Stratification and mixing dynamics in river waterholes: Insights from high-frequency observations

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Key points

- Stratification transitioned from weak and intermittent spring events to persistent and strong summer events under prolonged low-flow conditions.
- Water-column stratification was controlled by atmospheric forcing, mainly convective cooling and occasional wind during stagnant summer conditions.
- Persistent stratification led to a mean decline in deep-water oxygen concentration of 7% per day of stratification and caused frequent hypoxic conditions at 2m depths in the waterhole.

Abstract

Thermal stratification in river waterholes during zero- or low-flow conditions can suppress vertical mixing, promote hypoxia, and influence algal dynamics, posing significant risks to aquatic ecosystems during drought and summer low-flow periods. This study investigated thermal structure, mixing dynamics, and dissolved oxygen variability within a river waterhole in Deep Creek, Victoria, Australia, during low flow using high-frequency monitoring from a floating monitoring platform. Continuous measurements of temperature profiles, dissolved oxygen, meteorological forcing, radiation, and light attenuation were collected.

Results showed a clear seasonal transition from relatively weakly stratified spring conditions to persistent summer stratification under near-stagnant flow conditions. As flow velocities declined and light attenuation increased, stratification events became stronger, longer, and more frequent. Persistent stratification strongly influenced the dissolved oxygen distribution. Bottom-water dissolved oxygen progressively declined during prolonged stratification events and frequently reached hypoxic conditions (≤2 mg/L), while surface waters remained comparatively oxygenated except during a chain of stratification and mixing events in January, when dissolved oxygen in the upper layer also dropped below the critical stress threshold (≤4 mg/L). Event-scale analysis indicated that atmospheric forcing, particularly convective cooling and wind events were key driver of intermittent mixing during stagnant summer conditions. Rapid re-stratification commonly followed mixing events. Stratification occurred over the whole September-January record but was more persistent during summer. There was strong coupling between thermal stratification and hypoxia.

Keywords

Thermal stratification, hypoxia, waterholes, mass fish-kill, dissolved oxygen, rivers, drought refuge

Introduction

Oxygen is vital for sustaining aquatic ecosystems through support of aerobic respiration and ecological productivity (Blaszczak et al., 2023). Hypoxia, commonly defined as dissolved oxygen concentrations below 2 mg/L, is a serious environmental problem that can lead to severe ecological impacts, including mass fish-kill events (Guo et al., 2022). Frequent fish-kill events in Australian rivers have highlighted the vulnerability of river systems to rapid changes in water quality and dissolved oxygen during low flow (Baldwin, 2019; Balzer et al., 2025).

Thermal stratification is one of the major controls on hypoxic conditions in aquatic systems (Ladwig et al., 2021). During warm periods, surface waters heat more rapidly than deeper waters, producing a warmer and less dense surface layer above a cooler bottom layer (Boys et al., 2022). The resulting density gradient suppresses vertical mixing and restricts oxygen transfer from the atmosphere to deeper layers where oxygen consumption commonly occurs due to sediment oxygen demand and organic matter degradation (Becker et al., 2010).

Persistent thermal stratification in rivers was once considered uncommon because turbulent flow was assumed to maintain mixing throughout the water column. However, during drought and prolonged low-flow conditions (characterized by consecutive days with discharge below 5 ML/day), many rivers contract into isolated or weakly connected waterholes where flow velocities are greatly reduced (Anderson & Morison, 1989; Marshall et al., 2021). These deeper and slower-flowing sections are increasingly recognised as vulnerable to persistent thermal stratification and hypoxia (Townsend, 2006; Turner & Erskine, 1997).

Despite the ecological importance of river waterholes, the dynamics of stratification and mixing in these systems remain poorly understood (Zhai et al., 2023). In addition, river water-quality monitoring programs, particularly for temperature, commonly rely on low-frequency (e.g., monthly) or surface-only measurements. Such measurements may fail to resolve short-term and depth-dependent stratification and mixing events, potentially underestimating water-quality risks in river waterholes (Preite & Pearson, 2017).

To address this gap, a stationary floating raft platform was deployed within a stratified river waterhole to collect high-frequency measurements of temperature profiles, dissolved oxygen profiles, light penetration profiles, and the ambient meteorological conditions above the water surface. The objectives of this study were to:

1. Evaluate the temporal dynamics of thermal stratification and mixing within a river waterhole;
2. investigate the relationship between meteorological forcing and water-column stability; and
3. Assess the influence of persistent thermal stratification on the development of hypoxic conditions.

This study aims to improve understanding of thermal processes in low-flow river systems and to support monitoring and management strategies aimed at reducing hypoxia and fish-kill risk in river waterholes.

Material and methods

Study site

The study was conducted in a river waterhole in Deep Creek, a key tributary of the Maribyrnong River, northwest of Melbourne, Victoria, Australia. Deep Creek drains the central, northern, and eastern parts of the Maribyrnong catchment and supports several threatened and vulnerable aquatic species listed under the Environmental Protection and Biodiversity Conservation Act (Sinclair Knight Merz, 2012). During low-flow periods, these refuge pools provide important habitat for fish and other aquatic organisms. The study reach is located within the ~30 km drought refuge section of Deep Creek extending from Darraweit Guim to north of Lancefield (Figure 1). This study site is a ~600 m long river waterhole with a variable width ranging between 10 and 20 m. The selected site is located at a surface water monitoring station (Water Measurement Information System station no. 230232), which provides flow and rainfall data used in this study.

Waterholes are critical drought refuges for aquatic ecosystems and fish populations during dry periods (Sheldon, 2017). Sudden destratification events can, however, rapidly mix hypoxic bottom water throughout the water column, resulting in sharp declines in dissolved oxygen concentrations and increased ecological stress (Sheldon et al., 2022). This reach contains a series of permanent waterholes connected by shallower riffle sections (Cartwright & Gilfedder, 2015). Deep Creek's waterholes are commonly 1–2 m deep, although deeper sections also occur. Flow in Deep Creek responds primarily to rainfall events and declines substantially during summer, with the lowest flows typically occurring between December and May. Under prolonged drought conditions, sections of the creek may cease flowing entirely. Baseflow within permanent pools is sustained by groundwater discharge and bank storage release following flood events. Flow during dry periods has reportedly decreased from a historical median of approximately 19 ML/day to around 8 ML/day under current conditions (Cartwright & Gilfedder, 2015). Reduced pool volume during drought periods can increase water temperature and reduce dissolved oxygen concentrations. The selected study waterhole was chosen based on three criteria:

1. prolonged low-flow conditions,
2. minimal groundwater influence, and
3. sufficient depth (>3 m) to allow for the development and observation of distinct thermal layers.

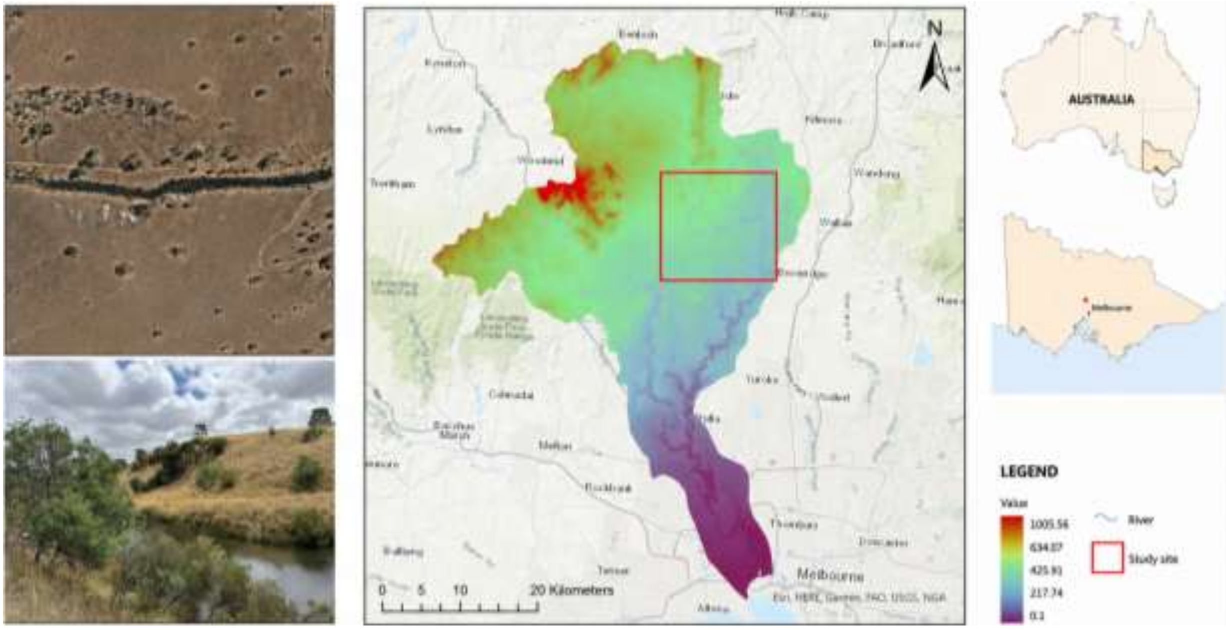

Figure 1 Deep Creek location, study site, and monitoring location

Instrumentation and observations

A stationary floating raft platform was deployed at the waterhole to enable continuous high-frequency (1-minute) monitoring of thermal structure, dissolved oxygen, light penetration, and meteorological forcing. The platform remained fixed within the waterhole and supported vertically distributed in-water sensors together with meteorological instrumentation and automated data logging systems. Table 1 summarises the measurements made.

A thermistor chain consisting of 12 temperature sensors (Therm-EP, ICT; accuracy ±0.05°C) was across the water column to capture the development and breakdown of thermal stratification, sampled at 1-minute intervals. Two dissolved oxygen sensors (OPTOD, AquaLabo; accuracy ±0.1 mg/L) were installed at different depths to monitor oxygen dynamics associated with persistent stratification and mixing events. In addition, three pyranometers (SP-110, Apogee; accuracy <3%) were used to quantify incoming shortwave radiation and light penetration within the water column.

Meteorological conditions above the water surface were monitored using a net radiometer (NR01, Campbell; accuracy ±10%) to measure incoming and outgoing shortwave and longwave radiation, together with a wind sensor (R.M. Young 05305, Campbell; accuracy ±0.2 ms^{-1}, ±3°) to measure wind speed and direction at 1-minute intervals. An adjacent monitoring station located on the river bank recorded air temperature and relative humidity using an EE181 sensor (Campbell; accuracy ±0.2°C and ±1.3–2.3% RH), atmospheric pressure using a PTB110 barometer (Vaisala; accuracy ±0.3 mb), shortwave radiation using a CS320-Apogee pyranometer (calibration uncertainty ±2.6%), and wind conditions using a 03002-L wind sensor (Campbell; accuracy ±0.5 ms^{-1}, ±5°). Meteorological variables collected at the riverbank station were also recorded at 1-minute intervals. Table 1 presents the instrumentation configuration of the monitoring raft. The depths of the underwater sensors varied relative to the stream bed due to changes in water level.

Table 1 Instrumentation distance from the water surface

Sensor type	Depth (m)
Temperature (Therm-EP)	0.05, 0.15, 0.28, 0.4, 0.61, 0.81, 1.13, 1.43, 1.8, 2.2, 2.72, 3.23
Dissolved oxygen (OPTOD)	0.33, 2.13
Downwelling shortwave radiation (SP-110)	0.2, 1.02, 2.02
Net radiation (NR01)	0.8 (above water surface)
Wind (RMY 05305)	2 (above water surface)

These measurements were used to investigate the relationship between atmospheric forcing and water-column stability using a bulk energy-balance approach. Figure 2 presents a schematic of the sensor configuration and pictures of the measurement platform and weather station. Maintenance was conducted weekly, including cleaning the dissolved oxygen and light sensors of biofouling.

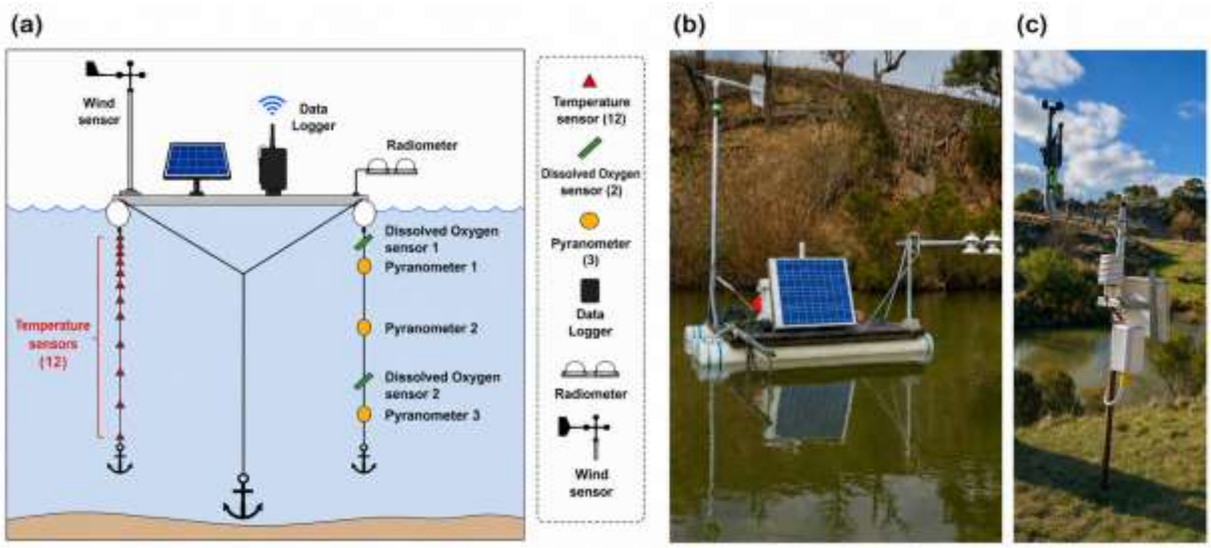

Figure 2 (a) Schematic illustration of the floating monitoring platform and sensor configuration, (b) field deployment of the floating monitoring platform, and (c) adjacent meteorological station

Results

Figure 3 shows the temporal evolution of the water temperature and dissolved oxygen conditions as a function of depth in the waterhole. Water temperature fluctuations are plotted relative to water-level variations referenced to the Australian Height Datum (AHD). Water levels varied between approximately 313.8 and 314.5 m AHD during the study period.

The vertical temperature gradient gradually increased from September to January, with surface waters warming more rapidly than the deeper layers. As temperatures increased, thermal stratification became progressively more pronounced, particularly during December and January when strong and persistent stratification developed. During this period, surface water temperatures commonly exceeded 25 °C, while the bottom waters remained substantially cooler, indicating a strong vertical temperature gradient. Diurnal heating and cooling cycles were also evident, with stratification strengthening during daytime heating and weakening overnight.

The dissolved oxygen record closely reflected these changes in thermal structure. Surface dissolved oxygen concentrations remained relatively stable for most of the monitoring period, generally between 8 and 11 mg/L except for one occasion in late January. In contrast, bottom dissolved oxygen concentrations progressively declined as stratification intensified. From late December onwards, bottom dissolved oxygen frequently dropped below the ecological stress threshold (< 4 mg/L) and periodically approached hypoxic conditions (< 2 mg/L). Occasional short-lived increases in bottom dissolved oxygen coincided with temporary weakening of thermal gradients, suggesting intermittent mixing and reoxygenation events. These recovery periods were brief, and oxygen depletion resumed rapidly once stratification was re-established. By mid- to late January, prolonged hypoxic conditions had developed in the bottom waters, with dissolved oxygen concentrations approaching near-zero values for extended periods. These conditions also contributed to a substantial decline in surface dissolved oxygen concentrations, which fell below ecological stress thresholds between 17 and 22 January, indicating that episodic hypoxic events can also occur in surface waters under warm, stratified, and low-flow conditions (Blaszczak et al., 2023; Zhai et al., 2023). Supersaturation of dissolved oxygen is evident at depth during periods of high water clarity, likely reflecting enhanced photosynthetic activity at depths where light availability and nutrient concentrations are favourable (Wetzel, 2001).

To quantify the stratification of the water column, water density was first calculated from measured temperature profiles using (Martin & McCutcheon, 1998):

$$\rho = 1000\left[1 - \frac{(T + 288.9414)(T - 3.9863)^2}{508929.2(T + 68.12963)}\right] \qquad (1)$$

Here ρ represents freshwater density ($\mathrm{Kg.\,m^{-3}}$) and T is water temperature (°C). Vertical density gradients between adjacent thermistors were then used to calculate the buoyancy frequency (N_C) to quantify the strength of water-column stratification following the approach of Zhao et al. (2022):

$$N_c = \frac{3600}{2\pi}\sqrt{\frac{g}{\rho_0}\frac{\partial\rho}{\partial z}} \qquad (2)$$

where N_c is the buoyancy frequency expressed in cycles per hour (cph), g is gravitational acceleration (9.81 $\mathrm{ms^{-2}}$), ρ_0 is the mean water density (kg $\mathrm{m^{-3}}$), and z is depth measured positively downward from the water surface (m)?

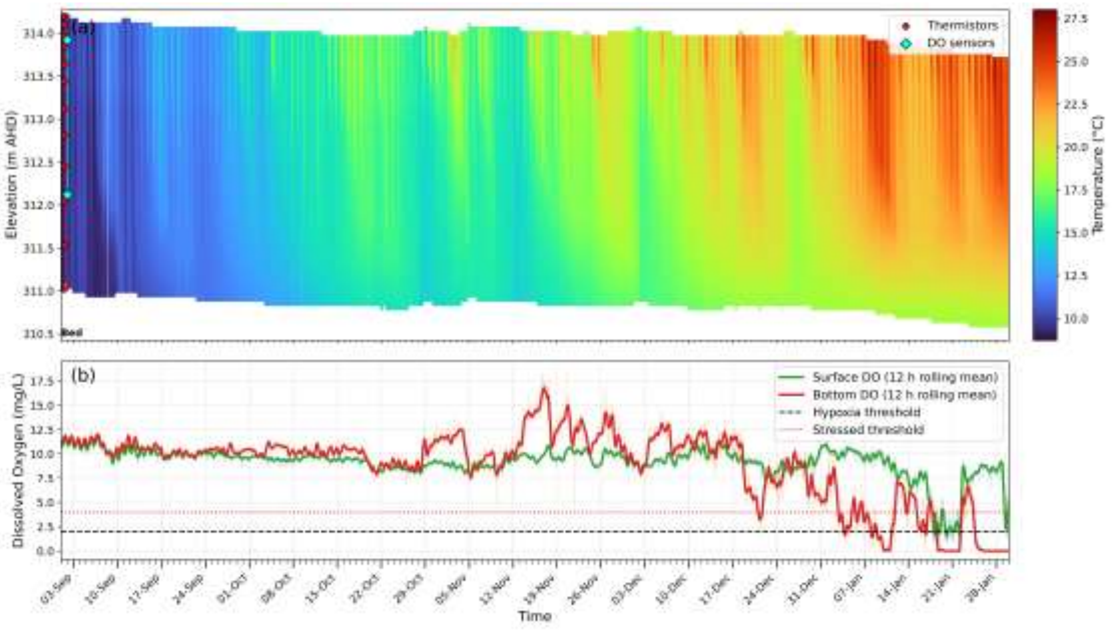

Figure 3 Temporal evolution of (a) water-column temperature profile, with temperature and oxygen sensor locations indicated, and (b) dissolved oxygen concentrations at 0.3 and 2.0 m depths between September 2025 and January 2026. Water surface elevation is indicated by the top of the coloured area in (a)

To identify stratification and mixing events, N_c was smoothed using a 10-minute rolling mean to reduce the effect of short-term variability. Persistent strong stratification events were defined as periods where N_c exceeded 15 cycles h^{-1} for at least 24 hours, allowing only short interruptions (<30 min) to avoid fragmentation of events caused by transient fluctuations. Mixing onset was then identified when the stratification intensity decreased below 70% of the representative event intensity for at least 3 hours. The automatically detected events were compared with manually identified stratification onset and mixing periods to assess the consistency of the classification approach. The automated method showed good agreement with manual classification and was found to better capture partial mixing events that may be overlooked by using the more conventional onset definitions for lakes. For instance, persistence-based criteria requiring the surface-to-bottom temperature difference to continuously exceed a 1°C threshold for 48 h (Woolway et al., 2014) are well suited for identifying sustained lake stratification, but may be less sensitive to the short-lived mixing and rapid re-stratification episodes observed during January in the waterhole, which coincided with severe surface hypoxic events.

Figure 4 highlights a clear seasonal transition in the dominant controls on water-column stability. During early spring, small but non-zero (cross-section averaged) flow velocities coincided with weak and short-lived stratification events, suggesting that a flow velocity around 0.01 -0.02 ms^{-1} or even smaller is sufficient to disrupt developing thermal gradients and maintain partial vertical mixing during cool conditions, consistent with the critical velocity framework of Mitrovic et al. (2006). The absence of persistent stratification during this period indicates relatively well-mixed conditions driven by hydrodynamic forcing (i.e. turbulence generated by flow).

As solar radiation increases through late spring and summer, persistent stratification becomes increasingly frequent, stronger, and longer in duration. At the same time, flow velocities approached zero, and the waterhole shifted towards a thermally stable state characterised by repeated re-stratification and only intermittent mixing events. Several mixing periods during summer occurred without measurable increases in flow velocity, indicating that hydrodynamic (mean stream flow) forcing alone could no longer explain the weakening of stratification.

Panels (b)-(d) of Figure 4 present temporal variations in air temperature, surface water temperature, wind speed, light attenuation, and flow velocity, with stratification and mixing onsets indicated. The vertical light attenuation coefficient (K_d) was estimated from subsurface light measurements using the Beer–Lambert relationship:

$$I(z) = I_0 e^{-K_d z} \quad (3)$$

Where $I(z)$ is the light intensity at depth z, I_0 is surface light intensity, K_d vertical light attenuation coefficient (m^{-1}). For each timestep, K_d was calculated from the slope of the linear regression between the natural logarithm of light intensity and sensor depth.

The results suggest that atmospheric forcing became increasingly important under stagnant summer conditions. Many mixing events coincided with rapid air-temperature fluctuations and elevated wind speeds, indicating that cool changes, nocturnal convective cooling, and, to a lesser extent, wind-driven turbulence, likely promoted temporary destratification. These events were generally short-lived, and stratification was rapidly re-established following mixing. This rapid re-stratification was particularly evident during January, when high solar radiation, warm surface-water temperatures, low flow conditions, and elevated light attenuation (k_d) Favoured strong near-surface heat retention and persistent thermal stability.

Previous studies have shown that stratification varies across daily and seasonal timescales and is influenced by meteorological forcing, morphology, turbidity, flow conditions, and wind sheltering (Bormans et al., 2004; Holgerson et al., 2022; Turner & Erskine, 2005). However, most previous studies focused primarily on flow-driven mixing controlled by the balance between buoyancy and interfacial shear stresses (Bormans & Webster, 1997; Mitrovic et al., 2025; Western et al., 1996). In contrast, this study highlights the important role of convective processes in driving mixing under prolonged low-flow conditions.

Figure 4 indicates a seasonal shift from flow-controlled mixing during Spring to atmosphere-controlled intermittent mixing during prolonged summer low-flow conditions. This is consistent with Wallace et al. (2017), who showed that thermal structure in dry-season waterholes is strongly controlled by atmospheric forcing, particularly solar radiation and wind speed. The increased resistance to sustained destratification observed during summer also aligns with broader studies showing that intensified stratification suppresses vertical exchange and promotes persistent water-column stability (Yan et al., 2024). Overall, these observations suggest that stability and persistence of stratification are governed by the balance between processes promoting stratification, such as solar heating and low-flow conditions, and those promoting mixing, such as wind forcing and surface cooling.

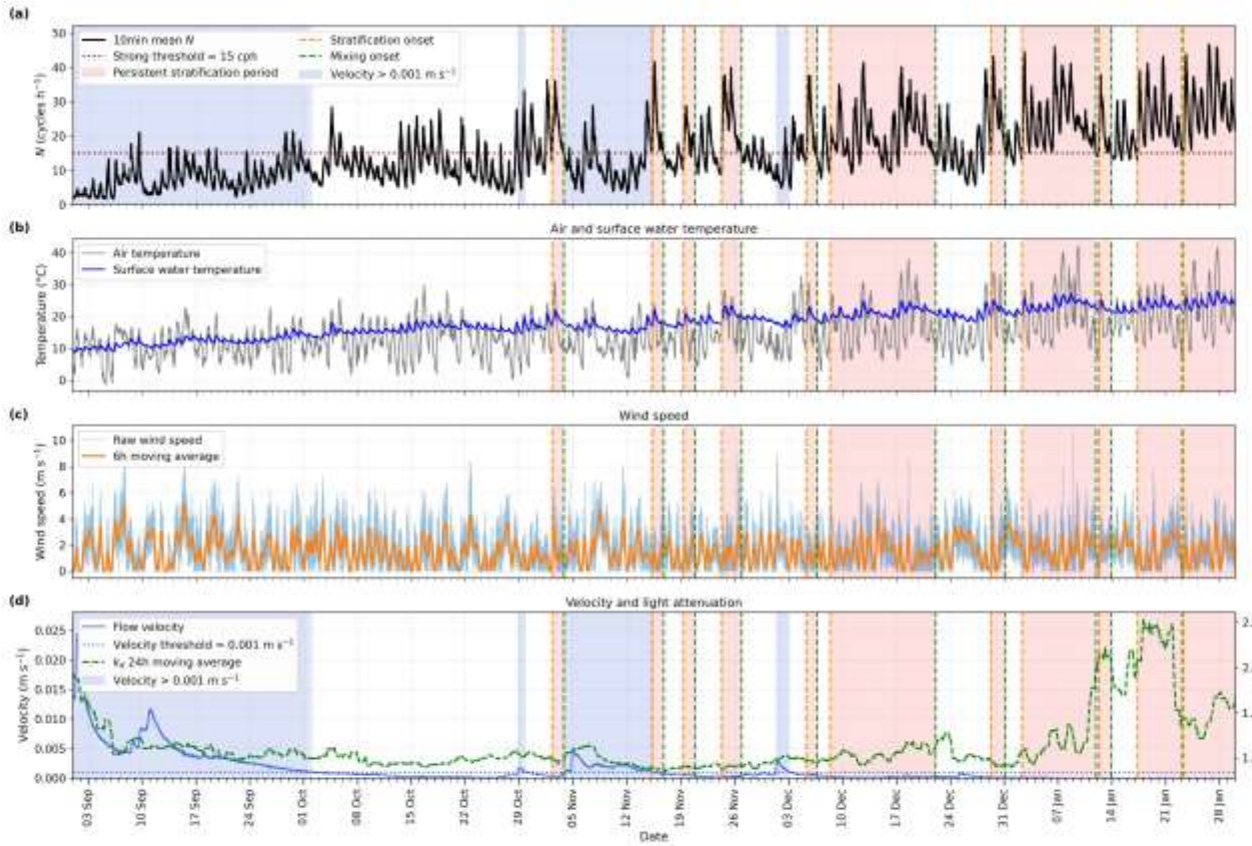

Figure 4 Temporal evolution of stratification strength (N_c) (a) and environmental drivers influencing stratification dynamics within the study waterhole including air and surface water temperature (b), wind speed (c), and flow velocity with light attenuation coefficient (k_d) (d). Major tick marks indicate 00:00 at 7-day intervals, with minor tick marks shown daily

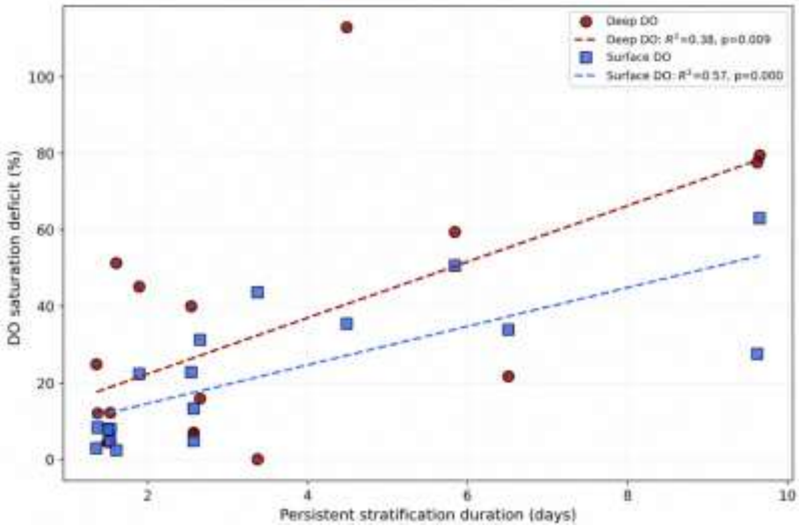

Figure 6 Relationship between persistent stratification duration and dissolved oxygen (DO) deficit in surface and bottom waters. Dashed lines represent linear regression fits for deep and surface DO depletion

Figure 6 shows the relationship between the duration of persistent thermal stratification and dissolved oxygen (DO) deficit in the surface and bottom waters. A positive relationship was observed for both depths, indicating that longer stratification events generally resulted in greater oxygen deficit. Linear regression analysis showed that bottom-water DO depletion increased at an average rate of 7% saturation day^{-1} ($R^2 = 0.38, p = 0.009$), while surface-water depletion increased at 5 % saturation day^{-1} ($R^2 = 0.57, p < 0.001$). The relatively low R^2 values are likely attributable to the limited number of observed events and the complex interplay of meteorological, hydrodynamic, and biogeochemical processes, including temporal delays in oxygen depletion responses. The larger and more variable depletion observed in bottom waters likely reflects the prolonged isolation of deeper layers from atmospheric reaeration and limited vertical mixing during persistent stratification. However, substantial oxygen depletion was also observed in surface waters during longer events, indicating that extended stratification progressively influenced the entire water column. These results indicate that extended periods of persistent stratification substantially increase the risk of hypoxic conditions within the waterhole. This result aligns well with findings from other aquatic systems where DO depletion increases with the increase of stratification duration (Mitrovic et al., 2025).

Conclusions

This study used high-frequency field observations to investigate thermal stratification, mixing dynamics, and dissolved oxygen variability within a river waterhole during low-flow conditions. There was a clear seasonal transition from relatively well-mixed spring conditions to persistent summer stratification. As solar radiation increased and flow declined, stratification events became more resistant to sustained mixing. Although intermittent mixing events temporarily weakened stratification and partially reoxygenated deeper waters, rapid re-stratification commonly followed these events.

Persistent stratification strongly promotes bottom-water oxygen hypoxia, with a deficit rate of about 7 %/day. Overall, the study highlights the strong coupling between persistent thermal stratification and hypoxia development in low-flow river waterholes and demonstrates the importance of high-frequency vertical monitoring for capturing short-duration mixing and oxygen depletion processes.

Acknowledgements

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