International Journal of Marine Science, 2026, Vol.16, No.3, 153-165
http://www.aquapublisher.com/index.php/ijms

Research Insight Open Access

Effects of Environmental Factors on the Health Status of Turbot (*Scophthalmus maximus*)

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International Journal of Marine Science, 2026, Vol.16, No.3 doi: 10.5376/ijms.2026.16.0012
Received: 02 Apr., 2026
Accepted: 05 May, 2026
Published: 18 May, 2026

Preferred citation for this article:
Zhu Y., and Li X.M., 2026, Effects of environmental factors on the health status of turbot (*Scophthalmus maximus*), International Journal of Marine Science, 16(3): 153-165 (doi: 10.5376/ijms.2026.16.0012)

Abstract As an important marine aquaculture species, the health status of the turbot (*Scophthalmus maximus*) is significantly influenced by various environmental factors during intensive farming. This paper systematically reviews the mechanisms by which key environmental factors-such as temperature, salinity, dissolved oxygen, and water quality-affect the physiological homeostasis, immune function, and disease susceptibility of turbot. The results indicate that temperature fluctuations can alter immune responses by inducing metabolic imbalances and changes in heat shock protein expression, thereby increasing susceptibility to pathogens. Salinity variations disrupt osmoregulatory systems, triggering stress responses and compromising disease resistance. Insufficient dissolved oxygen and the accumulation of ammonia-nitrogen lead to chronic stress and tissue damage, exacerbating health risks. Furthermore, environmental stressors enhance the pathogenicity of bacteria (such as **Vibrio** spp.), further promoting disease outbreaks. In high-density aquaculture systems, the coupled effects of multiple factors significantly amplify these health risks. Based on this analysis, the paper proposes strategies for environmental monitoring and integrated regulation to mitigate the adverse effects of environmental stress on turbot health and to enhance the stability and productivity of aquaculture systems.

Keywords Turbot; Environmental factors; Temperature stress; Osmoregulation; Disease outbreak

1 Introduction

Turbot aquaculture has become an important component of marine finfish production, driven both by declining wild stocks and by the species' favorable farming traits. Wild turbot catches have generally decreased since the mid-1990s, and aquaculture production has exceeded capture production since 2002, while the species remains valued for rapid growth, high survival, and efficient feed conversion. Over the past several decades, these attributes have supported the industrial development of turbot farming in Europe, and large-scale expansion in China has made turbot one of the world's most important cultivated flatfish species, with annual Chinese production reported above 60,000 tons in the first two decades after introduction (Fernández-González et al., 2022).

At the same time, the success of turbot culture depends strongly on environmental suitability, because this cold-water marine species performs best within relatively narrow physicochemical ranges. In European production systems, water temperatures of about 15°C-19°C and salinity of 25-35 psu are identified as key conditions for successful farming, highlighting the central role of site quality and water management in production outcomes (Fernández-González et al., 2022). More broadly across fishes, temperature, pH, salinity, and dissolved oxygen shape physiology, metabolism, behavior, and survival, with salinity shifts altering energy allocation and gill function, and low dissolved oxygen reducing growth, reproduction, and disease resistance (Mariu et al., 2023).

For marine fish, environmental factors are not only determinants of growth performance but also major regulators of stress and health status. Temperature increases can act as a direct physiological stressor, especially in ectothermic species approaching their upper thermal tolerance limits, and warming can also worsen oxygen-related constraints and broader population-level vulnerability (Alfonso et al., 2020). Turbot-specific studies show the same pattern at the organismal level: high stocking density suppresses growth, elevates cortisol and glucose, and weakens skin immune defenses, while elevated ammonia activates stress pathways and inhibits humoral immunity, indicating that environmental deterioration can rapidly translate into impaired welfare and disease susceptibility.

These concerns are especially relevant because environmental stress in turbot often operates through interacting physiological mechanisms rather than through simple single-factor effects. Elevated temperature in farmed turbot has been linked to increased disease-associated mortality above 20°C and to molecular signatures of oxidative stress and metabolic disturbance under pathogen challenge, while low salinity stress disrupts liver lipid metabolism and depresses key metabolic pathways (Liu et al., 2020). Against this background, the objective of this review is to synthesize current knowledge on how major environmental factors influence the health status of turbot, with emphasis on stress physiology, immune competence, and metabolic regulation. Such a synthesis is significant for improving environmental control in intensive culture systems, guiding health-oriented farm management, and identifying priorities for future research as climate change and production intensification continue to reshape the conditions under which turbot are farmed (Mariu et al., 2023).

2 Biological and Physiological Characteristics of Turbot (*Scophthalmus maximus*)

2.1 Growth traits and ecological adaptation

Turbot is a demersal flatfish distributed from the Northeast Atlantic to the Mediterranean, Baltic, and Black seas, and its life history reflects strong adaptation to benthic habitats. Larvae retain high dispersal potential through a pelagic phase, whereas postlarvae, juveniles, and adults are relatively sedentary, a combination that links local habitat specialization with broader population connectivity. Genomic evidence further indicates that ecological adaptation in this species has been shaped by environmental selection, with temperature and salinity emerging as major drivers and with candidate genes associated with osmoregulation, growth, and disease resistance identified in selected genomic regions.

The species also shows marked physiological and production-related variation in growth. Whole-genome analysis links turbot's adaptation to demersal and relatively cold environments to selection on genes involved in vision and membrane lipid metabolism, and reports sustained growth across approximately 13°C-20°C, indicating capacity to perform under cool and fluctuating thermal conditions. At the individual level, growth is strongly structured by sex and metabolic phenotype: females ultimately outgrow males under most thermal regimes, while fast-growing fish show up-regulation of anaerobic glycolytic pathways in white muscle, consistent with a higher metabolic rate supporting rapid somatic growth.

2.2 Immune system and stress response mechanisms

In turbot, stress responses involve endocrine activation together with tissue-specific immune modulation. Acute handling stress elevates cortisol and plasma K^+ after both aerial exposure and net confinement, but unlike the canonical response described for many other teleosts, turbot shows little or no parallel rise in plasma glucose, suggesting a distinctive stress physiology. Chronic crowding likewise acts as a potent stressor: high stocking density suppresses growth, increases plasma cortisol and glucose, and reduces mucus immune activities such as lysozyme, alkaline phosphatase, and esterase, indicating weakened barrier defense at the skin surface (Yang et al., 2020).

Environmental insults also connect the hypothalamic-pituitary-interrenal axis with oxidative stress and immune suppression. Under elevated ammonia exposure, juvenile turbot show increased CRH, ACTH, and cortisol together with reduced lysozyme, complement factors, and IgM, while liver antioxidant responses and heat-shock protein expression rise alongside lipid peroxidation, consistent with stress-induced immune inhibition and oxidative damage. Thermal stress appears to alter disease resistance through a related mechanism: at 21°C, challenged fish exhibit enhanced neutrophil-associated responses, sustained myeloperoxidase-linked oxidative stress, and disturbed NADPH and glucose metabolism, changes proposed to underlie thermo-linked epizootic risk in turbot culture.

2.3 Temperature and salinity tolerance ranges

Temperature and salinity jointly determine performance in juvenile turbot, and their interaction is more informative than either factor alone. In a three-month rearing study, growth, feed intake, and feed conversion efficiency were highest at 15‰ and lowest at full-strength seawater, with the overall optimal growth combination estimated at about 21.8°C and 18.5‰ (Jia et al., 2020). Osmoregulatory measurements support the same pattern: gill Na^+, K^+-ATPase activity, plasma chloride, and osmolality were lowest at intermediate salinity, implying reduced energetic costs of ion regulation under brackish to moderately saline conditions.

Tolerance limits nevertheless vary with life stage and endpoint. Sudden-change experiments in juveniles estimated incipient lethal limits at 7.06°C-26.54°C and 16.05-37.76 salinity, with highest short-term survival around 15°C-18°C and salinity 28-32, showing that survival tolerance is broader than the optimum for growth. Reproductive stages are more constrained: in Baltic turbot, fertilization success and viable hatch decline sharply below 7 psu, while egg survival is highest at 12°C-18°C and lower at 9°C and 21°C, indicating that successful reproduction near the species' brackish distribution limit depends on a narrower hydrographic window than juvenile persistence (Yang et al., 2020).

3 Key Environmental Temperature Effects on Health

3.1 Thermal stress and metabolic imbalance

Thermal stress rapidly disrupts energy metabolism in turbot and forces a shift toward compensatory catabolic pathways. In kidney tissue, exposure to 25°C-28°C increased cortisol, creatinine, *hsp70*, and *hsp90*, while also upregulating enzymes linked to aerobic metabolism and gluconeogenesis, including SDH, FBPase, MDH, cPEPCK, and G6Pase, indicating that maintenance of energy supply under heat load depends on metabolic reprogramming rather than simple metabolic depression (Yang et al., 2020). Consistent with this, integrated metabolome-transcriptome analysis showed that heat stress significantly affected pathways involved in steroid hormone biosynthesis, glycerophospholipid metabolism, sphingolipid metabolism, glycerolipid metabolism, and unsaturated fatty acid biosynthesis, suggesting that lipid homeostasis is also extensively remodeled during thermal challenge (Zhao et al., 2021).

At the whole-animal level, acute hyperthermal exposure at 27°C elevated serum cortisol, glucose, and respiratory frequency during the early stress phase, while hepatic glycogen, CAT, and GPx progressively declined and malondialdehyde increased, indicating rising oxidative cost and depletion of metabolic reserves as stress duration lengthened (Jia et al., 2020). Broader transcriptomic evidence supports this interpretation by showing that heat stress in turbot kidney enriches pathways related to fat metabolism, insulin signaling, apoptosis, FOXO, Jak-STAT, and P53 signaling, with hub genes such as AKT3, PIK3r2, and mdm2 implicated in coordinating the balance between metabolic adjustment and cellular injury under elevated temperature.

3.2 Heat shock protein (HSP) expression and immune modulation

Heat shock proteins are among the most responsive molecular indicators of temperature stress in turbot, but their regulation is tissue-specific and linked to broader stress physiology. Under rapid cooling from 18°C to 1°C, red and white blood cell counts and hemoglobin fell significantly, while cortisol, cholesterol, and triglycerides increased; in parallel, *hsp70* and *hsp90* expression changed across tissues, and the authors identified 8°C-5°C as a critical low-temperature stress zone for aquaculture management. Under heat exposure, *hsp70* and *hsp90* transcripts in liver increased significantly within 6-12 h, accompanying early endocrine disturbance and the onset of hepatocyte apoptosis, which supports the view that HSP induction forms part of an acute protective response that is activated before prolonged thermal damage becomes established (Figure 1) (Jia et al., 2020).

Mechanistically, heat-induced HSP regulation in turbot is not merely descriptive but has defined signaling features. In turbot kidney cells, heat stress activated ERK1/2 and HSF1 and induced *hsp90* expression, while ERK inhibition attenuated this response, identifying an ERK-HSF1-dependent pathway in the cellular heat shock response (Yang et al., 2020). At the genome-wide level, 16 *hsp70* genes have been identified in turbot, and several members-especially *hspa1a*, *hspa1b*, and *hspa5*-show strong responses across heat, salinity, parasitic, bacterial, and viral stress datasets, indicating that the HSP network participates not only in thermal protection but also in immune modulation across diverse environmental and infectious challenges (Zheng et al., 2023).

3.3 Temperature-related disease susceptibility patterns

Temperature strongly shapes disease susceptibility in turbot by altering both host defense capacity and pathogen performance. In megalocytivirus challenge experiments, fish held at 14°C-18°C showed undetectable or moderate viral replication and no mortality, whereas fish held at 20°C-24°C showed robust viral replication and 100% mortality; furthermore, shifting temperature downward during infection improved survival, while upward shifts

worsened outcomes (Jia et al., 2020). These results indicate that relatively small increases above the lower culture range can decisively transform infection from controlled to lethal, making temperature management a central component of viral disease prevention.

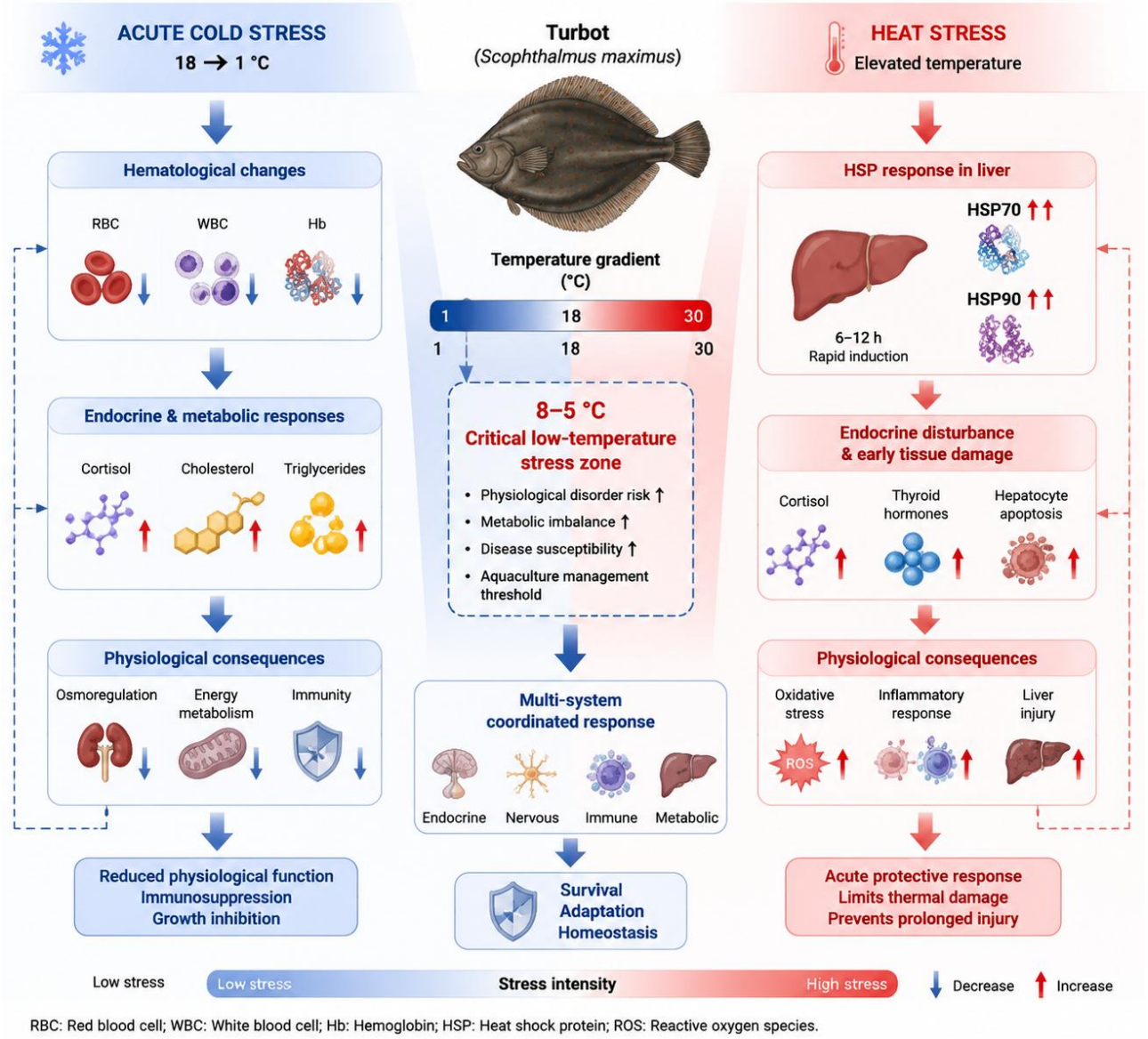

Figure 1 Integrated physiological and molecular stress responses of turbot under acute cold and heat exposure, highlighting hematological changes, endocrine disruption, and HSP70/HSP90 regulation

Evidence from pathogen-mimic challenge further suggests that high temperature can increase disease risk even when immune activity is stimulated rather than suppressed. Integrated transcriptomic and proteomic analyses showed that elevated temperature enhanced neutrophil-mediated responses but also intensified MPO-associated oxidative stress and disturbed NADPH and glucose metabolism, a combination proposed to contribute to thermo-linked epizootics in turbot (Liu et al., 2020). Related work on high water temperature and skin mucus immunity found significant temperature-dependent changes in immune-related factors at the mucosal surface, supporting the view that warming reshapes frontline defense systems as well as systemic stress pathways.

4 Salinity and Osmoregulatory Stress

4.1 Osmoregulation mechanisms in turbot

Turbot osmoregulation depends on coordinated responses across the gill, kidney, and other regulatory tissues, with ion transport and signaling pathways forming the core of adaptation to hypoosmotic challenge. Kidney

transcriptomics under salinity 5 versus 30 showed that inorganic ion channels and transporters, mineral absorption, and bile secretion contribute to iono-osmoregulation and cell-volume regulation, while tissue-specific analysis identified the kidney, gill, and spleen as major osmoregulatory organs. Complementary work further showed that low salinity activates PI3K-AKT signaling in osmoregulatory organs and that pathway inhibition suppresses ion-channel gene expression, indicating that signal transduction positively regulates ion transport during osmotic adjustment (Cui et al., 2020).

Hormonal and osmolyte-mediated regulation also plays a central role in salinity adaptation. Under low-salt stress, pituitary PRL expression and PRLR expression in gill and kidney increased and peaked at 12 h, while long-term low-salinity exposure maintained especially high PRLR expression in the gill, suggesting that PRL signaling is important in sustained hypoosmotic regulation (Liu et al., 2020). In parallel, the myo-inositol biosynthesis pathway showed adaptive expression with a 12 h turning point across tissues, and RNAi knockdown weakened gill osmotic regulation and shortened survival under salinity stress, showing that organic osmolyte metabolism is a functional component of turbot osmoregulation.

4.2 Effects of salinity fluctuations on physiological stability

Salinity fluctuations affect physiological stability by changing the energetic cost of ion regulation and altering core blood and enzyme indicators. In juvenile turbot reared for three months across 15, 25, and 33.5‰, gill Na+, K+-ATPase activity, plasma chloride, and osmolality were lowest at 15‰, and estimated optima for minimum osmoregulatory load clustered around intermediate salinities, supporting the view that moderate salinity reduces the energy expenditure required for homeostasis (Liu et al., 2020). This energetic dimension is reinforced at the molecular level by evidence that salinity challenge changes AMPK α1/α2 expression in gill in a time- and salinity-dependent manner and that AMPK expression is positively correlated with Na+, K+-ATPase activity, linking salinity stress directly to cellular energy sensing.

When salinity departs too far from normal seawater, stability is maintained only through broader metabolic and endocrine compensation. Low salinity caused major liver transcriptional changes, with 826 differentially expressed genes enriched in energy metabolism and especially lipid metabolism, and serum triglycerides decreased over time in freshwater exposure, indicating metabolic disturbance rather than neutral acclimation (Liu et al., 2020). Over longer exposure, low salinity at 10 ppt remodeled circadian organization of physiological markers by shifting or replacing normal daily rhythms of T3, T4, and ALT without clear oxidative damage, suggesting that chronic hyposmotic stress can preserve short-term balance but only by reorganizing endocrine and metabolic timing (Liu et al., 2026).

4.3 Interaction between salinity stress and pathogen infection

Salinity stress interacts with infection largely through its effects on host immunity and resistance rather than through direct pathogen measurements alone. In juveniles reared at 8, 20, 32, and 40, fish at salinity 20 had the highest 4-day LD50 after *Vibrio anguillarum* challenge and also showed the highest lysozyme, complement, and phagocytic activity, whereas immunity was poorest at salinity 40, indicating that intermediate salinity supports stronger disease resistance than more extreme salinity conditions. Additional response-surface analysis showed that salinity significantly shapes expression of immune markers such as *hsp70* and IgM in liver and kidney, with a significant temperature-salinity interaction for kidney IgM, supporting the conclusion that salinity modifies immune competence in a tissue-specific way (Cui et al., 2020).

Mechanistic studies suggest that improved osmotic capacity can indirectly strengthen resistance to salinity-associated health decline. Dietary or immersion myo-inositol increased gill myo-inositol content, extended survival under salinity stress, and enhanced osmoregulatory, antioxidant, and immune-related functions, with steroid-associated pathways occupying a central place in this response (Cui et al., 2020). Consistently, dietary myo-inositol also prolonged survival under low salinity by modulating cortisol synthesis, increasing Na+-K+-ATPase activity and ion-channel gene expression, whereas suppression of cortisol eliminated these benefits, indicating that endocrine support for osmoregulation can help buffer the immune and survival costs that accompany salinity stress.

5 Dissolved Oxygen and Water Quality Impacts

5.1 Hypoxia-induced physiological and behavioral changes

Turbot is notably sensitive to reduced dissolved oxygen, with measurable behavioral disturbance appearing before lethal thresholds are reached. During progressive hypoxia, abnormal swimming behavior was observed when dissolved oxygen fell below 5 mg/L, respiration frequency increased at the critical oxygen tension, and loss of equilibrium occurred near 1.30 mg/L; related work identified a critical oxygen tension around 3.34 mg/L and showed that turbots under severe hypoxia first respond through increased ventilation amplitude and frequency to maintain oxygen uptake (Maxime et al., 2000). This initial compensatory phase reflects a relatively strong short-term regulatory capacity, but it does not prevent endocrine and metabolic stress as oxygen availability continues to decline. At low oxygen tension, plasma cortisol, glucose, and lactate increase markedly, and severe hypoxia triggers rises in adrenaline and noradrenaline together with reduced arterial oxygen partial pressure, indicating activation of both the stress axis and anaerobic metabolism (Jia et al., 2021).

Longer hypoxic exposure shifts the response from acute compensation to impaired growth and tissue-level remodeling. Under chronic hypoxia at about 3.5 mg/L for 8 weeks, turbot showed reduced digestibility, feed efficiency, weight gain, and body indices, along with poorer muscle texture, lower nutrient content, and reduced myofiber number, indicating that oxygen shortage depresses both production performance and flesh quality (Guo et al., 2026). Similar long-term exposure elevated plasma cortisol and glucose, increased glycolytic and lipolytic enzyme activities, altered gill morphology, and upregulated *hif-1α*, *hif-2α*, and *hif-3α*, suggesting that chronic hypoxia induces coordinated endocrine, metabolic, and branchial adjustments to sustain gas exchange and energy balance (Jia et al., 2021).

5.2 Ammonia, nitrite, and toxic metabolite accumulation

Nitrogenous wastes are major toxicants in intensive turbot culture, and both ammonia and nitrite disrupt physiological homeostasis within short exposure periods. High ammonia exposure elevated CRH, ACTH, and cortisol while reducing GH, lysozyme, complement factors, and IgM, demonstrating simultaneous activation of the hypothalamic-pituitary-interrenal axis and suppression of humoral immunity. Nitrite caused a partly overlapping but distinct syndrome: at 0.4-0.8 mM, it increased GPT, GOT, ALP, *c3*, and C4, reduced IgM and lysozyme, and upregulated gill *hsp70*, *hsp90*, TLR-3, *tnf-α*, and *il-1β*, indicating blood physiological dysfunction together with inflammatory and immune disturbance (Jia et al., 2024).

The toxic effects of these metabolites also extend to oxidative damage, ion imbalance, and reduced oxygen transport. In ammonia-exposed fish, liver SOD and CAT activities, HSP expression, and MDA increased while GSH and IGF-1 decreased, supporting a mechanism involving oxidative stress and impaired growth regulation. Nitrite and nitrate further compromise oxygen-carrying capacity: nitrite raised methemoglobin, cortisol, glucose, and K+ while lowering hemoglobin and Na+, whereas chronic nitrate exposure increased plasma NO3-, NO2-, methemoglobin, cortisol, glucose, lactate, and K+ and decreased Hb, Na+, and Cl-, indicating hypoxic stress combined with osmoregulatory and metabolic disruption (Yu et al., 2021).

5.3 Water quality deterioration and chronic stress effects

Beyond acute toxicants, chronic deterioration of water quality in recirculating or high-density systems imposes persistent stress that reduces growth and damages multiple organs. Elevated nitrate over 60 days reduced survival and growth, caused dose-dependent gill and liver histopathology, lowered hemoglobin, increased methemoglobin, and dysregulated the GH/IGF-1, thyroid, and HPI axes, showing that nitrate acts as a chronic systemic stressor rather than a benign end-product of nitrification (Yu et al., 2021). Chronic nitrate exposure also injured the intestine by causing microvillus atrophy and lamina propria necrosis, downregulated tight-junction and mucin genes, and shifted the microbiota toward lower intrinsic flora and more potential pathogens, indicating weakened barrier function and long-term health deterioration (Yu et al., 2020).

Other chronic water-quality stressors in RAS show similarly broad impacts. Prolonged CO_2 exposure reduced specific growth rate, increased feed conversion ratio, damaged gill, liver, and intestinal tissues, and altered plasma

HCO3-, Na+, and Cl- as well as osmoregulatory and acid-base genes, indicating sustained disturbance of ion balance and buffering physiology even at relatively low CO_2 concentrations (Guo et al., 2023). Chronic handling or crowding stress also amplifies these effects at the whole-animal level: repeated handling elevated cortisol and progressively depressed innate immune indicators, while high-density exposure combined with ammonia intensified Na+ imbalance, Na+/K+-ATPase activity, and pro-inflammatory and heat-shock gene expression, showing that poor water quality and husbandry stress often interact rather than act in isolation.

6 Pathogen Dynamics under Environmental Stress

6.1 Bacterial infections

Bacterial disease is a persistent constraint in turbot aquaculture, and *Vibrio* spp. have been especially prominent across larval and grow-out systems. Early reports identified *Vibrio*sis as a common problem in turbot production and documented the involvement of several taxa, including *Vibrio anguillarum* serogroups O1, O2a, and O2b, as well as members of the *V. splendidus*/*pelagius* group. Farm-level microbiological surveys similarly found that *Vibrio* spp. were among the most prevalent bacteria recovered from diseased turbot, although lesion type could not always be linked to a single bacterial species.

More specific evidence shows that some *Vibrio* isolates are highly virulent under culture conditions. A *Vibrio pelagius* strain recovered from larval mass mortality was highly pathogenic to larvae and post-larvae, with an LD50 below 5 bacteria mL^-1 in larvae, and the isolate was also able to grow in sterile seawater at room temperature or 15°C, indicating both strong host pathogenicity and environmental persistence. In parallel, *Aeromonas salmonicida* is an important cause of furunculosis-related losses in turbot farming, and infection triggers early up-regulation of pro-inflammatory and innate immune genes such as *tnf-α*, *il-1β*, *il-10*, and *c3*, showing that even early-stage bacterial challenge rapidly perturbs host immune homeostasis (Fajardo et al., 2023).

6.2 Viral and parasitic outbreaks

Viral diseases in turbot range from larval neurologic syndromes to emerging hemorrhagic conditions in grow-out fish. A picornavirus-like agent was associated with encephalomyelitis in turbot larvae, with large numbers of virus particles observed in the brain and medulla and with outbreaks ending in extremely heavy, ultimately complete mortality in affected batches. More recently, turbot acute hemorrhage disease in China was linked to a novel circovirus, and diseased stocks showed rapid spread, mortality exceeding 90% within one to two weeks, and increasing viral copy numbers after experimental infection (Figure 2) (Jiang et al., 2024).

Parasitic diseases are also major components of pathogen dynamics in stressed turbot populations. *Enteromyxum scophthalmi* causes a severe enteric disease characterized by cachexia, high morbidity, and mortality, while early infection is marked by interferon-related responses together with down-regulation of complement and acute-phase genes, suggesting active immune evasion during colonization (Spinos et al., 2024). Likewise, *Philasterides dicentrarchi* causes scuticociliatosis with systemic tissue invasion, including severe encephalitis, hepatic necrosis, branchial lesions, and muscular degeneration, confirming that parasitic outbreaks in turbot can progress beyond surface infestation to multisystemic disease.

6.3 Environmental drivers of disease transmission and virulence

Environmental stress does not simply weaken the host; it also changes transmission opportunity and pathogen virulence. A classic fisheries disease framework showed that infectious outbreaks arise when susceptible fish encounter virulent pathogens under stress caused by temperature, eutrophication, sewage, metabolic wastes, industrial pollution, or pesticides (Spinos et al., 2024). In turbot specifically, simultaneous environmental and biological stress can act synergistically: low water depth elevated cortisol and metabolic disturbance, and the additional presence of *A. salmonicida* or *P. dicentrarch*i under these conditions further amplified several stress responses.

Temperature is one of the clearest environmental drivers of bacterial virulence and outbreak timing. In fish-pathogenic bacteria, temperature regulates virulence gene expression, and in *Aeromonas hydrophila* lower host-

relevant temperatures enhanced production of extracellular virulence factors and type III secretion-associated proteins, illustrating how virulence can increase under ectothermic infection conditions. Field and epidemiological observations support the same pattern in turbot systems: ulcerative disease outbreaks in juvenile turbot began after water temperature rose above 20°C, while broader aquaculture case studies have linked increasing temperature and salinity, especially when combined with reduced dissolved oxygen, to higher occurrence of *Vibrio*sis and other infectious diseases (Spinos et al., 2024).

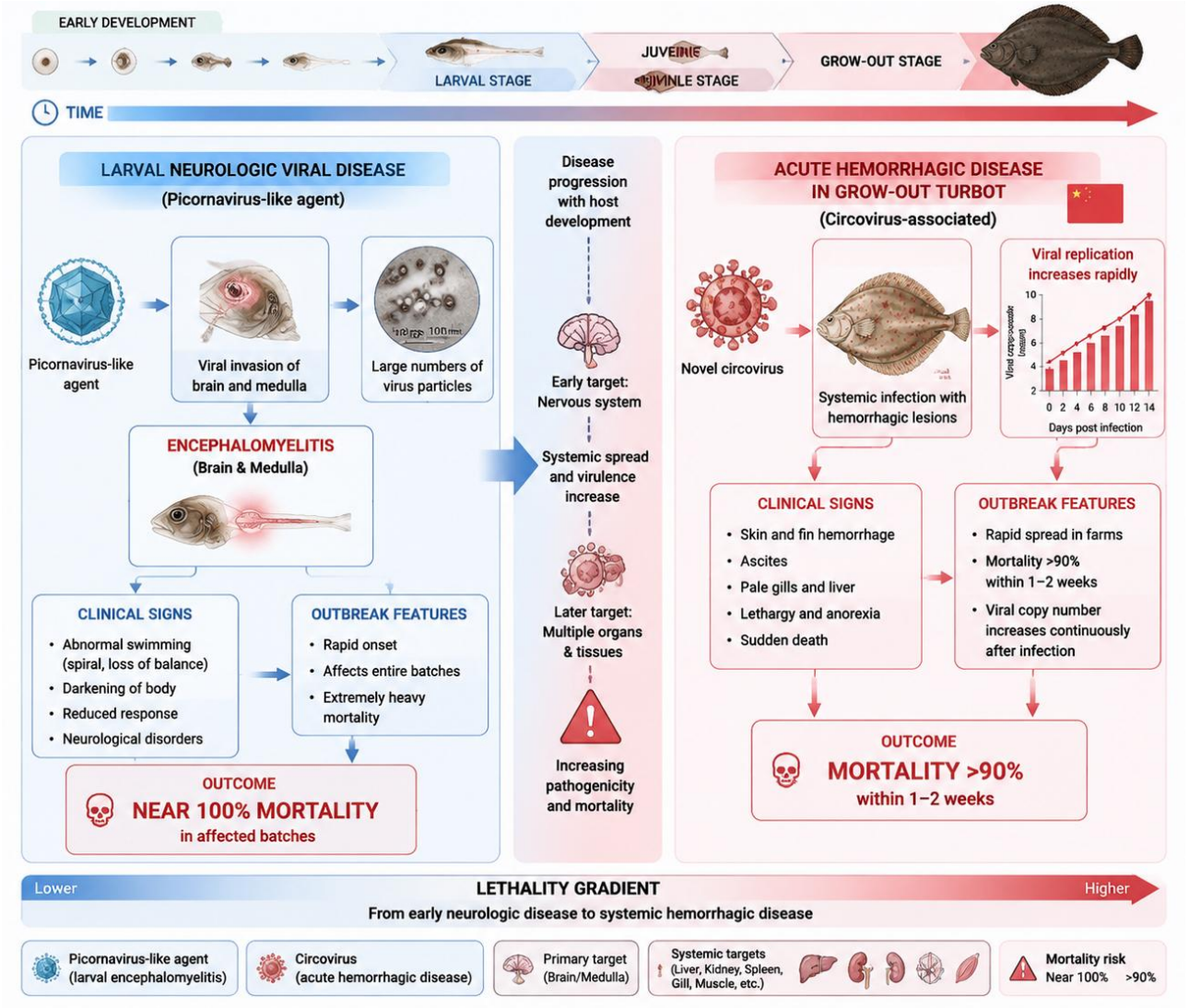

Figure 2 Progression of viral diseases in turbot from larval encephalomyelitis associated with picornavirus-like agents to acute hemorrhagic disease caused by a novel circovirus in grow-out stages

7 Feeding Environment and Nutritional Immunity

7.1 Temperature-dependent feeding behavior and digestion efficiency

As an ectothermic fish, turbot shows feeding and digestive responses that are tightly regulated by temperature, but these responses are not linear across the full thermal range. General fish evidence shows that temperature affects both feeding motivation and the capacity to digest and absorb nutrients, while turbot-specific trials indicate that feed intake increases from 14 to 18°C but declines significantly at 21°C under high-density recirculating conditions, suggesting that warming beyond the optimal range reduces appetite rather than continuously stimulating it. This pattern is consistent with the view that thermal effects on feeding are species-specific and depend on exposure intensity and duration, which is particularly relevant for a cold-water species such as turbot (Volkoff and Rønnestad, 2020).

Temperature also alters digestive efficiency by changing enzyme activity, gut processing, and the energetic scope available for postprandial metabolism. In fish generally, digestibility usually declines outside the optimal thermal window because digestive enzyme activities are temperature sensitive, and in turbot, pepsin activity increases from 14°C to 18°C before dropping sharply at 21°C, identifying 16°C-18°C as the practical optimum for feed intake, growth, and digestive performance in larger fish (Volkoff and Rønnestad, 2020). Older radiographic work further showed that gastric emptying time in turbot decreases as temperature rises, indicating faster meal processing at warmer temperatures, but this benefit should be interpreted together with the decline in appetite and enzyme performance once temperatures exceed the optimal range.

7.2 Nutritional regulation of immune competence

Nutritional status is a direct regulator of immune competence in turbot, because both adequate baseline nutrition and targeted supplementation can shape innate and adaptive defense functions. Broad aquaculture reviews agree that balanced nutrient supply is required for efficient host defense and that specific nutrients supplied above minimum requirement can improve fish health and disease resistance. In turbot, this principle is supported by vitamin D3 studies showing that dietary supplementation reduced mortality and spleen bacterial load after *Edwardsiella tarda* infection, while also elevating serum lysozyme activity, haemocyte reactive oxygen species production, and macrophage bactericidal capacity (Liu et al., 2021).

Other micronutrients similarly regulate turbot immunity through antioxidant protection and immune-gene modulation. Dietary vitamin E supplementation increased growth, lysozyme activity, phagocytic index, superoxide dismutase activity, and the expression of immune-related genes including *c3*, *tnf-α*, and *il-1β*, with the best overall response reported at 480 mg kg^-1. Vitamin C supplementation also enhanced non-specific immunity in juvenile turbot, particularly by increasing serum lysozyme and phagocytic capacity, whereas both deficient antioxidant supply and oxidized dietary lipid exposure suppressed head-kidney phagocyte function and increased mortality after *Vibrio anguillarum* challenge.

7.3 Feed quality deterioration under environmental stress

Environmental stress can degrade feed quality before ingestion, thereby weakening nutritional value and increasing toxicological risk. Storage studies show that humidity and temperature strongly influence the formation of mycotoxins in compounded fish feeds, and even short-term exposure to unsuitable storage conditions can deteriorate feed quality and promote fungal contamination. Warm, humid storage is particularly problematic because ochratoxin A was detected after treatment at about 25°C and >60% relative humidity, demonstrating how rapidly hazardous contaminants can develop in stored aquafeeds (Pietsch et al., 2020).

The biological consequences of feed deterioration extend beyond reduced nutrient density to impaired immunity and poorer production performance. Reviews on aquafeed contamination note that plant-based ingredients such as maize and oilseeds are favorable substrates for mycotoxigenic fungi and that mycotoxin exposure in fish is associated with reduced weight gain, poorer feed conversion, immune impairment, higher mortality, and possible carryover risks along the food chain. In turbot specifically, nutritionally damaged feed can also act through oxidative deterioration: fish given oxidized fish oil with antioxidant deficiency showed depressed phagocyte chemiluminescent response and higher mortality after bacterial challenge, confirming that environmentally induced feed spoilage can translate directly into reduced disease resistance (Pietsch et al., 2020).

8 Case Study: Environmental Stress-Driven Disease Outbreaks in Intensive Turbot Farming Systems

8.1 Overview of typical industrial recirculating aquaculture systems (RAS) and net pen systems

Industrial turbot farming is increasingly centered on land-based recirculating aquaculture systems because they permit intensive production under controlled indoor conditions and reduce exposure to external climatic variability. In turbot RAS, production water typically passes through linked compartments that include fish tanks, sedimentation units, biofilters, and ozone or protein-skimming chambers, allowing continuous control of temperature, dissolved oxygen, salinity, and nitrogenous wastes (Ahmed and Turchini, 2021). This engineering design supports high stocking density and stable production, which is one reason RAS has become widely used in turbot culture.

However, the same water-saving and high-density features that make RAS efficient can also concentrate biological and chemical risks when management is suboptimal. Reduced water exchange can permit the accumulation of growth-inhibiting factors, bacterial metabolites, and dissolved contaminants, while treatment performance may become unstable across season, hydraulic regime, and biomass load. Net pen systems differ in that they rely on natural water exchange rather than closed-loop treatment, so they generally experience greater direct exposure to fluctuations in temperature, oxygen, and other environmental drivers that can precipitate stress-linked disease events (Ahmed and Turchini, 2021).

8.2 Multi-factorial stress interaction (temperature-oxygen-ammonia coupling effects)

Disease risk in intensive turbot farming is rarely driven by a single variable, because temperature, oxygen, and ammonia interact at both physiological and production levels. Elevated temperature is a major stressor in this cold-water species and has been linked with rapidly increasing disease-induced mortality above 20°C, while high ammonia exposure activates the hypothalamic-pituitary-interrenal axis and suppresses humoral immune indicators such as lysozyme, complement, and IgM. This means that warming and ammonia accumulation can jointly weaken host resistance even before a specific pathogen is identified.

Oxygen availability modifies the severity of ammonia toxicity and therefore changes outbreak risk under intensive culture. In juvenile turbot, chronic un-ionised ammonia above 0.17 mg L^-1 reduced growth under normoxic conditions, whereas hyperoxia increased tolerance to ammonia, indicating that oxygen management can partly buffer toxic nitrogen stress (Gao et al., 2023). Evidence from another marine fish model points in the same direction mechanistically: ammonia exposure under higher temperature increased cortisol and *hsp70*, disrupted antioxidant balance, and reduced innate immune functions, supporting the view that coupled thermal and nitrogen stress can intensify physiological collapse in aquaculture systems.

8.3 Disease outbreak case analysis and management implications

Field and farm observations show that bacterial disease outbreaks remain the dominant health problem in intensive turbot production. A three-year epidemiological survey in China found that bacteria were isolated from 137 of 155 investigated disease cases, with Edwardsiella piscicida and *Aeromonas salmonicida* together accounting for about 71% of all cases, demonstrating that stress-sensitive bacterial pathogens dominate real farm losses (Gao et al., 2023). A separate RAS study detected potential pathogens such as Photobacterium damselae, Tenacibaculum discolor, Tenacibaculum soleae, and Serratia marcescens throughout multiple system compartments even in the absence of overt disease, indicating that intensive systems can harbor persistent background pathogen pressure.

Management implications therefore center on preventing environmental deterioration before it converts latent pathogen presence into clinical outbreak. During an Edwardsiella outbreak in turbot RAS, higher ozone treatment improved survival and reduced heterotrophic bacteria, *Vibrio* loading, and nitrite relative to lower-control conditions, suggesting that tighter water-quality control can reduce outbreak severity even when it does not fully remove infection pressure. At the population level, the marked decline in E. piscicida case proportion after vaccine introduction further indicates that outbreak control is strongest when environmental management is combined with targeted prophylaxis rather than relying on antibiotics alone, especially given the reported increase in antibiotic resistance over time (Gao et al., 2023).

9 Integrated Management Strategies for Health Improvement

Environmental monitoring and early warning systems are increasingly central to health management in turbot farming because intensive aquaculture depends on detecting water-quality instability before it causes physiological stress or mortality. In recirculating aquaculture systems, real-time monitoring of basic parameters such as dissolved oxygen, pH, temperature, turbidity, and salinity is already technically feasible, and IoT-based systems combined with artificial intelligence can generate warnings when conditions approach critical thresholds. This is especially important in turbot culture because emerging evidence shows that dissolved wastes such as phosphate can cause gill, liver, and spleen injury at high concentrations, reinforcing the need to expand monitoring beyond only traditional variables.

Early warning capacity is strengthened when environmental sensing is paired with anomaly detection, behavioral observation, and predictive analytics rather than relying on periodic manual measurements alone. Aquaculture monitoring platforms now support continuous data transmission, automated warning scores, and real-time visualization, while AI-based systems can detect deviations in fish behavior and water quality patterns quickly enough to support preventive intervention. In parallel, biosensor frameworks and biological early warning systems indicate that physiological or organism-level responses can provide a real-time signal of pollution or other stressors before overt disease develops, which is highly relevant for intensive turbot production under fluctuating environmental loads.

Water quality regulation in turbot systems should focus on preventing the accumulation of nitrogenous wastes, phosphate, and other dissolved metabolites while stabilizing hydraulic and feeding conditions that support biofilter function. In turbot RAS, treatment performance can vary with season, biomass, and recycle rate, and earlier farm-scale work showed instability in solids and nutrient removal under changing outdoor conditions, indicating that regulation must be adaptive rather than static. More recent turbot evidence also shows that feeding frequency affects ammonia and nitrite dynamics, with feeding twice daily producing the highest removal rates and the most stable water environment, likely because this regime better matches digestion and supports nitrification-linked microbial functions.

Biofiltration optimization should therefore integrate engineering control with husbandry management, because microbial treatment efficiency depends on both reactor design and the waste-loading pattern imposed by farming practice. General RAS evidence shows that recirculation can reduce ammonia by more than 80% and phosphate by more than 70%, but persistent high-ammonia zones can still emerge where aeration or local operation is suboptimal. For turbot specifically, continuous phosphate surveillance is now justified because juveniles tolerated 60 mg/L through compensatory tissue repair responses, whereas 120 mg/L caused apoptosis in gill tissue and broader hepatic and immune injury, meaning that dissolved nutrient control should target prevention of chronic sublethal toxicity as well as acute failure.

Probiotic and immunostimulant strategies offer a practical complement to environmental control by strengthening host resilience against the background pathogen pressure typical of intensive turbot farming. In juvenile turbot, dietary oregano oil and Bacillus coagulans improved growth, digestive capacity, and resistance to *Aeromonas salmonicida*, while host-associated Bacillus velezensis T20 improved intestinal antioxidant capacity, barrier function, microbiota composition, and survival after *Edwardsiella tarda* challenge. Native or host-adapted microbial candidates appear especially promising because prophylaxis is more effective than reactive treatment in early life stages, and beneficial strains can reduce mortality without depending on antibiotic-based control.

Adaptive farming strategies should combine these functional feeds with environmental adjustment of flow, hygiene, and routine operations to reduce chronic stress exposure. In turbot RAS, a flow velocity around 0.9 body lengths per second promoted feed intake, growth, and innate immune indicators, whereas higher velocity acted as a stressor and reduced growth, showing that hydrodynamic settings are a manageable component of health support. Newer dietary interventions also broaden the toolkit: multi-strain probiotics improved enzyme activity and intestinal structure, B. coagulans helped restore microbial homeostasis after antibiotics, and postbiotics from Cetobacterium somerae improved gut health and disease resistance, supporting a shift toward integrated, low-antibiotic health management in turbot aquaculture.

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