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Genotoxic Damage in Rainbow Trout *Oncorhynchus Mykiss* Exposed to Transport Stress

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Abstract

Transporting live fish is a common technique in the aquaculture industry. This research examined how 3-hour transportation stress affects the micronucleus frequency of rainbow trout (*Oncorhynchus mykiss*). The micronucleus test was used to assess micronuclei and nuclear abnormalities in peripheral erythrocytes. Fish were sampled before (control) and immediately after the 3-hour transport process (t0 group), 6 hours after the transport process (t6 group), 12 hours after the transport process (t12 group), and 24 hours after the transport process (t24). The research found that the greatest MN frequency was substantially detected in the t0 group ($p<0.001$). Additionally, the nuclear abnormalities (NAs) in the blood samples of t0, t6, and t12 groups are considerably greater ($p<0.001$) than those in the control group. An important rise in micronucleus frequency was seen following the transportation procedure, followed by a considerable drop at 12 and 24 hours post-departure, but not going back to the initial values. Micronuclei and nuclear abnormalities in peripheral erythrocytes serve as reliable markers of stressful circumstances in transported fish, as shown by the results.

Keywords

Oncorhynchus mykiss, fish transport, micronucleus test, nuclear abnormalities.

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Introduction

Transporting alive fish is essential in the aquaculture sector, however, it may lead to stress, impacting the physiological condition, and muscle quality, and perhaps resulting in mortality. Movement distress has impeded the growth of fish farming companies that depend on live movement. It is essential to establish a suitable transport method by monitoring and assessing the fluctuating stress reactions. Transporting fish may be a very stressful process due to factors such as capture, delivery, a variety of circumstances, and fluctuations in water (Robles et al., 2015) quality, especially during extended journeys (Martins et al., 2024). Transport-induced stress may elevate the rate of metabolism and gill breathing, leading to osmosis problems, and oxidative damage, with degradation of water quality due to increased excretion of CO_2 and ammonium (Yarahmadi et al., 2016; Turan & Ergenler, 2021a; Suljić & Kovčić, 2018; Sopinka et al., 2016).

Fish living in diverse environments may experience varying levels of stress due to varied environmental factors, which might impact their stress response via distinct pathways. For instance, temperature may influence the stress response dynamics by impacting reaction rates. Oxygen levels can impact metabolic capacity, affecting the extent and duration of the allostatic load. Social hierarchies among fish can lead to varied responses to a shared stressor (Ergenler & Turan, 2023). The digestive state (full or empty stomach) can influence stress tolerance during fish transport. Pathogen infection might modify the reactions and tolerances in comparison to healthy persons. Capturing, transporting, and handling cause stress for confined fish. Wild fish may encounter comparable disturbances, such as those caused by catch and release initiatives in recreational fishing. Cleaning, evaulating, and delivering immunizations might elevate the anxiety response in farmed fish. Additional adverse effects include overpopulation, oxygen deprivation, physical harm, anaesthetic or sedative side effects, and pressure fluctuations for deep-sea species. Research has shown significant increases in systemic cortisol and/or glucose concentrations in fish after purposeful handling and transportation, suggesting that these actions are intrinsically stressful (Turan & Turgut, 2020).

A threatened balance between adaptable and regressive stress occurs at the cellular level, typically reflected in the responses of the entire organism. Reactive oxygen forms signal inflammation as a common cellular response to disease or foreign substances. Genes involved with reactive oxygen species work hermetically way as part of the stressful response. Reduced oxidative stress promotes the generation of antioxidant defences. If these defences are unable to cope, it might lead to heightened gene expression with perhaps adverse effects. Stress-essential genes react to certain stress factors, while stress-induced genes play a role in metabolic and complex hormonal system activation. These genes impact intracellular disruptions and overall processes, necessitating an in-depth approach to comprehend the identification of stress and removal within a stressotope framework (Lushchak, 2016; Morgenroth et al., 2024).

The micronuclei test identifies chromosomal abnormalities in animals, such as fish, caused by stressors. This approach is used in environmental evaluations to identify genotoxic consequences. Erythrocyte nuclear abnormalities (NA) are another toxicity biomarker (Vijayakumar et al., 2019). Understanding this impact is crucial for understanding environmental factors' effects on fish erythrocyte stability (Kamshilova et al., 2013).

The recommended acclimatization processes for tropical fish, as outlined in the standards for appropriate management practices, vary from 10 to 60 minutes. Nevertheless, there is a deficiency in a universally accepted procedure, and the most effective methods and time for acclimatization remain uncertain. Additional details on acclimation methods are required in order to alleviate stress and facilitate the adjustment of animals to unknown conditions (Paixão et al., 2024).The transportation of fish can be a very challenging

procedure, especially during long journeys, characterized by many aspects, including catch, delivery, various conditions and changes in water quality (Arasu et al., 2024).That's why we wanted to explain the irregularity that the rainbow trout fish show in their erythrocytes during transfer using the micronucleus test technique (Dalzochio et al., 2016).

Material and Method

Rainbow trout with an average weight of 15.50±5.76 g were transported from Tunç Alabalık in Türkiye to the Aquaculture Research and Development Center at Iskenderun Technical. The transportation was conducted by a truck and took 3 hours. Fish were captured from the culture pond and transferred to a depuration tank for 16 hours to facilitate gastric emptying. Following transportation, the fish were moved to 500-L tanks equipped with constant aeration and a water recirculation system for their recuperation. Each bag of fish was placed in individual aquariums for further observation. Fish were sampled before (control) and at different time points after being transported: immediately (t0 group), 6 hours (t6 group), 12 hours (t12 group), and 24 hours (t24 group). At each sample point, 10 fish were euthanized using a phenoxyethanol solution with a concentration of 1.5 mg L^{-1} (Turan et al., 2016b). In addition, the water during the transfer is in optimum conditions.

In sampling station, water temperatures demonstrated to culture pond, 13.05±1.43 °C. The dissolved oxigen values 7.50±0.48, pHvalues 8.01±0.55, Hardness(µS/cm) 881±2.75. According to other parameters averages, NO3-N, NO2-N, NH4 were 2.03±1.02; 0.15±0.34; 0.02±0.012 mg/L relatively on culture pond. In addition toThe fish were then packed in 50-liter polyethylene containers with a 1:4 ratio of water to pure oxygen for transportation. Another sampling station, water temperatures demonstrated during transportation, 14.00±2.01 °C. The dissolved oxigen values 8.00±0.33, pH values 7.75±1.02, Hardness(µS/cm) 800±2.00. According to other parameters averages, NO3-N, NO2-N, NH4 were 3.00±1.00; 0.20±0.20; 0.05±0.1 mg/L relatively on transportation. The Aquaculture Research and Development Center at Iskenderun Technical's physicochemical values of the water during the transfer of the fish are as follows to 14.00±1.50 C. The dissolved oxigen values 7.45±0.52, pH values 7.50±3.0, Hardness(µS/cm) 830±1.50. According to other parameters averages, NO3-N, NO2-N, NH4 were 2.80±1.15; 0.18±0.50; 0.07±0.3 mg/L.

Micronucleus Test

The Micronucleus test assessed micronuclei and nuclear abnormalities in peripheral erythrocytes. Peripheral blood specimens were collected by heart puncture using a syringe containing heparin. The samples were immersed in absolute ethanol for 10 minutes and then treated with 5% Giemsa stain for another 10 minutes. The frequency of micronucleated cells was assessed using an illuminated microscope at 1000x magnification by examining a standard of 1000 blood cells in apiece fish. Carrasco et al., (1990) examined structural nucleus anomalies in peripheral smears, categorizing them into five basic groups.

Statistical Analysis

The data sets we obtained were subjected to normality (Shapiro–Wilk test) and homogeneity (Levene's test) tests before statistical analysis. Duncan used analysis of variance (ANOVA) and multiple comparison test to compare the differences of all parameters between stations (Dalzochio et al., 2016).

Results

Means and standard deviations of the micronuclei and nuclear abnormalities in *Oncorhynchus mykiss* obtained from control and the 3 h transport process groups were given in Table 1.

Table 1. Means (%) and standard deviations of micronuclei and different categories of nuclear anomalies of trout erythrocytes were obtained from the control and transport process groups

Groups	Micronucleus	Kidney	Binucleus	Notched	Lobbed	Budded
Control	3.100±0.361^{a}	2.900±0.721^{a}	2.200±0.100^{a}	5.733±0.208^{a}	5.500±0.010^{a}	5.400±0.100^{a}
t0	11.100±0.082^{b}	8.033±0.047^{e}	9.100±0.082^{f}	10.467±0.047^{e}	10.800±0.082^{f}	10.100±0.082^{a}
t3	10.167±0.125^{c}	7.267±0.205^{d}	8.167±0.125^{e}	8.733±0.205^{d}	8.867±0.047^{e}	8.400±0.082^{b}
t6	8.367±0.094^{d}	5.767±0.047^{c}	6.567±0.125^{d}	6.833±0.047^{c}	7.600±0.082^{d}	6.600±0.082^{c}
t12	5.600±0.141^{e}	4.900±0.082^{b}	5.267±0.205^{c}	6.067±0.094^{b}	6.267±0.047^{c}	5.733±0.170^{b}
t24	4.167±0.125^{f}	3.367±0.094^{a}	4.333±0.125^{b}	5.900±0.082^{ab}	5.867±0.125^{b}	5.500±0.082^{a}
P	***	***	***	***	***	***

*Values with different superscripts in each column indicate significant differences. Indicate the significance level between micronuclei and nuclear anomalies of trout erythrocytes obtained from control and transport process groups. (***, P<0.001). The data are given as arithmetic mean (%) ± SD.

As a result of the study, it is determined that the highest MN frequency is significantly observed in the t0 group ($p<0.001$). Besides, it is observed that the other nuclear abnormalities (NAs) in the erythrocyte samples of the t0, t6, and t12 groups are significantly higher ($p<0.001$) compared to the control group. A significant increase in micronucleus frequency was observed after the transport process but a significant decrease occurred after 12 and 24 h from departure even without returning to control levels. No fish mortality was observed in the transport process groups and the control during the experiment.

Furthermore, Ammonia nitrogen, pH, and dissolved oxygen are the most fundamental and important criteria for assessing water quality (Liang et al., 2020). The water quality can change rapidly when fish are being transported, especially at higher fish densities. However, in our study, it is believed that the water conditions in the tanks at the Fisheries Research and Development Center at İskenderun Technical University and the culture ponds from which the fish were taken are optimal.Additionally, efforts have been made to maintain optimal physicochemical parameters throughout the transfer process. However, it is believed that the abnormalities in the micronuclei are caused by the stress experienced by fish during the transfer process, rather than the physicochemical properties of the water.

Discussion

Optimized fish movement is essential for welfare fish production and research, since it encompasses the movement of breeding stock, regulation of reproduction, and the transfer of eggs and larvae. The growing popularity of sport fishing has created a higher demand for larger fish. Nevertheless, using unprofessional transportation techniques may have adverse effects on welfare to fish, resulting in stress, heightened susceptibility to infections, and perhaps even mortality. Producers may experience production delays and experience financial losses as a consequence. The duration of transportation might vary from 2 to 72 hours, with trucks and cars being the primary means of transportation. Commercial salmonid production may use many transportation methods, including coastal transport, road transfer with separated tanks, and helicopter

transport. Nevertheless, inadequate mobility may result in heightened anxiety, heightened susceptibility to illnesses, and perhaps even fatality. The effectiveness of transportation is strongly associated with the quality of animals being transported, which is connected to the use of optimum production methods, cultural parameters, water cleanliness, feeding regimens, and careful attention during harvesting. In order to achieve efficient transportation, it is important to possess understanding of the species, dimensions of the fish, and suitable methods of management. Acquiring a thorough knowledge of physiology, water quality, and transport strategies is essential in order to minimize animal stress and reduce mortality (Shabani et al., 2016). Transporting freshwater fish was an important step in aquaculture, however, it may subject the fish to stresses like ventilation, utilization, congestion, and isolation (Chandroo et al., 2005; OIE, 2015).

Transporting fish from one location to yet another increases plasma cortisol and glucose levels, and disrupts the equilibrium of sodium and chloride ions in their bodies (Sampaio & Freire, 2016). Plasma cortisol and blood lactate levels increase dramatically in fish subjected to gradual hypoxic (Herbert & Steffensen, 2005; Faught & Vijayan, 2016). Research demonstrates elevated levels of haemoglobin and red blood cell (RBC) count in fish blood when exposed to hypoxia. Low oxygen levels impact adenosine triphosphate (ATP) generation, resulting in decreased ATP production (Pollock et al., 2007; Zhao et al., 2014). In their study, they evaluated different temperatures and storage densities for transporting Rhamdia quelen fry in plastic bags for 24 hours. The authors have also determined that transportation at 25°C for more than six hours can cause stress in fish due to low oxygen content and high carbon dioxide concentrations. It has been suggested that the lack of optimal temperature during transportation increases mortality. In our study, it was observed that during the transfer of trout, the temperature was optimal, but the transfer process affected the erythrocytes, leading to anomalies.When subjected to oxidative stress, *C. punctatus* showed significant changes in their haematological parameters, micronuclei induction and pathogenic indicator activity of enzymes (Javed et al., 2016). Focus on at other transportation live fish literature tracking to (Du et al., 2016). The study focuses on the role of hypothalamus-hypophysia-interrenal ekseni genes in the development of *Coilia nasus,* specifically focusing on the role of cytokines like cortisol, urotensin I, and pro-opiomelanocortin in the development of stress. Although water quality parameters are optimum, but the results show that stress in C. nasus leads to significant changes in CRH and UI levels, with the POMC level being the most significant. The study also reveals that stress-related biomarkers are affected by stress levels, with lipid peroxidase and malondialdehit levels being the most significant. This study is similar to our study.The research showed a notable rise in micronucleus frequency following transportation, followed by a decline after 12 and 24 hours after departure, yet not reverting to initial values. The increase in the proportion of haemoglobin in the transported fish compared to their initial levels indicates that the alterations in the red blood cells are influenced by cortisol-induced micronuclei and nuclear abnormalities (Schreck & Tort, 2016).

Stress affects the genome, causing somatic mutations in blood cells, such as erythrocytes, altering their genetic characteristics and resulting in their dysfunction (Kelestemur & Ozdemir, 2010, Kamshilova et al., 2013). The low number of erythrocytes with micronuclei in trout erythrocytes after moving around to the aquaculture sector farm to the Aquaculture Research-Development Center (İskenderun Technical University) could be caused by the influence of native natural adrenaline generated as a consequence to moving stress.

Kamshilova et al., (2013) researched the impact of transportation on the presence of micronuclei in erythrocytes of starlet fish. They found that the transportation procedure led to an increase in the number of fish with micronuclei and the percentage of abnormal erythrocytes. Conducted a study on the transportation of Oreochromis niloticus tilapia fry, varying parameters such as time, density, temperature, dissolved oxygen, and salinity. The ideal fish transfer weight for tilapia fry is 2.4 ± 0.3 grams for every 80 grams of fry. They determined that it was achieved by conveying at a density of grams per litre, at 24 degrees Celsius, for up to

24 per mil hours. Yang et al., (2024) investigated the stress impact on sturgeon fish during transportation using ELISA kits and qPCR. The study examined levels of cortisol, epinephrine (EPI), adrenocorticotropic hormone (ACTH), corticotropin-releasing hormone (CRH), and gene expression of glucocorticoid receptor (GR), heat shock protein 90 (HSP90), and heat shock protein 70 (HSP70). They found that transplanting disturbed the equilibrium of homeostasis and led to fluctuations in gene expressions (Luz et al., 2024).

Refaey & Li, (2018) investigated the basal levels of transferred and non-transferred fish, as well as serum cortisol, glucose, total cholesterol, and triglyceride concentrations, along with aspartate aminotransferase activity at 0 and 1 hours. They observed a significant increase in these substances. The study revealed a substantial decrease in the total protein content of the blood in transported fish. Researchers observed an increase in total antioxidant capacity (T-AOC), malonaldehyde concentration, glutathione peroxidase, and catalase activities in fish after 6 hours of transit. These levels recovered to normal after 72 hours. Showed that yellow catfish (*Brycon cephalus*) need 24 to 72 hours to recover from transportationstres. Our team (2021) reported that DNA damage in hybrid tilapia subjected to the short-transport process. We found that a rise in DNA harm metrics in the gill cells suggests a mutagenic influence of the transport process on the DNA damage of gill cells (Turan & Ergenler, 2021c). We also examined how the transport mechanism impacts the frequency of micronuclei in the erythrocytes of carp (*Cyprinus carpio*). The research found that the t0 group had the greatest MN frequency. Additional nuclear abnormalities (NA) were substantially more prevalent in the blood samples of the t0, t6, and t12 groups compared to the control group (Turan & Ergenler, 2021a), with a p-value of less than 0.01. Our findings areconsistent with prior studies. Previous studies suggest that variations in physiological healing under transportation stress are likely influenced by factors such as fish species, reactions to stress, transportation time, stress levels, and transportation conditions (Refaey & Li, 2018).

Conclusions

Effective transportation of animals may mitigate stress, heightened susceptibility to diseases, and potential mortality. The quality of the animals is intricately connected to their breeding circumstances, which include ideal production procedures, culture parameters, water purity, feeding regimens, and precise harvested care. Comprehending the characteristics of the species, the size of the fish, and the tactics used for management is essential for ensuring efficient transportation. An in-depth knowledge of anatomy, water quality, and transport strategies may effectively reduce animal stress and mortality. The study showed that rainbow trout O. mykiss showed signs of stress following a 3-hour trip, as shown by significant increases in micronucleus frequency and nuclear abnormalities before the journey began. After 12 hours, a decline in stress markers was seen, with micronucleus frequency returning to control values during transit, indicating rapid adaptation of this organism to stress circumstances. Micronuclei and nucleus anomalies in periphery blood cells serve as crucial markers for anxiety for relocated trout.

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Author Contribution

Abdülsamed Tunç: Conceptualization, methodology, formal analysis, investigation, writing—original draft. **Funda Turan:** Conceptualization; project administration; methodology; writing, original draft; reviewing and editing. **Ayşegül Ergenler:** Conceptualization, methodology, formal analysis, investigation, writing original draft All authors provided feedback on earlier drafts of the work. All authors have reviewed and endorsed the final article.

Conflict Interests

Conflicting objectives The researchers open up any conflicts of interest.

Data Availability Statement

The data that supports the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Compliance with Ethical Standards

Every necessary international, national, and institutional guideline for the treatment and handling of animals has been complied with.

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