir madde haline dönüştürülme sürecindeki tüm hücre içi kimyasal süreçleri (anabolizma ve katabolizma) kapsayan işlemler basamağıdır. Hücre enerjisi ya besinlerden ya da vücutta depo halinde bulunan karbonhidrat, lipit ve proteinlerin aerobik katabolizmasından üretilmektedir. Metabolik reaksiyonlar içerisindeki oksidasyon süreciyle enerji ortaya çıkmaktadır. Aerobik metabolizma sırasında, elektron taşıma zincirinin sonunda oksijen tüketilir ve Krebs döngüsü yoluyla karbondioksit üretilir. Diğer yandan enerji glikoz sayesinde laktat üretimiyle anaerobik olarak da üretilebilmektedir. Ara metabolizmanın temel amacı vücudun ihtiyacı olan enerjiyi sağlamaktır. Krustaselerde gözsapı nöropeptitlerinin birçok fonksiyonlu üyesi olan hiperglisemik hormon (CHH) ve 5-HT enerji dengesinde önemli rol oynamaktadır (Chen, Toullec ve Lee, 2020).

Periferik serotonin memelilerde stres, açlık, agresyon ve üreme gibi birçok sürecin gerçekleştirilmesinde enerji temininde büyük bir öneme sahiptir. Serotonin ayrıca insülin ve glikojen sentezini etkileyip hipoglisemiye ya da hiperinsülinemiye neden olabilir (Haleem ve Mahmood, 2021). Kısaca, 5-HT'nin omurgalılarda kısa süreli enerji sağlamada, uzun vadede enerjinin korunması ve desteklenmesi amacıyla çeşitli fizyolojik adaptasyonların düzenlenmesinde önemli bir rol oynadığı ifade edilmektedir. Bununla birlikte, su canlıları üzerine yapılan çalışmaların yeterli olmadığı ve daha fazla araştırma yapılmasına ihtiyaç duyulduğunu belirtmek gerekir.

Literatürde 5-HT'nin enerji homeostazi üzerine etkileri ile ilgili kabuklularda çok az sayıda araştırma mevcut olup, bu araştırmalar genellikle antidepresanların enerji homeostazi üzerine etkileri ile sınırlıdır. Fluoksetin hidroklorür ile maruz bırakılan fantom karidesi (*Palaemon pandaliformis*)'nde metabolizma hızı ve amonyak atılımının arttığı, dolayısıyla da rutin metabolizmanın yükseldiği belirtilmiştir (Rezende, Almeida, Henriques ve Barbieri, 2021). Adenozin trifosfatın bir türevi cAMP'a bağımlı olan Protein kinaz A glikojen, şeker ve lipit metabolizmasını düzenler. Kırmızı bataklık kerevitinin agonistik davranışlarında cAMP-PKA sinyal yolunun önemli bir rol oynadığı bildirilmiştir Genellikle agonistik davranışı takiben gerçekleşen kavgada cAMP konsantrasyonundaki artışın veya azalışın kaybedeni veya kazananı belirlediği bildirilmiştir (Momohara, Minami, Kanai ve Nagayama, 2016). Yapılan bir başka çalışmada, aydınlık/karanlık, aydınlık/aydınlık ortamlarda tutulan ve 5-HT enjekte edilen mermer kerevit olarak isimlendirilen *Procambarus virginalis* negatif fototaksiden pozitif fototaksiye geçmiştir. 5-HT kaynaklı pozitif fototaksiye, azalan cAMP seviyesinin neden olduğu belirtilmiştir. Özetle, bu yolun agresyon sırasında enerji sağlamaktan sorumlu olduğu belirtilmiştir.

Kısaca, yapılan çalışmalar enerjinin düzenlenmesinde 5-HT'nin rolünün önemini açık bir şekilde göstermektedir.

Vücudun enerjiye ihtiyaç duyduğu zaman ortaya çıkan ATP (Adenozin trifosfat) tedariğindeki zorluklar laktat üretimini uyararak ATP hemostazını desteklemek amacıyla hayvanlarda kısa, orta ve uzun vadede hücresel adaptasyonlara yol açar. Laktat omurgalı ve omurgasızlarda temel enerji kaynağı, glukoneojenik öncü ve endokrin benzeri etkilere sahip bir sinyal molekülüdür (Brooks, 2020). Beyin normal sinaptik fonksiyonları sürdürebilmek için laktata ihtiyaç duyar. Hayvansal organizmalarda hipoksik durumlardan çıkma sürecinde nöronlar laktata ihtiyaç duyar. Aynı zamanda laktatın beyinde sinyal verme rolü olduğu da belirtilmektedir (Proia, Di Liegro, Schiera, Fricano ve Di Liegro, 2016).

Kabuklularda büyüme balıklarla karşılaştırıldığında, sabit kalsifiye yapılara sahip olmamaları, büyüyebilmek için kabuk değiştirmek zorunluluğu ve abiyotik faktörler gibi eksternal faktörlerin etkilerinden dolayı oldukça karmaşık bir süreci kapsamaktadır (Chang, Sun, Chen ve Yeh, 2012). Genel olarak kabuk değiştirme sıklığı bir büyüme faktörü olarak görülmekte ve kabuk değiştirme sıklığının artması potansiyel olarak büyümenin arttığı anlamına gelmektedir. Bununla birlikte kabuk değiştirerek büyümenin sağlanması vücut ağırlığı olarak değil de vücut boyutunun büyüklüğünün artması olarak ifade edilebilir. Kısaca kabuk değiştirmeksizin dekapodlar büyüyemez. Kabuk değişimini engelleyen hormon (MIH, Moult inhibiting Hormone) Krustase hiperglisemik hormon (CHH), Nöropeptit Y ve diğer nöropeptitler, Metil Farnesot (MF) ve Kabuk değiştirme hormonu (E, Ecdyson) kabuk değişim sürecini düzenleyen en önemli hormonlardır. Son yıllarda gelişen Yüksek verimli dizilim teknolojisi sayesinde ilgi genler, proteinler ve bunların yolakları açık bir şekilde tanımlanmıştır. Bununla birlikte araştırmalara konu olabilecek bir

hayli açık alan bulunsa da yapılan ilerlemeler göz ardı edilemez. Yukarıda değinildiği gibi, serotonin (5-HT) gibi bioaminler birçok hormonu ve nöropeptitleri etkileyerek kabuk değişiminin indüklenmesinde ve büyüme sürecinin düzenlenmesinde etkin rol oynarlar. Beslenme karmaşık bir fizyolojik süreci kapsamaktadır. Sağlıklı canlılarda beslenmenin artması potansiyel olarak büyümenin indüklenmesine neden olur. Krustaselerde Serotonin (5-HT) ki direkt olarak beslenmeyi ve iştahı etkileyerek büyümenin gerçekleşmesini hızlandırabilir. Bu molekül aynı zamanda enerji denge yolunu ve sistemik enerji hemostazını düzenleyip büyüme sürecindeki enerji ihtiyacının maksimize edilmesinde önemli bir görev üstlenir.

Sainath ve Reddy (2010), tatlısu yengecinde (*Oziotelphusa senex senex)* kabuk değişiminin indüklenmesi, büyüme ve gelişme üzerine serotonin ve melatonin enjeksiyonunun etkilerini araştırdıkları çalışmalarında serotoninin herhangi bir etkisinin olmadığını belirtmişler, hatta serotonin enjekte edilen yengeçlerde kabuk değişiminin olumsuz etkilendiğini rapor etmişlerdir. Aktaş, Ciğer, Genç, Genç ve Çavdar, (2014) beyaz karides *Litopenaeus vannamei* diyetlerine farklı dozajlarda serotonin ilave edilmesinin kabuk değişimi, büyüme ve gelişme parametrelerine etkilerini test etmişler, kabuk değiştirme sıklığının 20mg/kg serotonin dozaj katkılı yemlerle beslenen karideslerde önemli ölçüde azaldığını, yani kabuk değişimini indüklenmediğini, büyüme ve gelişme açısından herhangi bir etkisinin ise olmadığını belirtmişlerdir. Araştırmacılar bu durumun serotonin'in Y organ tarafından salınan kabuk değiştirmeyi inhibe eden hormon seviyesinin artmasından kaynaklandığını ifade etmişlerdir. Mattson ve Spaziani (1985), Huberman (2000) 5-HT'nin MIH salınımını uyardığı ve bu durumun Y organı tarafından üretilen Ecdysteroidlerin sentezini ve kan içerisine salınmasını engellediği bildirilmektedir.

Dekapod yetiştiriciliğinde agresyon ve kanibalizm kaynaklı yaralanmalar, eksik veya kırık uzuvlar dolayısıyla bunlara bağlı yaşama oranının düşük çıkması genel olarak ortaya çıkan yaygın bir durumdur. Bu durum elde edilen ürünün miktarının ve kalitesinin düşmesine ve pazarlamada problemler çıkmasına neden olmaktadır. 5-HT katkı maddelerine karides, yengeç gibi tüm krustase türlerinin nasıl tepki verdiklerinin belirlenmesi, uygun doz ve maliyetin ortaya çıkarılması gibi konuların çözüme kavuşması triptofanı daha akılcı bir madde haline getirebilir. 5-HT sentezinde rolü olan enzimlerin yemlere ilave edilmesi veya enjeksiyon yolu ile verilmesi de temel araştırma konularındandır. Yetiştiriciliği yapılan birçok krustase türünde yumurta ve yavru üretiminde tek taraflı veya çift taraflı gözsapı kesimi yaygın olarak kullanılan bir tekniktir (Aktaş, Kumlu ve Eroldoğan, 2003, Nagaraju, 2011). Gözsaplarında bulunan X organ - Sinüs bezi kompleksinin ortadan kaldırılması ile kabuk değişimini engelleyen MIH hormonu ve MOIH seviyesi minimuma indirgenmektedir. Gözsapı kesimini takip eden süreç içerisinde hemolenfte Metil Farnesot ve Ecdysteroid sentezi ve salgılanması artmaktadır. Bu durumda kabuk değişimi, çiftleşme ve gonad gelişimi sürecinin hızlanması ve üremenin indüklenmesi sağlanmaktadır. Gözsapı kesimi ile her ne kadar gonad gelişimi ve yumurtlama indüklense de elde edilen yumurtaların sayısında, larvaların kalitesinde ve anaçların yaşama oranında ilerleyen süreçte düşmeler yaşandığı kaydedilmiştir (Aktaş ve Kumlu, 1999, Esmaeili, Kadri ve Kumar, 2025). Gözsapı kesiminde yaşanan olumsuzlukları gidermek amacıyla çeşitli alternatif metotlar geliştirilmiştir. Bu teknikler arasında su sıcaklığı-fotoperiyot uygulaması (Aktaş vd., 2003), hormon ve nörotransmitter enjeksiyonu gibi metotlar araştırma konusu olmuştur. Gözsapı kesimine alternatif olabilecek test edilen bu metotlar içerisinde en fazla ilgi 5-HT enjeksiyonu ile ilgili olup, birçok farklı karides, yengeç ve kerevit türünde araştırma konusu olmuştur (Tablo 1). Kabuk değişimi ile yakından ilişkili olan serotoninin göz saplarının

nörosekretör hücrelerinde varlığı birçok araştırmacı tarafından bildirilmiştir (Bellon Humbert ve Van Herp, 1988; Rodrigoz Sosa, Picones, Calderon Rosete, Islas ve Arechiga, 1997). Yapılan araştırmalarda serotoninin kabuk değiştirme ve üreme üzerine etkilerinin çok yönlü olduğunu ve birçok türde kabuk değişimini ve üremeyi indüklediğini söylemek mümkündür.

Krustaselerin embriyonik ve larval gelişim sürecinde de 5-HT'nin önemli etkilerde bulunduğu çeşitli çalışmalarda rapor edilmiştir. Tatlısu karidesi, *Macrobrachium rosenbergi* ile yapılan bir çalışmada, 5-HT ve dopamin düzeyinin erken embriyo döneminden son gelişme dönemine kadar, hatta portakal sarısı renkli yumurta aşamasından gri-siyah yumurta aşamasına kadarki döneme kadar arttığı bildirilmiştir (Tinikul vd., 2016). 5-HT'nin krustaselerde embriyodan larval döneme ve larval dönemlerden metamorfoz geçirerek postlarval dönemlere geçişte etkili olduğu bildirilmektedir (Cristei, 2014). Aynı şekilde Amerikan ıstakozu, *Homarus americanus*'un (Beltz ve Kravits, 2002), Avustralya kereviti, *Cherax destructor* (Rieger ve Harzsch, 2008) ve kırmızı kral yengeç, *Paralithodes camtschaticus* 'un embriyonik dönem başlangıcında ve larval dönemlerinde stomagastrik sinir sisteminde 5-HT varlığından söz edilmektedir. Bunlara ilaveten 5-HT'nin kaslarda ve barsaklarda CHH gibi diğer nörohormonların sentezini ve salınımını uyardığı, bu durumun embriyonik süreç boyunca büyümenin indüklenmesini de sağladığı bildirilmektedir (Nagaraju, 2011). Özetle, 5-HT krustaselerde kabuk değişimi, büyüme, rejenerasyon ve gelişimde önemli bir rol oynamaktadır.

Hayvanlarda yem alımının artması ve buna bağlı olarak sindirim ve emiliminin artması, büyüme performansının artması anlamına gelmektedir. Besin alımı ise beslenme davranışı ve iştah tarafından belirlenmektedir. Bunlar ise merkezi ve periferik endokrin yollar arasındaki etkileşim ile düzenlenmektedir. İştah davranışı hareket etmeyi, çevredeki besinin algılanmasını,

hareketlerin yavaşlatılmasını ve yiyecek aramayı içeren bir süreçtir. Tüketim davranışı ise besinin yakalanmasını ve tüketmeye başlamayı kapsar (Tierney, 2020). Hepatopankreas ya da orta barsak (midgut) olarak bilinen sindirim bezi çeşitli sindirim enzimlerinin (Lipaz, amilaz, esteraz ve kitinaz gibi) salgılanmasından ve sindirilen yemin emiliminden sorumludur. Hepatopankreas ya da midgut içerisinde EC (enterekromafin hücre) hücreleri dağınık olarak bulunmakta ve çeşitli biyolojik ve fizyolojik süreçlerin düzenlenmesinde kritik görevler üstlenen nörotransmitterleri ve nöropettitleri salgılarlar. Sindirim bezine yayılmış olarak bulunan bu hücreler periferik 5-HT üretimini sağlarlar (Guo ve Xi, 2022). EC hücreleri canlı tarafından yutulan ve sindirilen besin maddelerini ve mikrobiyal metabolitleri tespit ederek gerekli cevapları verebilme kapasitesine sahiptirler. Gut mikrobiomu periferik 5-HT üretimini düzenleyip üretebilir. Barsak –Beyin aksisine en önemli katkı sağlayan faktörlerden bir tanesi barsak mikrobiyotası ve EC hücreleri arasındaki interaksiyondur.

Tablo2. Serotonin, 5 HT uygulamasının krustaselerde temel etkileri

Tür	Serotonin	Uygulama dozu/ uygulama yolu	Temel etki ve bulgular	Kaynak
Litopenaeus vannamei Pasifik beyaz karidesi	Serotonin	20 mg/kg Yem	Kabuk değiştirme sıklığında **azalma**, büyüme ve gelişme üzerinde olumlu etkiler.	Aktaş vd., (2014)
Penaeus semisulcatus Yeşil Kaplan Karidesi	Serotonin	50 µg g^{-1} Vücut ağırlığı, enjeksiyon	Gonad gelişimi ve yumurtlamayı indükleme	Aktaş ve Kumlu (2005)
Litopenaeus vannamei Pasifik beyaz karidesi	Serotonin	50 µg g^{-1} Vücut ağırlığı, enjeksiyon	Gonad gelişimi ve yumurtlamayı indükleme	Vaca ve Alfaro, (2000)
L. vannamei Pasifik beyaz karidesi	Serotonin	14 µL -, 25 µg/g vücut ağır. Enjeksiyon	Gözsapı kesilen karideslerde Spermatofor kalitesi ve miktarı artmamış Kesilmeyenlerde Gonad gelişimi ve yumurtlamayı indükleme	Sainath ve Reddy, (2010)
L. stylirostris Mavi Karides	Serotonin	25 µg g^{-1} vücut ağırlığı, Enjeksiyon	5-HT ve spiperonun birlikte enjeksiyonunun sadece olgunlaşmayı ve yumurtlamayı tetiklemekle kalmayıp, aynı zamanda olgunlaşmayı teşvik eden feromonların suya salınımını sağlama	Quesada-Avila vd.., (2024)

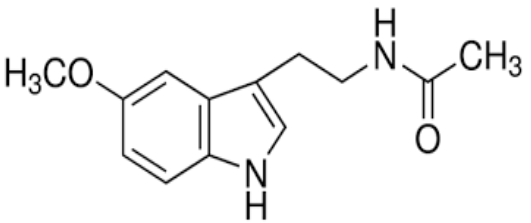
Procambarus clarkii Kırmızı bataklık kereviti	Serotonin	----	GSH salınımını uyardığı dolayısıyla üremenin tetiklenmesi rolü	Sarojoni vd, (1995)
P. monodon Siyah kaplan karidesi	Serotonin	50 µg/g vücut ağırlığı, enjeksiyon	Gonad gelişimi ve yumurtlamayı indükleme	Wongpraset vd., (2006)
Uca pugilator Kemancı Yengeç	Serotonin	1.25×10^{-9}–1.25×10^{-7} mol/yengeç	Uzuv kopması sonrası rejenerasyon hızını ve ilk kabuk değişimi süresini kısaltmıştır. GSH sentezi ve salınımı artmış	Richardson vd., (1991)
Travancoriana schirnerae Tatlısu yengeci	Serotonin	10µg g^{-1} Deneme süresince 4 kez	Gonad gelişimini indükleme	Dineshan ve Devi, (2022)
Pontastacus leptodactylus Türk kereviti	Serotonin	10µL enjeksiyon $2,5x10^{-8}$ mol/gram vücut ağırlığı.	Ovaryum gelişimini indükleme	Bal ve Harlıoğlu, (2022)
M. rosenbergii Tatlısu karidesi	Serotonin	2.5×10^{-5}, 2.5×10^{-6} mol /karides 1 µg g^{-1} vücut ağırlığı	Sinir sisteminde CHH ekspresyonunun düzenlenmesinde önemli bir rol oynar. Yumurtalıkta Scyol-CHH ekspresyonunu yukarı doğru düzenler. Yumurtalık gelişimine ve oosit olgunlaşmasına olumlu etkiler	Meeratana vd., (2006)

P. indicus Beyaz karides	Serotonin	50 µg/g vücut ağırlığı	Hem vitollogenes süreci ve hem de sonrasında mayoz bölünmenin başlaması ve oosit olgunlaşması üzerine etkisi	Tomy vd., (2016)
Barytelphusa guerini Tatlısu yengeci	Serotonin	10^{-8},10^{-9}, 10^{-10} mol 100 µL/Yengeç	Gonad gelişimini uyaran ve gonad gelişimini baskılayan hormonların düzenlenmesinde etkilidir.	Prasad vd., (2014)
L. vannamei Pasifik beyaz karidesi	Serotonin +spiperone	50 µg g^{-1} vücut ağırlığı	Gonad olgunlaştırma ve yumurtlama üzerine olumlu etkiler.	Alfaro vd., (2004)
P. clarkii Kırmızı bataklık kereviti	Serotonin	100 µL enjeksiyon, 15 µg/g vücut ağırlığı	Testis olgunlaşması, Androjenik bezin gelişimini olumlu etkiler.	Liu vd., (2014)
P. monodon Siyah kaplan karidesi	Serotonin	50 µg g^{-1} vücut ağırlığı	İnhibitörler ile birlikte yumurtalık gelişimini previtellojenik aşamadan olgunlaşmış oositlere kadar hızlandırır.	Mani vd., (2019)
Scylla serrata (Çamur yengeci)	Serotonin	2 µg g − 1 vücut ağırlığı	Yengeçlerde dolaşımdaki metil fernosat ve ekdisteroid seviyelerini yükselterek kabuk değiştirme ve üreme süreçlerini uyarır.	Girish vd., (2017)
M. rosenbergii Tatlısu karidesi	Serotonin	2.5×10^{-5}, 2.5×10^{-6} mol	Karideslerde gonad gelişimine pozitif etki	Tinukul vd., (2009)

Melatonin

Melatonin, (5-Hidroksitriptamin) İşlevi, Yapısı ve Metabolizması

Melatonin ilk kez sığır epifiz bezi özütünde tanımlanmıştır (Lerner, Case, Takahashi, Le ve Mori, 1958). Bu tarihten sonra biyosentezi, metabolizması, fizyolojik fonksiyonları konusunda çok sayıda çalışma yürütülmüştür. 15-20 yıl öncesine kadar yalnızca hayvanlarda epifiz bezi tarafından üretildiği düşünülen melatoninin, yapılan çalışmalar neticesinde muz, kiraz, ceviz vb. gibi yenilebilen birçok bitki türünde de varlığı belirlenmiştir (de la Puerta vd., 2007). Sonuç olarak, melatoninin hemen hemen her bitki ve hayvan türünde bulunan bir molekül olarak karşımıza çıktığını söylemek mümkündür (Şekil 3).



Şekil.3. Melatoninin Kimyasal Yapısı (N-acetyl-5-methoxy-tryptamine)

Melatoninin karakteristik özelliklerinden biri, sentezinin, ilgili hayvanın alışkanlığından bağımsız olarak, ışık/karanlık döngüsü içinde karanlıkta gerçekleşmesidir. Bu nedenle genellikle kronobiyotik molekül ya da karanlık hormonu olarak tanımlanmaktadır. Organizmanın fizyolojisi için oldukça önemli olan çevresel ipuçlarından birisi ışık/karanlık fotoperiyot sinyalidir. Çünkü birçok süreçte fotoperiyoda yanıt verilmektedir. Işık ile ilgili bilgi organizmaya melatonin aracılığıyla iletilmektedir. Kısaca, fotoperiyodik sinyaller ile iç ortam arasındaki bağlantı melatonin sayesinde yürütülmektedir. Çeşitli fizyolojik süreçlerin

gerçekleştirilmesi ışık/karanlık döngüsüne göre düzenlenip senkronize edilmektedir. Melatonin omurgalılarda sirkadiyen ritimlerin, mevsimsel üremenin, uykunun, yaşlanmanın, ruh halinin, davranışın ve termoregülasyonun düzenlenmesinde, osmoregülasyonda, besin alımında vb. birçok fonksiyonda rol aldığı bildirilmektedir (Sainath, Swetha ve Reddy, 2013). Kısaca melatoninin çevresel sinyallerin alımında ve senkronizasyonunda, bu sinyallerin canlının fizyolojisi ve davranışlarıyla bütünleştirilmesinde potansiyel bir biyolojik ipucudur.

Omurgasızlarda melatoninin varlığına dair ilk kanıt bir çekirge türü olan *Locusta migratoria'*da elde edilmiştir. Bu canlının gözlerinde gece süresince melatonin düzeyinin oldukça yüksek olduğu bildirilmiştir (Vivien-Roels ve Pevet, 1986). Takip eden yıllarda çok sayıda çalışmada melatonin varlığından sözedilmiştir (Sainath vd., 2013)

Omurgalılarda melatoninin biyolojik önemi hakkında artan bilgiler bileşiğin biyokimyasına olan ilgiyi artırmıştır. Melatonin iki fonksiyonel gruptan oluşan bir indolamindir. O-metil ve N-asetil kalıntıları bu gruplar hem biyolojik etkileri hem de oksidasyon kimyası için dikkate alınmaktadır. Melatonin amfifilik bir yapıya sahiptir; bu da yüksek lipid ve su çözünürlüğünü gösterir.

Melatonin biyosentezi omurgasızlarda, bitkilerde ve hayvanlarda benzerdir (Hardeland, Pandi Perumal ve Cardinali, 2006). Melatonin triptofandan hidroksilasyon, dekarboksilasyon, N-asetilasyon ve O-metilasyondan oluşan bir dizi olay zinciriyle sentezlenir. Dolaşım sisteminden alınan triptofan, triptofan hidroksilaz enzimi aracılığıyla hidroksile edilerek 5-hidroksitriptamine (5-HT) dönüştürülür. 5-Hidroksitriptofan, aromatik bir amino asit dekarboksilaz enzimi tarafından dekarboksilasyona uğrayarak serotonin oluşturur. N-asetiltransferaz tarafından serotoninin N-asetilasyonu, N-asetilserotonin sentezine

olanak tanır. Son olarak, hidroksiindol-O-metil transferaz tarafından gerçekleştirilen O-metilasyon, N-asetil serotonini melatonine dönüştürür.

Melatoninin krustaselerde büyüme, solunum, üreme ve sindirim gibi birçok fizyolojik süreçte görev aldığı bildirilmektedir (Tablo3). Çok yönlü bir molekül olan ve çok yönlü işlevler sergileyen melatoninin belirli reseptörler aracılığıyla etki ettiği kabul edilmektedir. Melatoninin nükleer reseptörlerle, hücre içi proteinlerle etkileşimi ve antioksidan etkileri bu bileşiğin genel birçok etkisini açıklayabilir. Memelilerde melatoninin reseptörlerle etkileşimi iyi anlaşılmasına rağmen krustaselerde bu konu üzerine yapılan çalışmalar oldukça yeni ve azdır.

Melatonin genel olarak doğal antioksidan olarak bilinir (Tan, 1993). Melatoninin antioksidan etkinliği çeşitli patolojik durumlarda kapsamlı bir şekilde incelenmiştir (Tan vd., 2003; Rodríguez vd., 2004). Ayrıca çok sayıda çalışma melatoninin antioksidan savunma sistemindeki etkisini doğrulamış, süperoksit dismutaz ve glutatyon peroksidaz gibi belirli antioksidan enzimleri düzenleyerek veya hidroksil radikali, peroksil radikali, nitrik oksit, hidrojen peroksit gibi reaktif oksijen türlerinin temizleyicisi olarak hareket ederek etkisini göstermiştir (Reiter, Tan ve Allegra, 2002). Bu nedenle, melatoninin hem reaktif oksijen hem de reaktif azot türlerini nötralize etme yeteneğine sahip serbest radikallerin doğrudan bir temizleyicisi olarak hareket ettiği ve böylece savunma mekanizmasını iyileştirdiği belirtilmektedir (Sainath vd., 2013).

Kabukluların merkezi sinir sisteminde (MSS) bulunan biyojenik bir amin olan melatonin, dekapodlarda yumurtalık olgunlaşmasını destekleyen önemli bir nöroendokrin düzenleyici görevi üstlenmektedir. Esasen, vitellogeneze (yumurta sarısı oluşumu) ve oosit gelişimine yol açan hormonal bir zinciri tetikleyen bir yukarı akış nörotransmitteri olarak işlev görür.

Kabuklu canlılarda rejenerasyon (uzuv yenilenmesi) ile kabuk değiştirme süreçleri birbiriyle yakından ilişkili olan fizyolojik bir olaydır. Ototomi yoluyla bir uzvunu kaybeden yengeçlerde, kopma bölgesinde öncelikle bir kütikül tabakası oluşmakta; ardından gerçekleşen ardışık kabuk değişimleri sayesinde bu yapı zamanla gelişerek yeniden işlevsel bir uzva dönüşmektedir. Benzer bulgular, *Periplaneta americana* üzerinde gerçekleştirilen çalışmalarda da ortaya konmuştur (Richter, Böhm ve Leubert, 1999).

Araştırmalar, bu süreçte ekdison (kabuk değiştirme hormonu) ve asikotropik hormonun kritik rol oynadığını göstermektedir. Süreç boyunca melatoninin beyinden asikotropik hormon salınımını uyardığı; bu hormonun ise kabuk değiştirme bezlerinde ekdison üretimini tetiklediği bildirilmektedir. Sonuç olarak, böceklerde melatoninin kabuk değiştirme mekanizmasında önemli bir düzenleyici işlev üstlendiği söylenebilir.

Dekapod krustaselerde üremenin düzenlenmesinde birbirine zıt yönde etkiye sahip iki nörohormonun rol oynadığı bilinmektedir (Aktaş ve Kumlu, 1999). Bu hormonlardan birincisi gözsaplarında bulunan ve (X organ) nörosekreter hücreler tarafından sentezlenip sinüs bezi kompleksinde depolanan, uyarılmalarla salınan gonad gelişimini engelleyen hormondur (Panouse, 1943). Diğeri ise beyinde ve thorasik ganglionda sentezlenen gonad gelişimini teşvik eden hormondur (Fingerman, 1987). Bu peptitlere ilaveten, Y organı tarafından üretilen metil farnesoat üremenin düzenlenmesinde rol oynamaktadır. 10^{-7} mol melatonin enjeksiyonunun tatlısu yengeci *O. senex. Senex*'te üremeyi tetiklediği belirlenmiştir (Sainath ve Reddy, 2010). Araştırıcılar, tüm bunlara ilaveten, gözsapı kesimi yapılmayan ve melatonin enjekte edilen yengeçlerin kan dolaşımında metil farnezoat ve ekdisteroid seviyesinin arttığı ifade etmişlerdir. Yıldırım ve Aktaş, (2016) *Litopenaeus vannamei* ile yapmış oldukları çalışmada 1 mg/kg düzeyinde yemlere eklenen

melatonin katkılı yemlerle beslenen karideslerin kontrol grubu karideslerine göre daha fazla kabuk değiştirdiğini belirtmişlerdir. Araştırıcılar 0-5 mg/kg yem olacak şekilde farklı düzeylerde yeme ilave edilen melatoninin ağırlıkça ve boyca kazanım üzerine herhangi bir etkisinin olmadığını bildirmişlerdir. Melatoninin genel olarak filogeni boyunca büyüme ve yenilenmeyi engellediğine ve melatonin aracılı engelleme olaylarının büyüyen dokularda mitoza doğrudan etki yoluyla gerçekleştiğine inanılmaktadır. Kabuk değişimini engelleyen ya da inhibe eden (MIH) hormon, bu işlevi nitrik oksit vasıtasıyla, canlıdaki nitrik oksit sentez aktivitesini azaltarak gerçekleştirmektedir (Sainat vd, 2013). Yapılan çalışmalar göz önüne alındığında, melatoninin kabuk değişimi ve büyüme süreci üzerine etkilerinin net olarak anlaşılabilmesi için daha fazla çalışma yapılmasına ihtiyaç vardır.

Sürekli değişen bir ortamda glikoz homeostazının korunması yaşamın temel bir sürecidir. Kabuklu deniz hayvanlarında hiperglisemik hormonun (CHH) glikoz homeostazı için hayati öneme sahip olduğu iyi bilinmektedir. Bu bağlamda, CHH'nin kimyasal yapısı, etki mekanizması ve hedef bölgeleri çeşitli kabuklu deniz hayvanlarında kapsamlı bir şekilde incelenmiştir. Kabuklularda X-organ/sinüs bezi (XO/SG) kompleksinden sirkadiyen bir düzende hiperglisemik hormonun salınmasının ışık/karanlık hemolenf şeker seviyelerinin nedenlerinden biri olabileceği öne sürülmüştür. Melatonin, organizmaya fotoperiyot bilgisi ileten kimyasal bir haberci olarak oldukça değerlidir ve seviyeleri kabuklularda döngüsel olarak dalgalanır.

Biyolojik ritmiklik, organizmaların fizyolojik organizasyonunda temel bir olgu olarak kabul edilmektedir. Bu çerçevede sirkadiyen ritimler, davranışsal ve fizyolojik süreçlerin süreklilik gösteren döngüsel paternleri şeklinde tanımlanmaktadır. Yeryüzündeki canlıların büyük bir kısmı, metabolik aktivitelerini ışık/karanlık döngüsüne uyumlu biçimde sürdürmektedir. Nitekim

çeşitli krustase türlerinde gerçekleştirilen çalışmalar; günlük renk değişimleri, oksijen tüketimi, lokomotor aktivite, hemolenf glukoz regülasyonu, pigment translokasyonu, asetilkolinesteraz aktivitesi ve antioksidan savunma sistemlerinin ritmik özellikler sergilediğini ortaya koymaktadır. Bu bağlamda melatonin, fotoperiyodik bilginin organizmaya aktarılmasında ve çevresel uyaranlara karşı adaptif fizyolojik yanıtların oluşturulmasında kritik bir mediatör olarak işlev görmektedir. Örneğin, kırmızı bataklık kereviti olarak bilinen *Procambarus clarkii* üzerinde yapılan çalışmalarda, hemolenf glikoz ve laktat düzeyleri ile nörotransmitter salınımının melatonin aracılı sirkadiyen ritimler sergilediği gösterilmiştir (Tilden vd., 2003).

Kabuklu deniz canlılarında oksijen tüketiminin, çevresel uyaranlara ve fotoperiyoda bağlı olarak günlük ritmik değişimler gösterdiği bilinmektedir. Bu ritmik dalgalanmalar, aerobik metabolizmadaki değişimlerin bir yansıması olup antioksidan savunma sistemini de etkileyebilmektedir. Bu kapsamda, *Procambarus clarkii ve Procambarus digueti* üzerinde gerçekleştirilen çalışmalarda, hemolenf ve hepatopankreas kompleksinde glutatyon bağımlı enzim aktiviteleri ile glutatyon düzeylerinde günlük değişimlerin meydana geldiği ortaya konmuştur (Geihs vd., 2010; Maciel vd., 2010). Ayrıca Geihs vd., (2010) östarin bir yengeç türü olan *Neohelice granulata*'da melatoninin oksidatif stresin düzenlenmesinde etkili olduğunu bildirmiştir. Tüm bu bulgular, melatoninin oksidatif homeostazın düzenlenmesinde önemli bir rol üstlendiğini açıkça göstermektedir.

Tablo 3. Melatonin uygulamasının krustaselerde temel etkileri

Tür	Melatonin	Uygulama dozu/ uygulama yolu	Temel etki ve bulgular	Kaynak
Eriocheir sinensis Çin eldiven yengeci	Melatonin	20 µL/yengeç	Melatonin oksidatif hasarı onarır, ACP, AKP ve Na^+-K^+-ATPaz aktivitesini stabilize eder, sitokrom C içeriğini artırır, hemositlerin apoptoz oranını ve fagositoz aktivitesini geri kazandırır.	Zhang vd., (2019)
Eriocheir sinensis (Çin Yengeci)	Melatonin	Enj., 0.01- 5 g/L konsantrasyon, 2mL/yengeç	Hemosiyanin düzeyini ve Antioksidant kapasitesini artırdığı	She Han vd., (2019)
Pontastacus leptodactylus Türk kereviti	Melatonin	20 mg/kg yem	Jüvenillerde büyüme performansı ve yaşama oranı artmıştır	Özkök vd., (2021)
Litopenaeus vannamei Pasifik beyaz karidesi	Melatonin	1mg/kg yem	Kabuk değişimini uyardığı, Et yağ oranının azalmasına neden olduğu, canlı ağırlık kazancını olumsuz etkilediği	Yıldırım ve Aktaş, (2016)
Oziotelphusa senex senex Tatlısu yengeci	Melatonin	10^{-7} mol.	Ovaryum olgunlaşmasını indüklemiştir.	Sainath ve Reddy, (2011)
Uca pugilator *Kemancı yengeç*	Melatonin	10 µMol enjeksiyon	sirkadiyen bilginin iletiminde filogenetik bir role sahip olduğu.	Tilden vd., (2003)

Neohelice granulata	Melatonin	200 - 20,000 pmol yengeç$^{-1}$	CHH gen ekspresyonunu inhibe etmiş ve göz sapı ve reseptöre bağlı hiperglisemiye neden olmuştur.	Maciel vd., (2014)
Procambarus clarkii Kırmızı bataklık kereviti	Melatonin	50 mg/kg yem	Büyümenin desteklenmesi, spesifik olmayan bağışıklıklarını ve antioksidan kapasitesinin artırılması, hepatopankreas ve bağırsaktaki sindirim enzim aktivitesinin artması ve sirkadiyen saatle ilgili gen ekspresyonunu düzenleme.	Chen vd., (2025)
S. spinigera	Melatonin	35 mg/kg yem	Büyüme performansını ve üreme fonksiyonunu artırma	Hossain vd., (2025)
C. destructor	Melatonin	75–81 mg/kg yem	Büyüme performansında iyileşme, hepatopankreasın antioksidan kapasitesini ve hemolenf bağışıklık parametrelerinin artması.	Yang vd., (2023)
Eriocheir sinensis (Çin Yengeci)	Melatonin	50.80 ± 7.26 mg/kg yem	Solungaç yapısı ve solunum düzenlenmesinde işleve sahip olan bir antioksidant.	Yang vd., (2021)
Litopenaeus vannamei Pasifik beyaz karidesi	Melatonin	59,59–71,97 mg/kg yem	Büyüme performansını iyileştirdiğini, yağ asidi β oksidasyonunu artırdığı ve hepatopankreasta yağ birikimini önleme	Ye vd., (2024)
Macrobrachium rosenbergii Tatlısu karidesi	Melatonin			Yang vd., (2021)

SONUÇ

L-triptofan, karmaşık ve çok yönlü metabolik yolları aracılığıyla birçok canlı grubunun fizyolojisi için vazgeçilmez bir moleküldür. Besinsel alınımı ve metabolik dönüşümler, hem merkezi sinir sisteminde hem de periferik sistemde homeostazın sürdürülmesi için kritik öneme sahiptir. Bu nedenle, krustaselerde triptofan metabolizmasının anlaşılması, çeşitli hastalıkların patofizyolojisinin aydınlatılması, intestinal sistem üzerine etkilerinin açıklanabilmesi, direkt veya dolaylı olarak regenerasyon, kabuk değişimi ve üreme üzerine etkilerinin detaylandırılması krustase yetiştiriciliğinde besin ve besleme konularında yeni stratejilerin geliştirilmesine, hastalıkların teşhis ve tedavi edilmesinde büyük önem taşımaktadır.

Mevcut literatür bilgileri dikkate alındığında dekapodların balıklardan daha farklı bir hormonal sinyal mekanizmasına sahip oldukları söylenebilir. Dolayısıyla krustaselerde bir nörotransmitter olarak serotoninin kritik bir işleve sahip olduğu, başka hiçbir nörokimyasal maddenin bu denli çeşitli rollere sahip olmadığını söylemek mümkün gözükmektedir. Omurgalılarda 14-15 adet farklı serotonin reseptörü varlığı baz alındığında krustaselerde yalnızca 3 farklı reseptör tanımlanması ve bu reseptörlerin çok geniş bir etki mekanizmasına ve farklı lokalizasyonlara sahip bulunması farklı dekapod türlerinde elde edilen sonuçların tutarsız olmasına ve hatta işlevleri ile ilgili spekülasyonlara yol açmaktadır. Tüm bunlara ilaveten, dekapod krustase türleri üzerine serotoninin etkileri spesifik bazda detaylı olarak çalışılmamış ve bu alanda oldukça büyük bir boşluk bulunmaktadır. 5-HT'nin antioksidanlar, bağışıklık ve bağırsak mikrobiyotası gibi pek çok alandaki olası rollerine dair bilgilerimiz henüz emekleme aşamasındadır. Mevcut çalışma bulguları ve sonuçları serotonin hormonu ve nörotransmitterin krustaselerde tüm fizyolojik süreçlerde önemli

görevler üstlendiğini ortaya koymaktadır. Nöropeptidomik analizler ve genom analizi gibi alternatif tekniklerin gelişimine paralel olarak gelecekte serotonerjik sistemle ilgili bazı yeni nöropeptitlerin keşfedilme olasılığının yüksek olduğu ifade edilebilir.

Dekapod krustaselerde melatoninin; büyüme, glikoz homeostazı, kabuk değişimi, uzuv rejenerasyonu ve üreme süreçlerinin düzenlenmesinde önemli rol oynadığı, ayrıca antioksidan özellik gösterdiği ve biyolojik ritimlerin kontrolünde etkili olduğu anlaşılmaktadır. Bununla birlikte, mevcut çalışmaların büyük ölçüde omurgalılar temel alınarak yürütüldüğü ve kabuklular açısından sınırlı kaldığı ifade edilebilir. Son dönemlerde, gerek su ürünleri yetiştiriciliği sektörünün genişleyen potansiyeli gerekse temel biyolojik mekanizmaların modellenmesinde sağladıkları metodolojik avantajlar doğrultusunda, kabuklu organizmalar üzerindeki araştırmalarda niteliksel ve niceliksel bir ivmelenme kaydedilmektedir. Buna karşın; büyüme ve üreme süreçlerini kontrol eden nöroendokrin moleküllere odaklanan moleküler düzeydeki yaklaşımlar derinleştikçe, organizmanın fotoperiyot, termal rejim ve trofik girdiler gibi çevresel uyaranları nöroendokrin-çevresel eksende fizyolojik yanıtlara dönüştürme mekanizmalarını açıklamak üzere holistik (bütüncül) yaklaşımlara duyulan ihtiyaç belirginleşmektedir. Bu entegrasyonun sağlanmasında melatonerjik sistemin spesifik biyolojik rollerinin tam anlamıyla karaktere edilmesi kritik bir önkoşuldur. Nitekim melatoninin kabuklu fizyolojisindeki potansiyel uygulama alanları ve fonksiyonel spektrumu henüz tam olarak aydınlatılamamıştır. Sonuç olarak, kabuklu fizyolojisinde melatoninin işlevsel rolüne ilişkin mevcut literatür, bu konunun nihai bir kabule ulaşmaktan uzak olduğunu ve ileri düzey araştırmalara açık bir alan teşkil ettiğini göstermektedir.

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