

Spatio-Temporal Distribution of Brachyuran Zoea in Vembanad lake, South India: Influence of Salinity Zones and Seasonal Variations

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Abstract

This study investigates the spatio-temporal distribution of brachyuran larvae in Vembanad Lake, a tropical estuarine ecosystem. The research categorizes the estuary into three salinity zones: polyhaline, mesohaline, and oligohaline. It analyzes larval abundance in relation to key environmental factors, including temperature, salinity, pH, and dissolved oxygen. Data collected across multiple seasons showed that larval abundance peaked during the pre-monsoon season and significantly declined during the monsoon season. The mesohaline zone exhibited the highest larval abundance, indicating that it was a crucial habitat for brachyuran larval development. The findings emphasized the impact of seasonal freshwater influx during the monsoon, which transformed the estuary into a freshwater system, posing significant challenges for marine larvae. These results contributed to the understanding of larval distribution in tropical estuaries and have implications for the conservation and management of these critical habitats.

Keywords: Brachyuran zoea, meroplankton, larval abundance, estuarine ecology, Vembanad Lake

Introduction

Brachyuran zoeae are meroplanktonic, surviving in pelagic trophic realms and representing ecologically sensitive life stages, distinct from benthic megalopa larvae and adults. The pelagic larval stages of

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benthic crustaceans contribute to genetic dispersal and the colonization of uninhabited areas (Koettker & Freire, 2006). Larval recruitment and dispersal in brachyuran larvae can vary considerably across taxa and are generally achieved through retention and larval export strategies (Sandifer, 1975; Charmantier, Giménez, Charmantier-Daures, & Anger, 2002). In the former strategy, larval development is restricted to the adult habitat, while in the latter, early larval stages disperse far from adult habitats and may return at later larval stages. The early life stages of most marine species are influenced by environmental factors such as temperature, salinity, and currents, which transport larvae horizontally over long distances (Archambault & Bourget, 1999; Calderón-Aguilera, Marinone, & Aragón-Noriega, 2003; Vieira & de Calazans, 2015; Epifanio & Cohen, 2016). Additionally, larval survival can be significantly threatened by predation pressure.

Vembanad Lake, the largest estuary on the southwest coast of India, is a transitional ecotone between the sea and land, encompassing mangroves, mudflats, swamps, and marshes, which provide suitable habitats for many decapod crustaceans as breeding and nursery grounds (Mogalekar et al., 2015). Although several studies (Madhupratap & Haridas, 1975; Varghese & Krishnan, 2009; Cleetus, Asha, Suson, & Nandan, 2016; Ravi, Raju, Sreekumar, & Varghese, 2020) in this region have broadly focused on zooplankton diversity and composition, information on the larval distribution and species composition of brachyurans is limited, leaving their specific ecological roles and distribution patterns largely unexplored. To address this gap, this study was undertaken with two objectives: (1) to examine the seasonal and geographical distribution of brachyuran zoea in Vembanad Lake, and (2) to delineate the relationships between major hydro-

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graphic variables and the abundance of brachyuran zoea. These findings will provide insights into the environmental factors influencing the distribution and abundance of brachyuran zoea, helping to identify areas critical for their conservation and management.

Materials and Methods

Vembanad Lake (Lat. 9° 29'-10° 10' N; Long. 76° 13'-76° 31' E) (Fig. 1), a tropical positive estuary situated along the southwest coast of India, is the largest brackish water system in Kerala. While the rivers Pamba, Achankovil, Manimala, Meenachil, and Muvattupuzha contribute to freshwater inflow, the lake remains connected to the sea through a permanent opening near Cochin. The study stations were classified into three distinct zones-polyhaline (18-35 PSU), mesohaline (5-18 PSU), and oligohaline (0.5-5 PSU)-based on the Venice classification system. Hierarchical clustering was performed using Euclidean distance and the average linkage method, with salinity as the primary variable, to group stations with similar salinity regimes (Taupp & Wetzel, 2014).



Fig. 1. Map showing study area

Surface (~0.5 m) plankton samples were collected monthly from October 2015 to September 2017 at 10 sampling stations in Vembanad Lake during the early hours. A plankton net with a 200 μ m mesh size (60 cm in diameter) and a flow meter (General Oceanics, Model Number 2030 R) were used to quantify the amount of water filtered during 10minute horizontal tows at 1 knot, to assess brachyuran zoeal abundance. The equation for the flow meter to calculate the volume of water filtered through the net is given below:

DISTANCE in meters =

Difference in COUNTS (final-initial) (x) Rotor Constant 999,99

VOLUME cubic meters =

 $\{3.14159 x (x) (Net Mouth Radius)2\}$ (x) Distance

Samples were immediately preserved in absolute alcohol and subsequently analyzed in the laboratory. Additionally, surface water samples were collected to measure major hydrographic parameters. Salinity (PSU) and temperature (°C) were measured using a handheld refractometer and thermometer, respectively, while pH and dissolved oxygen (mg/L) were determined using a portable water quality analyzer (Eutech PCD 650).

Zooplankton samples were sub-sampled using a Folsom plankton splitter and standardized to 100 mL. Brachyuran larvae were enumerated and initially sorted using a stereo microscope (Leica D300), followed by identification to the family level under a compound microscope (Leica ICC50) based on standard literature (Kakati, 1988; Ko, 1995, 1997; Josileen & Menon, 2004; Rice & Tsukimura, 2007; Bento & Paula, 2018).

The brachyuran larval abundance was expressed as number of larvae per 100 m³. In the context of seasonal studies, the period from October to January was taken as post-monsoon, February to May as premonsoon, and June to September as monsoon (Ravi et al., 2020). Analysis of Variance (ANOVA) was performed to analyze the significance of spatiotemporal distribution and abundance. Before conducting ANOVA, we tested the assumptions of normality using the Shapiro-Wilk test and homogeneity of variance using Levene's test. In cases where the normality assumption was violated (p<0.05), we applied the non-parametric Kruskal-Wallis test as an alternative. For significant Kruskal-Wallis results, post-hoc test was performed to identify pairwise differences between seasons. To determine the relationship between major water quality parameters and brachyuran zoea, a Variance Inflation Factor (VIF) test was conducted prior to Canonical Correspondence Analysis (CCA). The CCA was performed using the PAST software ver. 4.03 (Hammer, Harper, & Ryan, 2001).

Results and Discussion

Surface water conditions exhibited distinct seasonal variations in the polyhaline region of Vembanad Lake over the study period (Fig. 2). The temperature ranged from 27.5°C to 31.75°C, with the premonsoon season (29.92±1.07°C) recording the highest value and the monsoon season (28.29±0.65°C) the lowest. Salinity varied from 1 to 36 PSU, peaking in the pre-monsoon season (31.21±2.26 PSU) and reaching its lowest in the monsoon season (7.44±4.43 PSU). The Shapiro-Wilk test indicated that temperature data deviated from normality in the premonsoon and monsoon seasons (p<0.05), while the post-monsoon season satisfied the normality assumption (p>0.05). Similarly, salinity data exhibited non-normal distribution in the monsoon season (p<0.05), whereas the pre-monsoon and postmonsoon seasons met the normality assumption (p>0.05). In addition, Levene's test for both temperature and salinity revealed significant results (p<0.05), suggesting heterogeneity of variances among seasons. Given these violations of ANOVA assumptions, we employed the non-parametric Kruskal-Wallis test, which indicated significant differences in both temperature (p=0.003) and salinity (p<0.001) across seasons. Further, Tukey's post-hoc test revealed significant seasonal differences, with salinity varying across all seasons and temperature differing between the pre-monsoon and the other two seasons.

pH levels ranged from 6.95 to 7.90, with the highest recorded in the pre-monsoon season (7.76±0.06) and the lowest in the post-monsoon season (7.53±0.25). Dissolved oxygen showed slight fluctuations, reaching its highest in the post-monsoon season (5.06±0.19 mg/L) and its lowest in the pre-monsoon season (4.31±0.70 mg/L). Remarkably, pH and dissolved oxygen remained relatively stable over the two-year study period in the polyhaline region of Vembanad Lake, highlighting the intricate seasonal dynamics of these key water parameters in the ecosystem.

In the mesohaline region of Vembanad Lake, surface water exhibited distinct seasonal patterns over the study period (Fig. 3). Surface water temperature ranged from 27°C to 34.9°C, with the highest recorded in the pre-monsoon season (31.82±1.67°C) and the lowest in the monsoon season (30.14±0.79°C). Salinity ranged from 0 to 33 PSU, reaching its peak in the pre-monsoon season (17.06±3.85 PSU) and its lowest in the monsoon season (1.38±1.59 PSU). Surface water pH showed minimal variation, with the highest recorded in the monsoon season (7.33±0.25) and the lowest in the post-monsoon season (7.27±0.19). Dissolved oxygen concentrations ranged from 1.22 to 7.72 mg/L, with the highest recorded in the post-monsoon season (5.63±0.33 mg/ L) and the lowest in the pre-monsoon season (4.77±0.71 mg/L).



Fig. 2. Mean (±SD) seasonal variations in temperature, salinity, pH and DO in polyhaline region during the study period

In the mesohaline region, the Shapiro-Wilk test for salinity indicated a deviation from normality in the post-monsoon and monsoon seasons (p<0.05), while the pre-monsoon season met the normality assumption (p>0.05). Additionally, Levene's test showed significant variance heterogeneity (p<0.05). Consequently, the Kruskal-Wallis test was applied, revealing a highly significant difference in salinity across seasons (p<0.001). For temperature, the data followed a normal distribution across all seasons (p>0.05), but Levene's test (p<0.05) indicated unequal variances, warranting the use of a nonparametric test. The Kruskal-Wallis test showed a significant difference in temperature among seasons (p<0.01). Similarly, pH data met the normality assumption in all seasons (p>0.05), but Levene's test (p<0.01) revealed significant variance differences. Despite this, the Kruskal-Wallis test did not indicate significant differences in pH across seasons (p>0.05). For dissolved oxygen (DO), the Shapiro-Wilk test showed non-normality in the post-monsoon and pre-monsoon seasons (p<0.05), while the monsoon season exhibited normal distribution (p>0.05). Unlike other parameters, Levene's test for DO indicated homogeneity of variance (p>0.05). The Kruskal-Wallis test detected a significant difference in DO among seasons (p<0.05). Based on these results, nonparametric tests were applied where assumptions of normality and homogeneity of variance were violated and Tukey's post-hoc test revealed significant seasonal differences, with pre-monsoon differing from post-monsoon and monsoon in salinity and temperature, and from post-monsoon in dissolved oxygen.

In the oligohaline region of Vembanad Lake, surface water temperature ranged from 27.1°C to 34.2°C (Fig. 4). The pre-monsoon season (31.89±1.69°C) recorded the highest mean temperature, while the monsoon season (30.00±0.84°C) had the lowest. Salinity ranged from 0 PSU to 10 PSU, with the highest recorded in the pre-monsoon season (3.91±2.08 PSU) and the lowest in the monsoon season (0.03±0.06 PSU). During the study period, pH ranged from 6.0 to 8.7, with the pre-monsoon season (7.43±0.29) recording the highest and the monsoon season (6.98±0.38) the lowest. Dissolved oxygen concentrations showed a seasonal decline, peaking in the post-monsoon season (5.99±0.60 mg/L) and reaching its lowest in the monsoon season (5.01±0.62 mg/L).

In the oligohaline region, the Shapiro-Wilk test for salinity indicated non-normal distribution across all three seasons (p<0.01), and Levene's test further confirmed significant variance heterogeneity (p<0.01). Consequently, the Kruskal-Wallis test was performed, revealing a significant difference in salinity



Fig. 3. Mean (±SD) seasonal variations in temperature, salinity, pH and DO in mesohaline region during the study period

among seasons. For temperature, normality was violated in the monsoon season (p<0.01), whereas the post-monsoon and pre-monsoon seasons met the normality assumption (p>0.01). Levene's test showed homogeneity of variance (p>0.05), but the Kruskal-Wallis test indicated a significant seasonal difference (p<0.05). Regarding pH, the Shapiro-Wilk test indicated non-normality in the monsoon season (p<0.05), while the post-monsoon and pre-monsoon seasons showed normal distribution (p>0.05). Levene's test suggested homogeneity of variance (p>0.05), and the Kruskal-Wallis test detected significant differences in pH among seasons (p<0.05). For dissolved oxygen (DO), normality was violated in the pre-monsoon season (p<0.05), whereas the post-monsoon and monsoon seasons met the normality assumption (p>0.05). Levene's test indicated homogeneity of variance (p>0.05), and the Kruskal-Wallis test showed a significant difference in DO across seasons (p<0.05). Tukey's post-hoc test revealed significant seasonal variations, with premonsoon differing from post-monsoon and monsoon in salinity and pH, from post-monsoon in temperature, and monsoon differing from both seasons in dissolved oxygen.

In the polyhaline region, the abundance of brachyuran zoea exhibited a distinct seasonal pattern during the study period. The highest abundance was recorded in the post-monsoon season (1023.67±170.82 No./100m³), while the lowest was observed during the monsoon season (148.81±56.16 No./100m³) (Fig. 5a). The Shapiro-Wilk test indicated that larval abundance data significantly deviated from normality across all three seasons (p<0.05). Additionally, Levene's test confirmed heterogeneity of variance (p<0.01). Given these violations of parametric assumptions, the Kruskal-Wallis test was conducted, revealing significant differences in larval abundance among seasons (p<0.01).

A noticeable increase in brachyuran zoeal abundance was observed during the study period in the mesohaline region of Vembanad Lake. The premonsoon season (2850.62±1429.11 No./100m³) recorded the highest abundance, while the monsoon season (14.59±7.62 No./100m³) exhibited the lowest abundance (Fig. 5b). In the mesohaline region, normality was violated in the post-monsoon and pre-monsoon seasons (p<0.01), while the monsoon season met the normality assumption (p>0.05). Levene's test indicated significant variance heterogeneity (p<0.05). Consequently, the Kruskal-Wallis test was applied and detected significant seasonal variation in larval abundance (p<0.05).

In the oligohaline region, the highest abundance was recorded during the pre-monsoon season (151.12±33.68 No./100m³), while the lowest was



Fig. 4. Mean (±SD) seasonal variations in temperature, salinity, pH and DO in oligohaline region during the study period

observed during the monsoon season $(7.39\pm2.44$ No./100m³) (Fig. 5c). In the oligohaline region, normality was violated in the pre-monsoon and post-monsoon seasons (p<0.05), while the monsoon season met the normality assumption (p>0.05). Levene's test indicated significant variance heterogeneity (p<0.05). The Kruskal-Wallis test further confirmed significant differences in larval abundance among seasons (p<0.05). Tukey's post-hoc test revealed significant seasonal abundance variations, with monsoon differing from pre-monsoon and post-monsoon in the polyhaline region, and pre-monsoon differing from monsoon and post-monsoon in the mesohaline region.

A total of 6,492 brachyuran larvae were collected, of which 6,001 larvae were identified to the family level, corresponding to five families: Macrophthalmidae, Hymenosomatidae, Sesarmidae,



Fig. 5. Mean (± SE) seasonal variations in abundance of brachyuran zoea in polyhaline, mesohaline and oligohaline region during the study period

Pilumnidae and Portunidae. Brachyuran larvae that could not be assigned to a family were grouped as "Unidentified". In the polyhaline region, Macrophthalmidae (52%) was the dominant family, followed by Pilumnidae (27%), Portunidae (5%) and Sesarmidae (4%) (Fig. 6). Within the polyhaline zone, Macrophthalmidae larvae were prevalent during the pre-monsoon and post-monsoon seasons. In contrast, Pilumnidae and Portunidae larvae were most abundant only during the post-monsoon season, whereas Hymenosomatidae larvae were extremely rare, occurring only in this region during the monsoon season (Fig. 7).

Within the mesohaline zone, the following families were found: Macrophthalmidae (41%), Pilumnidae (23%), Sesarmidae (15%), and Hymenosomatidae (14%) (Fig. 6). Larvae of Macrophthalmidae, Pilumnidae, Hymenosomatidae, and Sesarmidae were observed throughout the pre-monsoon and post-monsoon seasons, with higher abundance during the pre-monsoon. In this region, Portunidae larvae were rarely observed during the post-monsoon season (Fig. 7).

Hymenosomatidae (68%) was the most prevalent family in the oligohaline zone, followed by Sesarmidae (19%) and Macrophthalmidae (6%) (Fig. 6). In this region, Sesarmidae larvae were found during both the pre-monsoon and post-monsoon seasons, while Macrophthalmidae and Pilumnidae larvae were observed only during the pre-monsoon season. Hymenosomatidae larvae were present throughout the year, with greater abundance during the pre-monsoon and post-monsoon seasons (Fig. 7).

While all other identified larvae were distributed throughout the lake with no discernible change in composition by estuarine zone, Portunidae larvae occurred only occasionally and sporadically. The majority of the larvae belonged to the zoea I stage, although Hymenosomatidae, Pilumnidae, Macrophthalmidae and Sesarmidae represented larvae up to the zoea III stage.

Canonical Correspondence Analysis (CCA) was used to examine the relationships between brachyuran larval distribution and various water quality parameters across the polyhaline, mesohaline, and oligohaline regions of Vembanad Lake across different seasons. In the polyhaline zone (Fig. 8a), CCA Axis 1 explained 89.5% of the variance, while CCA Axis 2 accounted for 10.5%. Portunidae showed a strong association with salinity and



Fig. 6. Percentage composition of different brachyuran larval families in three different zones of Vembanad Lake

dissolved oxygen. In the mesohaline zone (Fig. 8b), CCA Axis 1 explained 98% of the variance, whereas Axis 2 accounted for 2%. Sesarmidae and Macrophthalmidae were closely associated with specific salinity, temperature, and pH conditions characteristic of this transitional habitat. The oligohaline region (Fig. 8c) exhibited a distinct ecological dynamic, with CCA axis 1 explaining 83.5% of the variance and axis 2 accounting for 16.5%. In this region, the distribution of brachyuran larvae was influenced by lower salinity levels and fluctuating temperature conditions.

The study of the spatio-temporal distribution of brachyuran zoea in Vembanad Lake has provided significant insights into the dynamics of larval abundance in relation to key environmental factors. The observed seasonal and salinity-based patterns of larval abundance establish a strong foundation for understanding the complex interactions between biological processes and environmental conditions in a tropical estuary. While this study focuses on the larval abundance, distribution and diversity, previous research on adult brachyuran crabs has documented their diversity in the region. Roy and Nandi (2007) reported 18 brachyuran species from Vembanad Lake, while Devi, Joseph, and Khan (2015) recorded 24 species from the Cochin backwaters, representing 16 genera and 8 families. More recently, Apreshgi, Kumar, Mullasseri, and Abraham (2024) identified four brachyuran families, viz.,

Portunidae, Grapsidae, Sesarmidae, and Ocypodidae from the Puthuvype mangroves. These studies on adult crabs complement the present findings by providing a broader understanding of the life cycle and habitat preferences of brachyuran species in the estuarine ecosystem.

The highest abundance of brachyuran larvae during the pre-monsoon season, followed by a marked decrease in the monsoon season, aligns with the typical reproductive and developmental cycles of many brachyuran species. This seasonal variation can be attributed to several factors, including changes in salinity, temperature, and nutrient availability, all of which are known to influence larval development and survival (Murphy & Iken, 2014; Simith, de Souza, Maciel, Abrunhosa, & Diele, 2012). The pre-monsoon period, characterized by stable salinity and relatively high temperatures, likely provides optimal conditions for larval hatching and growth. The reduction in larval abundance during the monsoon season corresponds to the significant dilution of salinity in the estuary due to heavy rainfall and freshwater runoff, as indicated by previous studies on tropical estuaries (Madhu et al., 2007; Cleetus et al., 2016).

The categorization of the estuary into polyhaline, mesohaline, and oligohaline zones based on salinity gradients provides a useful framework for analyzing larval distribution. The highest larval abundance observed in the mesohaline region suggests that this zone offers a favourable environment for the survival and development of brachyuran larvae. This finding aligns with studies on other estuarine systems, where mesohaline zones are often characterized by moderate salinity levels that support a diverse and abundant planktonic community, serving as a food source for larvae (Magris & Fernandes, 2011; Venkataramana, Gawade, Bharathi, & Sarma, 2023).

The oligohaline region of Vembanad Lake remains a freshwater zone throughout the year, largely due



POMN PRMN

Fig. 7. Seasonal distribution of larval families in three different zones of Vembanad Lake; POMN (Postmonsoon), PRMN (Pre-monsoon) and MN (Monsoon)

to the Thanneermukkom bund, which is closed during the pre-monsoon period. This closure restricts saline water intrusion from the Cochin estuary, particularly from mid-November to May, when river discharge from major rivers is minimal (Jacob, Revichandran, & Kumar, 2013). As a result, the oligohaline region experiences low salinity levels even during the dry season. However, this sustained freshwater environment is less favourable for brachyuran larvae, which generally thrive in medium to high salinity conditions. Consequently, brachyuran larval abundance in this region remains lower compared to other regions of the estuary, as the continuous low salinity limits their breeding and larval development.

Salinity, on the other hand, appears to have a more complex relationship with larval abundance. While the mesohaline zone exhibited the highest larval abundance, the polyhaline zone also supported a significant number of portunid larvae, particularly during the post-monsoon season. This pattern may be explained by the reproductive season of marine brachyuran crabs. *Portunus pelagicus*, an important commercial species in Vembanad Lake, has its peak breeding season during November–December along the southwest coast (Pillay & Nair, 1971).

Mangroves and mudflat intertidal areas are the primary habitats for crab species, belonging to the family Macrophthalmidae, Pilumnidae (Chhapgar, 1957; Davie, 1989), Sesarmidae (Giddins, Lucas, Neilson, & Richards, 1986; Ng, Rani, & Nandan, 2017) and Hymenosomatidae (Chuang & Ng, 1994). The latter are also found in fresh, brackish, and marine waters.

The presence of the zoea I stage in the samples indicates that breeding occurs inside the estuary. On the other hand, the presence of later zoeal stages suggest that the larvae remain within the estuary to grow and develop (de Lima, de Oliveira, & Martinelli-Lemos, 2019). The absence of the megalopal stage in the present samples supports the hypothesis that megalopae are found on or near the bottom waters (Sandifer, 1975; Mense & Wenner, 1989; DeVries, Tankersley, Forward, Kirby-Smith, & Luettich, 1994).

The present results on surface water quality, including temperature, salinity, pH, and dissolved oxygen, provide essential insights into the environmental conditions that influence larval distribution. Temperature, salinity, and dissolved oxygen were



Fig. 8. The Canonical Correspondence Analysis (CCA) triplot examined relationships between the water quality variables and brachyuran zoea in (a) Polyhaline, (b) Mesohaline and (c) Oligohaline regions of the Vembanad lake

the most influential factors controlling the distribution and abundance of crustaceans in estuarine environments (Geetha & Nandan, 2014; Yamamoto et al., 2015; Epifanio & Cohen, 2016; Kembaren, Zairion, Kamal, & Wardiatno, 2018; Devi, Joseph, & Korath, 2021). Temperature and salinity are particularly important, as they directly affect the physiological processes of brachyuran larvae (Magris & Fernandes, 2011; de Lima, Butturi-Gomes, Pantoja, & Martinelli-Lemos, 2022). The correlation between higher temperatures and increased larval abundance during the pre-monsoon season suggests that temperature plays a key role in enhancing larval growth and development.

The transformation of the estuary into a freshwater system during the monsoon season, as suggested by previous studies (Madhu et al., 2007; Cleetus et al., 2016; Harikrishnan, Jose, Nidhin, & Anilkumar, 2021), has significant implications for the ecology of brachyuran larvae. The complete freshwater influx likely disrupts the existing salinity gradient, leading to a homogenization of the estuarine environment. This drastic change in salinity can have a profound impact on the survival of marine organisms, including brachyuran larvae, which are generally adapted to brackish water conditions. The observed decline in larval abundance during the monsoon season supports this hypothesis, as the larvae may either experience increased mortality or migrate out of the estuary in response to changing salinity conditions. Additionally, the low-salinity conditions during the monsoon season are unfavourable for breeding in some brachyuran species (Pillay & Nair, 1971).

In summary, the spatio-temporal distribution of brachyuran larvae in Vembanad Lake is strongly influenced by a combination of seasonal changes, salinity gradients, and environmental factors such as temperature and salinity. The mesohaline zone emerged as a critical habitat, providing a breeding and nursery ground for many brachyuran crabs, while the monsoon season posed significant challenges due to the influx of freshwater. These findings contribute to a deeper understanding of the ecological processes governing larval distribution in tropical estuaries and provide valuable insights for the conservation and management of these vital ecosystems.

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