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Aquatic Sciences and Engineering aims to contribute to the literature by publishing manuscripts at the highest scientific level on all fields of aquatic sciences. The journal publishes original research and review articles that are prepared in accordance with the ethical guidelines.

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Research Article

Fishery, Growth, and Mortality of Threatened Asian Sunfish, *Horabagrus brachysoma* (Gu¨nther 1864) in Five Rivers of Western Ghats Hotspot, India

Chelapurath Radhakrishnan Renjithkumar[1], **Kuttanelloor Roshni**[2]

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ABSTRACT

Community-based data monitoring (CBM) systems play a vital role in small-scale inland fisheries, offering valuable insights into the catch patterns of various fish species over extended periods. This study adopted a participatory approach to investigate the fishery, demography, and exploitation patterns of the 'Vulnerable' Catfish (*Horabagrus brachysoma*) in five river systems within the Western Ghats biodiversity hotspot of India. The catches of this species from various landing centres across the Western Ghats' rivers varied between 0.73 t to 3.15 t annually. These fish typically measured between 11.00 and 41.10 cm in Total Length (TL) and weighed between 26.00 g to 470.00 g in Total Weight (TW). Analysis of annual length frequency data provided by local fishers, the estimated growth parameters of *H. brachysoma* are estimated as; asymptotic length ($L\infty$) between 316.05 and 421.05 mm, growth coefficient (K) between 0.58 yr $^{-1}$ and 1.10 yr $^{-1}$ from different rivers. The total mortality (Z) was calculated to range between 1.25 yr $^{-1}$ and 2.91 yr $^{-1}$ while the fishing mortality (F) was estimated between 0.62 yr $^{-1}$ and 2.09 yr $^{-1}$. The estimated fishing mortality rate of *H. brachysoma* in the Periyar River, at 2.09 yr $^{-1}$, is alarmingly high and among the highest recorded for this species. The calculated exploitation rate (E) ranging from 0.49 to 0.72 yr $^{-1}$ exceeds the anticipated optimum exploitation levels (0.5). This suggests that the populations of *H. brachysoma* in the river systems of the Western Ghats are experiencing overexploitation. Various conservation measures such as fishing closures during spawning seasons, restrictions on mesh sizes, non-fishing zones and quota systems should be implemented.

Keywords: Yellow catfish, Western Ghats, Vulnerable, Fishery, mortality, conservation

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INTRODUCTION

According to the Food and Agriculture Organization (FAO, 2010), a staggering 90% of the world's exploited fish stocks remain unassessed, leaving many in the 'uncertain risk' category (Costello et al., 2012). Unassessed fish stocks, often supporting artisanal and subsistence inland fisheries, play a vital role in providing livelihoods and employment opportunities for rural communities, who depend on these resources for their well-being and food security (Lynch et al., 2016; Smith et al., 2005). Inland fish and fisheries are a crucial component of global food security, providing local and affordable sources of essential nutrients, including protein, fatty acids, oils, and micronutrients, to hundreds of millions of people worldwide, particularly in developing countries (Funge-Smith & Bennett, 2019). Inland fisheries are often marked by small-scale, household-level, and subsistence-oriented practices, where fishing is conducted on a local scale to meet the basic needs of the community (Youn et al., 2014). In developing countries, inland fisheries play a vital role in supporting the livelihoods of over 60 million people, serving as a critical source of both food security and income (Cooke et al., 2016). Global inland fisheries face a myriad of threats, posing significant risks to the livelihoods and well-being of millions of people worldwide (Smith et al., 2005). Collecting precise data on inland fisheries production poses challenges due to the dispersed nature of most inland fisheries activities, often leading to underreporting or no reporting at all to government agencies (Allan et al., 2005). Despite the important contributions, inland fish and fisheries generally remain economically and socially undervalued and biologically underappreciated because accurate information about these small scale highly dispersed fisheries is inherently difficult to acquire (Youn et al., 2014).

Effective management of freshwater fish population is crucial for both food security and minimizing biodiversity loss (Tickner et al., 2020). This may be done by the management of exploitation (reduction of bycatch, minimizing fishing effort and size limits regulation), management of fish habitat (river flow regulation and management of aquatic vegetation) and the use of fisheries enhancements (river ranching and use of artificial reefs) (Lorenzen et al., 2016). Fish stock assessment models are invaluable tools for fishery managers providing critical insights into the dynamics of fish population and their response to various external pressures, including commercial fishing, predation and environmental changes (Hilborn et al., 2020; Sun et al., 2020). Accurate data for length-based stock assessments can be obtained through targeted sampling at commercial fish landing sites or fisheries-cruise surveys, eliminating the need for complete removals from the target stock (Shephard et al., 2021). This approach enables researchers to gather essential information on fish populations, such as size distribution and abundance, without causing unnecessary harm to the stock or disrupting the ecosystem, thereby supporting sustainable fisheries management. However, in inland habitats where local knowledge of fishers is essential, researchers and fisheries managers may find it difficult to obtain and validate data for stock assessment (Valbo-Jørgensen & Poulsen, 2000). Community-based data monitoring (CBM) systems offer an alternate approach in such small-scale and artisanal fisheries, which are playing an increasingly important role in sustaining local food supply and food security (Lam et al., 2019; Oviedo & Bursztyn, 2017). Local communities frequently monitor fish catch patterns in their fishing practises throughout the year, and this knowledge can be captured as quantitative indicators (Shephard et al., 2021).

Despite their importance, the growth patterns and exploitation dynamics of threatened freshwater species harvested from the Western Ghats have received limited research attention, with only a few studies addressing these critical aspects (Prasad et al., 2012; Raghavan et al., 2018; Renjithkumar et al., 2020; Shanmughan et al., 2021). The freshwater fisheries of the Western Ghats region in India are inextricably linked to the well-being of local communities residing along rivers and reservoirs because it provides a source of food and livelihood for local community (Prasad et al., 2012; Rajeev et al., 2011). The freshwater fisheries in this region are facing unprecedented threats due to "open-access" nature of the fisheries, the use of destructive fishing gears, overfishing of resources and poor enforcement of rules and regulations (Raghavan et al., 2011). The Asian Sun catfish or the yellow catfish, *Horabagrus brachysoma* (Gu¨nther 1864) belongs to the family Horabagridae and is an important food fish exploited by traditional fishers from rivers, lakes, backwaters, and associated inland canal systems in the Western Ghats region of Kerala, Karnataka, and Maharashtra states (Raghavan et al., 2016). The species is a nocturnal, opportunistic, omnivorous feeder that adapts its diet based on prey availability (Padmakumar et al., 2009; Prasad & Ali, 2008; Sreeraj et al., 2006). Its peak breeding season occurs from June to July, coincides with the south-west monsoon in peninsular India (Bindu et al. 2012; Chandran & Prasad, 2014). It is an important food fish widely harvested across its range by traditional fishers using gill nets operated from dugout canoes, as well as cast nets, drag nets, stake nets, and hook-and-line (Bindu, 2006; Prasad et al., 2012; Sreeraj et al., 2007). The fishery for *H. brachysoma* appears to be unsustainable in many rivers of the Western Ghats due to overexploitation of stock and habitat destruction (Raghavan et al., 2016) and the IUCN has classed the species as 'Vulnerable' due to an overall population drop (Raghavan & Ali, 2013). It has been suggested that without effective management plans, excessive fishing effort and subsequent overexploitation in certain rivers (Prasad et al., 2012; Prasad, 2008) could lead to the collapse of the fishery. Management of the fishery of yellow catfish is hindered due to the lack of successful fisheries management programmes in Kerala's WG waters, where the largest share of exploitation takes place (Prasad et al., 2012). A study on the population dynamics of *H. brachysoma* in the River Periyar suggests several management strategies to protect the species such as restricting gear (minimum mesh size of 160–180 mm for gill nets), enforcing a minimum size limit (200 mm) and implementing closed seasons from May to August. (Prasad et al., 2012). There are no reliable estimates of the *H. brachysoma* fishery, growth, mortality, and exploitation in its native ranges in the Western Ghats except from Prasad et al. (2012) studied the population characteristic of the species from a single river system in Southern India. The current study examines the fishery, growth, and exploitation status of vulnerable *H. brachysoma* exploited from different river systems (Pampa, Achenkovil, Muvattupuzha, Periyar, and Chalakudy Rivers) in the Southern Western Ghats of India.

MATERIALS AND METHODS

Study area

The study was conducted at the major fish landing centres along five major rivers in central Kerala, India: Periyar (244 km), Chalakudy (146 km), Pampa (176 km), Muvattupuzha (121 km), and Achenkovil (128 km) Rivers, which are located in the central region of Kerala state. The five fish landing sites were strategically selected due to the high occurrence of *H. brachysoma* in the daily catches of local fishers. Bhoothanthankettu, Periyar (10°08′12.75″ N; 76°39′51.27″ E); Pulikakadavu, Chalakudy (10°14′28.53″ N; 76°19′50.27″ E); Mulakulam, Muvatupuzha (09°51′15.00″ N; 76°29′19.12″ E); Prayikkara, Achenkovil (09°15′44.04″ N; 76°32′28.72″ E) and Edatuva, Pampa (09°21′53.29″ N; 76°28′35.05″ E) (Fig. 1) were the landing centres selected for the study.

Data collection

For data collection, we selected 10 fishers (two fishers from each of rivers) and provided training in essential fisheries data collection methods. This training covered procedures for measuring and re-

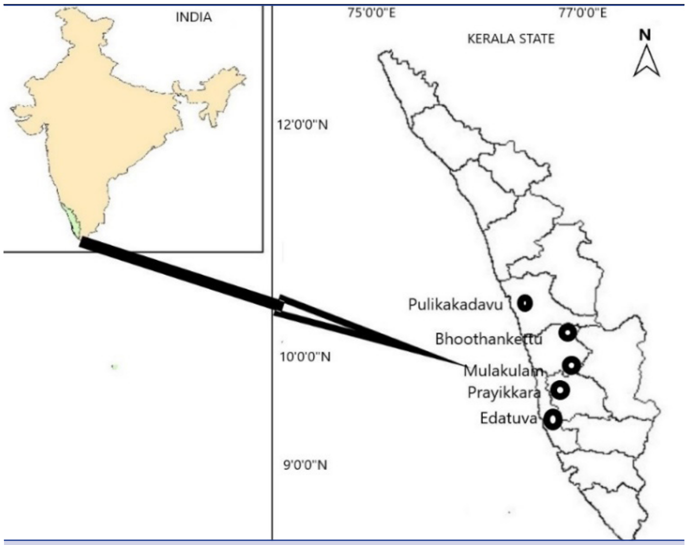

Figure 1. Map showing the landing centre of different rivers of Western Ghats were *H. brachysoma* collected.

cording fish length and weight. Total length (TL) was measured to the nearest 0.1 mm using a digital sliding calliper, and total body weight (BW) was measured to the nearest 0.1 g using an electronic weighted scale. These 10 local fishers served as data assistants for a year (April 2020 to March 2021), providing monthly data on the length and weight of *H. brachysoma* specimens. Monthly catch data of *H. brachysoma* harvested at the five rivers were also recorded with assistance from the data collectors. The total catch (kg) from each haul of gill nets and hook & lines from each river were recorded by the data team. To ensure data accuracy, the authors conducted random monthly visits to each of the five rivers. These visits validated the fishers' data collection techniques, evaluated the quality of data entries and provided technical assistance as needed. At the end of the study, we consolidated the data recorded by the data collectors in datasheets and logbooks.

The primary fishing gears used for catching yellow catfish were gill nets, as well as hook and lines. Gill nets used for fishing typically ranged in length from 75 to 150 metres, with mesh sizes varying from 30 to 60 mm. Daily landings from each type of gears were calculated using the formula: W = (w/n) x N, as described by Kurup et al. (1993), where: W = total weight of fish landed, w = total weight of fish from sampling gear, n = number of gears sampled, N = total number of similar gears operated. The monthly catch was estimated by multiplying daily catch by the total number of fishing days in a month, assumed to be 25 days. To calculate the annual exploited quantity, the monthly landings were summed over a 12-month period, providing a comprehensive estimate of the total annual catch.

1938 samples of *H. brachysoma* were collected from Periyar (n=383), Chalakudy (n=407), Pampa (n=448), Muvatupuzha (n=351), and Achenkovil (n=349) Rivers for demographic analysis. The length frequency data were categorized into 25 mm class intervals.

Growth, mortality and exploitation

Length frequency distributions were used to estimate the growth, mortality and exploitation pattern of *H. brachysoma*. The von Bertalanffy growth function parameters including asymptotic length (L_∞) and growth coefficient (K) were estimated with the ELEFAN-1 (electronic length frequency analysis) module in FiSAT-II (FAO-ICLARM Stock Assessment Tools II) software (Gayanilo et al., 2005). The growth parameters were obtained by von Bertalanffy growth formula (VBGF) was fitted using $L_t = L_\infty\,[1-\exp^{-K(t-t_0)}]$, where L_t is the growth at time t, L_∞ is the asymptotic length, K is the growth coefficient, t is the age of fish and t_0 is the theoretical age at length zero. The age at zero length (t_0) was calculated from Pauly's (1983) equation: $\text{Log}(-t_0) = -0.392 - 0.275\,\text{Log}\,L_\infty - 1.038\,K$. Longevity ($t_{max}$) of the population was determined as $t_{max}=3/K$ (Pauly 1984), and growth performance index (phi prime,φ) was estimated using the equation $\log K + 2\log L\infty$ (Moreau et al. 1986). Growth performance equation can be utilized to compare growth rates among species and assess growth performance potential under different environmental stresses (Pauly 1994). Total mortality (Z) was estimated from the length-converted catch curve method (Pauly 1984) and natural mortality (M) was determined using Pauly (1980) empirical equation: $\text{In}\,M= -0.0152-0.279\,\text{Log}\,L_\infty + 0.6543\log K + 0.463\,\text{Log}\,T$, where, L_∞ is the asymptotic length in mm, K is the growth constant in year^{-1} and T is the mean annual temperature (26-30°C). Fishing mortality (F) was derived from the equation $F=Z-M$ (Ricker 1975) and exploitation rate (E) was calculated as $E=F/Z$. The probability of capture was determined by backward extrapolating the descending limb of the length-converted catch curve using the FiSAT II software package (Gayanilo et al., 2005). The probabilities of capture obtained from the catch curve analysis were used to estimate the length at first capture (L_{50}%) through a logit function (Pauly 1984). Recruitment pattern was assessed by reconstructing the recruitment pulses from a time series of length-frequency data (Gayanilo et al., 2005). A length-structured virtual population analysis (VPA) was carried out to estimate survivors, natural mortality and fishing mortality in each length group. The relative yield per recruit (Y/R) and relative biomass per recruit (B/R) analysis were estimated using knife selected method given by Beverton & Holt (1966), which help to understand whether populations are overexploited, E_{max} (exploitation rate with maximum yield) and E_{50} (exploitation that retains 50% of the biomass).

RESULTS AND DISCUSSION

The annual exploited fishery of *H. brachysoma* from the five rivers of Southern WG was estimated to be 7.17 t. High landing was observed in Pampa River (3.15±0.099 t) and low landing in Muvatupuzha (0.57±0.013 kg) (Fig. 2). Monsoon season (June – September) contributed the bulk of landing (3.26±0.097 t) followed by post

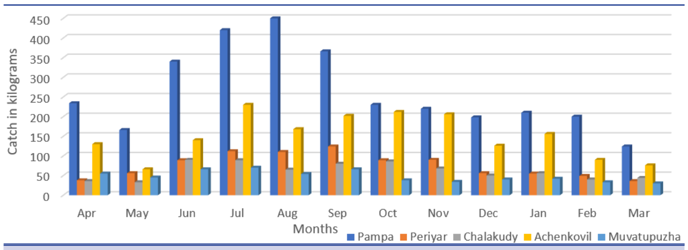

Figure 2. Monthly variations in the landing of *H. brachysoma* from different river systems of Western Ghats.

monsoon season (October- January) with 2.26±0.074. *Horabagrus brachysoma* is an important food fish, is extensively exploited from Western Ghats rivers by traditional fishers (Bindu, 2006; Sreeraj et al., 2007; Renjithkumar et al., 2011, 2016). Gill nets and hook and lines are the main gears used for catching. Depending on the net size, about 3-6 gill nets and 1-2 hook and lines are actively engaged in yellow cat fishing in each landing centre of the rivers. During the monsoon season, the floodplain fishery (locally known as *Oothapiditham*) yields a significant catch of mature *H. brachysoma*, with approximately 100 kg harvested weekly using traditional fishing traps, gill nets, and electric fishing methods (Shaji & Laladhas, 2013). The landing of the species from Kerala state rivers ranges from 3.67 t to 17.1 t (Renjithkumar et al. 2011, 2016) whereas annual landings in Vembanad Lake ranged between 2 and 439 t (Bindu, 2006; Kurup et al., 1995; Sreeraj et al., 2007). The fishery of this species in many rivers seems unsustainable due to excessive fishing effort with over fishing such as growth fishing (capturing individuals before they reach a size significant enough to contribute to the spawning stock) and recruitment fishing (exploiting the spawning stock itself) (Prasad et al., 2012; Raghavan & Ali, 2013; Raghavan et al., 2016).

The frequency distribution of length groups showed considerable variation in the length range of *H. brachysoma* exploited from various rivers of Western Ghats region (Fig 3). Although this species can reach a maximum length of 420 cm (Bindu & Padmakumar, 2019), individuals of this size were not encountered in the present study. The largest specimen recorded in this investigation was 410 mm, significantly smaller than the reported maximum length. The length range of *H. brachysoma* populations in Chalakudy and Muvatupuzha Rivers are much smaller than other rivers. In Chalakudy and Muvatupuzha Rivers the maximum length of fishes was recorded in the size class 330 mm and 308 mm respectively. The highest length of *H. brachysoma* in Pampa was 406 mm, while the maximum lengths in Achenkovil and Periyar River were 384 mm and 350 mm, respectively. Although only 29% of the exploited *H. brachysoma* in the River Pampa were < 200 mm TL, the proportions increased to 31% in the River Achenkovil, 42% in the River Periyar, and > 50% in the Rivers Chalakudy and Muvatupuzha. The size at first maturity for the *H. brachysoma* is known to be 175 mm and 188 mm TL in males, and 168 mm and 185 mm TL in females (Bindu et al., 2012; Chandran & Prasad, 2014). Length-frequency analysis reveals that a significant proportion of yellow catfish are being caught in small-scale fisheries before they reach sexual maturity, indicating a potential threat to the sustainability of the population. Harvesting small fish before they reach maturity leads to a slow population recovery and reduced fish catches and decreased profits (Isaac & Ruffino, 1996; Myers & Mertz, 1998).

Restructured form of the length frequency data of exploited *H. brachysoma* populations from five river presented as output of ELEFAN I shows that the growth curves for different populations differ considerably (Fig.4). The asymptotic length ($L\infty$) ranged from 316.05 mm in Achenkovil to 421.05 mm in Pampa, while the growth coefficient (*K*) ranged from 0.58 y^{-1} in Chalakudy to 1.10 y^{-1} in Muvatupuzha (Table 1 and Fig. 5). The demographic characteristics of *H. brachysoma* have been understudied, with only a few investigations, including Prasad et al. (2012) in the Periyar River, therefore the comparison between geographic populations becomes difficult. The growth coefficient (*K*) values for the *H. brachysoma* population in the five rivers were significantly lower compared to the Periyar River population (4.60 y^{-1}) in the Southern Western Ghats (Prasad et al., 2012). The variations in differences in growth parameters of *H. brachysoma* observed across different rivers can be attributed to a combination of factors including stock variances, ecological conditions of the habitat, feeding habits, and environmental parameters. The high growth coefficient (*K*) and low longevity were recorded in Periyar and Muvatupuzha Rivers when compared to Chalakudy, Pampa and Achenkovil Rivers. It indicated that Periyar and Muvatupuzha Rivers yellow catfish populations acquired asymptotic length ($L\infty$) quickly which agree with Pauly & Munro (1984), that species having shorter life have higher '*K*' values and reach their $L\infty$ within one or three years of life history. Potential longevity of *H. brachysoma* was the highest (5.17) in Chalakudy and lowest in lowest (3.3) in Muvatupuzha (Table 1). Estimates of growth performance index (φ) recorded in this study (4.29 to 4.52) were lower (4.99) than those observed for Periyar River (Prasad et al., 2012).

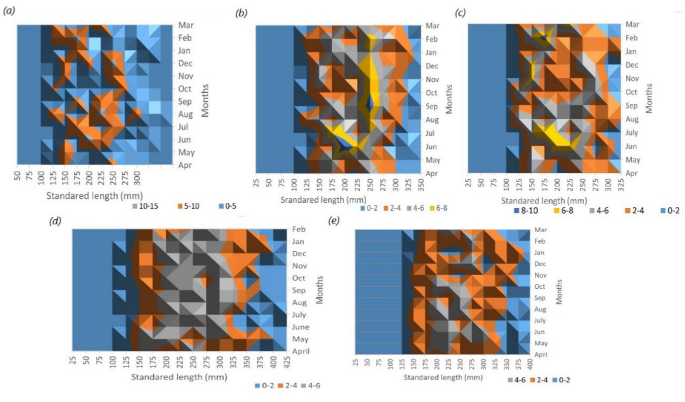

Figure 3. Distribution of length frequencies of *H. brachysoma* across five rivers in the Western Ghats (a) Chalakudy (b) Periyar (c) Muvatupuzha (d) Pampa (e) Achenkovil.

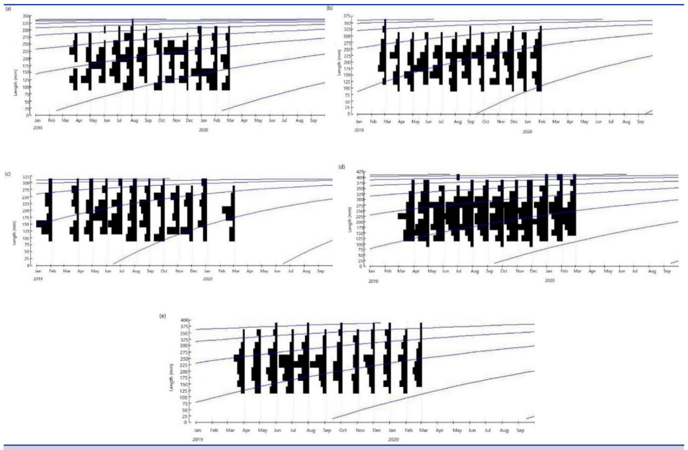

Figure 4. von-Bertalanffy growth curves for *H. brachysoma* from different rivers on the Western Ghats (a) Chalakudy (b) Periyar (c) Muvatupuzha (d) Pampa (e) Achenkovil.

The decrease in the fish population size can be attributed to by two primary factors: natural mortality (*M*) (disease, predation, pollution and other environmental stressors) and fishing pressure (*F*). Fishing mortality (*F*) was highest in Periyar (2.09 y^{-1}) and lowest in Achenkovil (0.62 y^{-1}), whereas natural mortality (*M*) was lowest in Pampa (0.62 y^{-1}) and highest in Muvatupuzha (0.99 y^{-1}) Rivers (Table 2). The fishing mortality rate of *H. brachysoma* was higher than the natural mortality rate in four rivers (Periyar, Muvatupuzha, Chalakudy and Pampa) indicating a rather significant fishing pressure on the species. The mortality rate of species is crucial for developing exploitation strategies to harvest and manage the fishery resources optimally. The ratio of total mortality (*Z*) to growth coefficient (*K*) is a key indicator of population dynamics. A ratio greater than 1.0 ($Z/K > 1.0$) suggests a mortality-dominated population, where deaths exceed growth, leading to a declining population. In contrast, a ratio less than 1.0 ($Z/K < 1.0$) indicates a growth-dominated population, where growth outpaces mortality, resulting in an increasing population (Etim et al., 1999). *H. brachysoma* populations in all the rivers studied were dominated by mortality (Z/K = 1.88–3.58). This is a highly alarming situation where overfishing gradually reduce recruitment, resulting in severe population losses in the near future (Rajeev et al., 2018). In an ideally managed fishery, the fishing mortality rate (*F*) should be balanced with the natural mortality rate (*M*), resulting in an exploitation rate (*E*) of approximately 0.5 y^{-1} (Gulland, 1970).

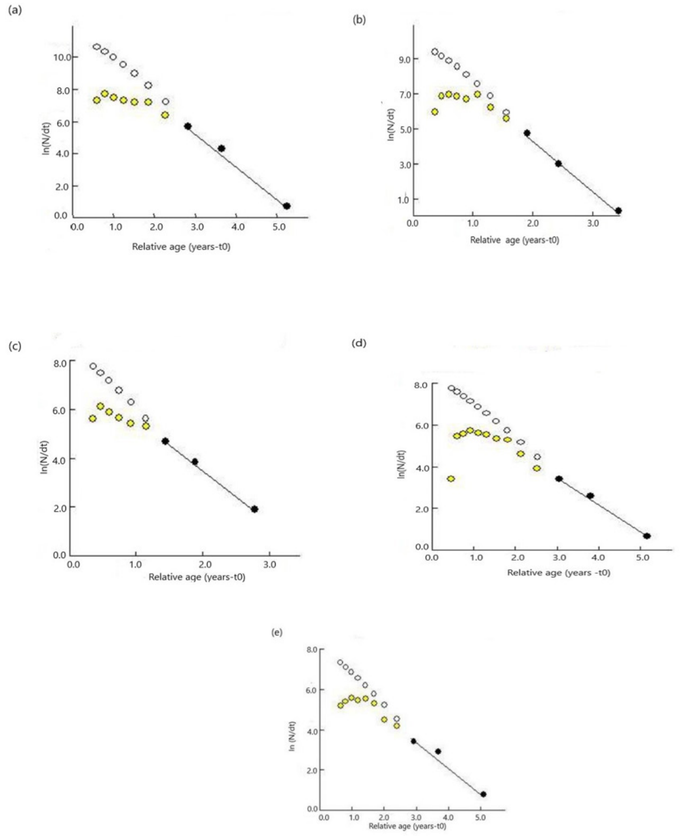

Figure 5. Length converted catch curve for the estimation of mortality of *H. brachysoma* from different rivers on the Western Ghats (a) Chalakudy (b) Periyar (c) Muvatupuzha (d) Pampa (e) Achenkovil.

The exploitation rates (*E*) of yellow catfish in four rivers (Chalakudy, Pampa, Periyar, and Muvatupuzha) are greater than sustainable threshold of 0.5, whereas the exploitation level in the Achenkovil River nearly equals to $E_{0.5}$, indicating uncertain future for native yellow cat fish population if management attention is not taken. Yellow catfish exploitation level (*E*) in the Chalakudy, Periyar, Muvatupuzha and Pampa were higher (0.70, 0.72, 0.52 and 0.53) than the expected optimal exploitation level ($E_{50,}$), indicating that these populations are overexploited. When compared to the Muvatupuzha and Pampa Rivers, populations of *H. brachysosma* in the Chalakudy and Periyar Rivers had higher levels of exploitation and fishing mortality. The size at first capture (*Lc*) calculated from the probability of capture was 197.86 mm –288.22 mm (Table 3). *Lc* was 78% of *L*∞ in Rivers Chalakudy and Periyar, but it was it was 61– 63.5% of *L*∞ in the Muvatupuzha, Pampa and Achenkovil. The harvest of smaller individu-

Table 1. Growth parameters of *Horabagrus brachysoma* from different rivers on the Western Ghats.

Rivers	Asymptotic length (*L*∞, mm)	Growth coefficient (*K*) year^{-1}	Growth performance index (φ)	Longevity (*3/K*) years
Chalakudy	342.30	0.580	4.29	5.17
Periyar	368.55	0.890	4.52	3.70
Muvatupuzha	316.05	1.100	4.45	3.30
Pampa	421.05	0.590	4.40	5.08
Achenkovil	394.80	0.600	4.37	5.00

Table 2. Mortality rates (year−1) and exploitation level of *Horabagrus brachysoma* from different rivers on the Western Ghats.

River	Total mortality rate (*Z*)	Natural mortality rate (*M*)	Fishing mortality rate (*F*)	Exploitation rate (*E*)
Chalakudy	2.08	0.63	1.45	0.70
Periyar	2.91	0.82	2.09	0.72
Muvatupuzha	2.07	0.99	1.08	0.52
Pampa	1.30	0.62	0.68	0.53
Achenkovil	1.25	0.63	0.62	0.49

Table 3. Length of first capture (Lc), E_{10}, E_{50} and E_{max} of *Horabagrus brachysoma* from different rivers on the Western Ghats.

River	Length at first capture (*Lc*) mm	E_{10}	E_{50}	E_{max}
Chalakudy	268.42	1.000	0.442	1.000
Periyar	288.22	0.753	0.406	0.834
Muvatupuzha	197.86	0.718	0.399	0.802
Pampa	267.63	0.707	0.403	0.821
Achenkovil	242.16	0.707	0.398	0.794

als suggests that specimens are being caught even before they reach sexual maturity, which contributes to future recruitments. Virtual population analysis (VPA) revealed that the species experienced considerable natural mortality in Achenkovil and Pampa Rivers at a young age, but the fishing mostly targeted comparatively larger sized individuals in Chalakudy, Periyar, and Muvatupuzha Rivers (Fig. 6). Exploitation levels estimated using relative yield per recruit (*Y′/R*) and relative biomass per recruit (*B′/R*) analysis using knife-edge selection were found to range between 0.398- 0.442 (E_{50}) and 0.794-1.00 (E_{max}) respectively in various river system (Fig. 7). The current level of exploitation was found in between 62% and 87% of the maximum exploitation (E_{max}) from the five river systems.

Conservation management measures

The current study is likely the first study on community-based data monitoring systems in small-scale inland fisheries in Western Ghats of India and it shows that yellow catfish are under heavy exploitation pressure and native populations are vulnerable to collapse in the absence of immediate management interventions. Monitoring, Control, and Surveillance (MCS) systems in the Western Ghats play a crucial role in regulating fishing activities, ensuring compliance with conservation measures, and protecting aquatic ecosystems from overfishing and illegal practices. Controlling the overall harvest of yellow catfish could be the most significant management method for conserving the *H. brachysoma* population, however reducing fishing effort in an artisanal subsistence fishery like India is nearly difficult. As a result, management interventions for the protection of *H. brachysoma* in Western Ghats rivers should be based on a combination of technical measures such as restrictions on fishing gear and mesh size limits, closed seasons, non-fishing zones, and the introduction of catch quotas. There is currently no upper size limit for landing *H. brachysoma* in Western Ghats rivers. Implementing an upper size limit stimulate stock recovery and improve the sustainability of its fishery. The length at first maturity of *H. brachyso-*

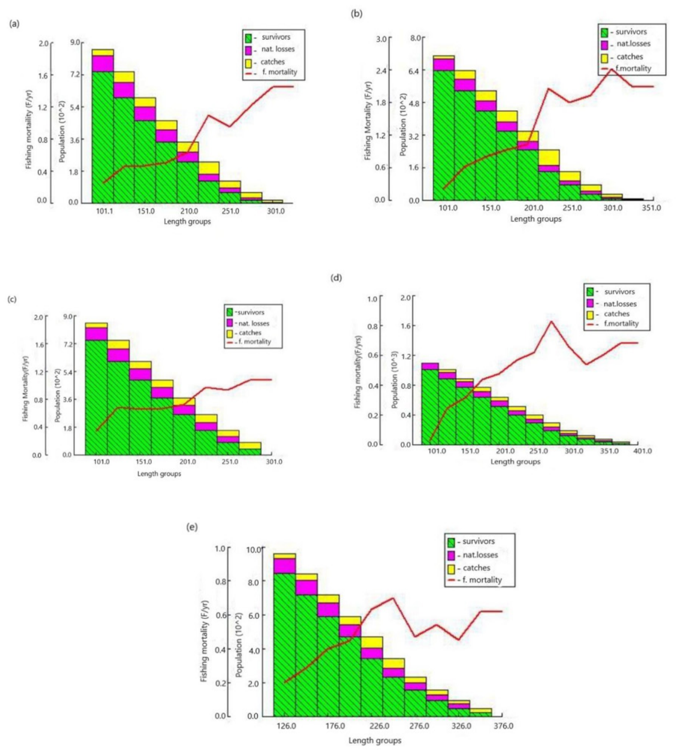

Figure 6. Length-structured virtual population analysis (VPA) of *H. brachysoma* from different rivers on the Western Ghats (a) Chalakudy (b) Periyar (c) Muvatupuzha (d) Pampa (e) Achenkovil.

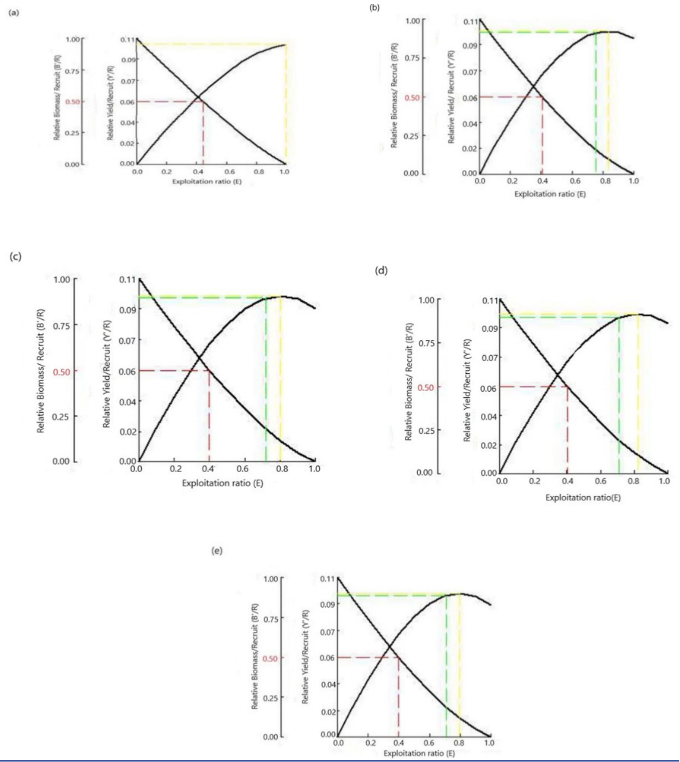

Figure 7. Relative yield per recruit (*Y′/R*) and relative biomass per recruit (*B′/R*) plots of *H. brachysoma* from different rivers on the Western Ghats (a) Chalakudy (b) Periyar (c) Muvatupuzha (d) Pampa (e) Achenkovil.

ma was estimated to be 175-188 mm in males, and 168 - 185 mm in females (Bindu et al., 2012; Chandran and Prasad, 2014). A minimum catch size limit of 200 mm can be enforced in the rivers to prevent recruitment overfishing. Allowing them to attain this size ensures they have the opportunity to spawn at least once, aiding in the conservation of the species. In addition to setting size limits, restrictions on net mesh size should be implemented.

Heavy exploitation of juveniles of *H. brachysoma* was occurred due to unethical fishing practices such as fishing traps and electrocution (Shaji and Laladhas 2013). Presently, there are no regulations in place to prohibit the use of electric fishing in the monsoon floodplain fishery in Kerala, which results in the unintended mortality of juvenile fish and could disrupt population dynamics and future stock recruitment. Currently, the fishers use gill nets with mesh sizes ranging from 20 and 60 mm, resulting in the capture of small-sized juvenile fishes before they reach maturity. Gill nets should have a minimum mesh size of 40-80 mm to prevent the capture of immature juveniles. Restriction on the limits on mesh size or size of the fish to enforce is challenge for developing management measures for small-scale subsistence fisheries in Western Ghats Rivers. To protect the yellow catfish population during its critical breeding period, a four-month closed season (June-September) should be implemented in Kerala state, coinciding with the Southwest monsoon months when they spawn. In cooperation with local fishermen, a temporary shutdown of the fishery during the spawning season (closed season) should be developed. This measure aims to safeguard the spawning stock and support the enhancement of recruitment levels. In the Western Ghats, establishing protective areas and no-fishing zones (NFZs) for *H. brachysoma* can be especially effective in regions with unique habitats, such as Thattekad and Pooyamkutty along the Periyar River, as well as Athirappilly and Vazhachal along the Chalakudy River. Catch quotas for each fishing fleet will be developed in consultation with local fishermen, scientists, and fisheries managers for each river. To prevent the indiscriminate exploitation of catfish in the rivers of central Kerala, the Department of Fisheries, Government of Kerala in collaboration with the Kerala State Biodiversity Board (KSBB) and Kerala Forest and Wildlife Department should establish a strict prohibition on fishing. To ensure compliance, a penalty of Rs. 50,000 and imprisonment for up to six months should be imposed on offenders. Effective enforcement and strict monitoring of existing legislation can significantly contribute to reducing the harvesting of mature catfish from natural waters. Ultimately, engaging local communities in conservation efforts is crucial for the long-term protection of catfish populations.

Statements & Declarations

Compliance with Ethical Standard: This is an observational study. The Cochin University of Science and Technology (CUSAT) Research Ethics Committee has confirmed that no ethical approval is required.

Conflicts of Interest: The authors declare that they have no conflict of interest.

Consent to Publish: The author's obtained consent from all individual participants for whom identifying information is included in this article.

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Data availability statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Research Article

Phenotypic and Genotypic Antibiotic Resistance of Bacteria Isolated from Ready-to-eat Salted Seafood

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ABSTRACT

Safe food production faces significant challenges from both disease-causing bacteria and antibiotic-resistant bacteria, as both pose serious risks to public health and food safety. This study investigated the presence of antibiotic-resistant bacteria in salted seafood (lakerda) samples obtained from fishermen and fish markets. Phenotypic analysis revealed that lakerda samples collected from fishermen contained bacteria with multi-antibiotic resistance, including *Pseudomonas fluorescens, Staphylococcus haemolyticus,* and *Staphylococcus equorum*. *Carnobacterium maltaromaticum*, *Carnobacterium mobile*, and *Vibrio hibernica* species were isolated in larkerda samples sold by fish markets. It was determined that among isolated bacteria, *V. rumoiensis* did not contain any of the genotypically tested genes. However, *P. fluorescens* carried *blaTEM, qnrB, qnrS, blaZ,* and *msrA*; *S. haemolyticus* harbored *blaTEM, tetK, dfrD, blaZ, msrA, msrB,* and *mecA*; *C. maltaromaticum* possessed *blaTEM, qnrA, qnrB, qnrS, strA-strB, aphAI-IAB*, and *mecA*; *C. mobile* included *blaTEM, blaZ, msrA, dfrD,* and *mecA*; and *V. hibernica* carried *blaTEM, blaZ, mecA,* and *VanA*. In addition, *S. pasteuri* and *S. equorum* had the *mecA* resistance gene. In conclusion, public health needs to provide hygiene conditions in the preparation of lakerda, determine the ways of transmission, take precautions, and raise awareness of producers and consumers.

Keywords: Food safety, lakerda, antibiotic resistance, resistance genes, microbiology

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INTRODUCTION

Food selection is of the utmost importance in increasing the number of healthy individuals and making future generations healthier. For this reason, consuming high-quality and safe food should be the priority. In addition to the treatment costs of diseases caused by malnutrition, the fact that individuals are out of production during the disease processes harms the country's economy. In developed countries, governments pursue policies on the importance of food, the sustainability of food resources, and the delivery of safe food to consumers (Koç & Uzmay, 2015).

Antibiotic resistance, a critical issue, is the condition when antibiotics, crucial in treating or preventing bacterial diseases, lose their effectiveness in killing bacteria or halting their reproduction (World Health Organization, 2017). The escalating causes of antibiotic resistance, including the indiscriminate use, poor dosage, and overuse of antibiotics in livestock and agriculture, are alarming. Bacteria in the natural flora, indicator bacteria, and pathogenic bacteria are acquiring resistance to various antibiotics. Moreover, the rise in antibiotic resistance increases the risk of transferring antibiotic-resistance genes from bacteria in the natural flora to pathogenic bacteria (Urban-Chmiel et al., 2022). This urgent issue demands our immediate attention and action.

The bacterium's genetic material can be transferred to another bacterium of the same species or even to bacteria of different species.

Thus, bacteria can show multiple antibiotic resistance (Sharma et al., 2014). As a result, the antibiotics used to treat diseases cannot be utilized as targeted. As reported in the Global Action Plan report prepared in England, it is projected that bacteria resistant to antibiotics will become strong enough to kill one person every three seconds in the world by 2050. The same report also stated that the fight against resistant bacteria will be worth 100 trillion USD (O'Neill, 2016). In Türkiye, an estimated 7,607 deaths in 2021 were directly attributable to bacterial antimicrobial resistance, while 30,490 deaths were associated with infections where resistance played a contributing role (Measuring Infectious Causes and Resistance Outcomes for Burden Estimation, 2024).Salting is one of the oldest methods of food preservation. The practice of salting fish was carried out in the ancient Egyptian civilization for the first time. Salt is used in different stages of seafood storage. The purpose of salting applied to seafood is to bind the water in the product by the salt, thereby reducing water activity and thus preventing spoilage. The antiseptic effect of the chlorine in salt is also among the positive effects. Lakerda is a traditional product that uses the salting method in aquatic products and is mainly made from toric and bonito fish. The salting process, done initially to prevent the fish from spoiling, is now used to create a different flavor (Aksu et al., 2013). Technological applications (such as heat treatment or pressure application) that will inhibit the existing microorganism load to a large extent are not carried out during the production stage (Turan et al., 2009; Duyar et al., 2020). Before the presentation, salted foods are often consumed directly without any processing, such as cooking, to reduce or remove the microorganism load (Turan et al., 2009; Duyar et al., 2020).

Ensuring and preserving the health of individuals in society is a shared goal across all political views (Erbaydar, 2003). Monitoring and preventing microorganisms that could threaten human health in ready-to-eat foods is crucial. The present study is of utmost importance as it aims to identify the presence of antibiotic-resistant bacteria in lakerda products and investigate the carriers of resistance genes. This research is vital to monitoring antimicrobial resistance and evaluating its risks to human health.

MATERIALS AND METHODS

Materials

In the study, 30 samples of packaged lakerda, obtained from three different fish markets and three different fishermen in Türkiye, were used to isolate antibiotic-resistant bacterial isolates. Care was taken to ensure that commercial products were not close to their expiration dates and that the samples taken from the fishermen were lakerda prepared from the new fishing season. The samples were brought to the laboratory in styrofoam boxes (+ 4 °C). Lakerda samples were kept in the refrigerator (+ 4 °C) until microbiological analysis, which was conducted within a maximum of 7 days.

Bacterial isolations and identification

10 g of samples taken from lakerda samples for microbiological analysis were homogenized for one minute in 90 ml of peptone water. Decimal dilutions of the homogenate (10^{-1} to 10^{-6}) were then prepared. Inoculations were done from these dilutions according to the spreading and pour plate methods. The media were incubated at the appropriate temperature, and incubation times were shown in Table 1 for the growth of microorganisms in the incubator. Different types of colonies were developed on Tryptic Soy Agar (TSA), TSA+10% Sodium chloride (NaCl), and DeMan, Rogosa and Sharpe (MRS) media. Then, each isolate was inoculated separately in suitable liquid media. After the growth of bacteria on the medium, they were stored in sterile cryogenic tubes at -80°C in the presence of 30% glycerol.

Table 1. Media used for isolation of bacteria and incubation temperatures.

Parameters	Media	Temperature (°C)	Incubation time	Reference
Total heterotrophic viable bacteria	TSA	22°C	5 days	This study
Total halophilic bacteria	TSA +10% NaCl	30°C	10 days	Brillantes et al., 2002; Ruginescu et al., 2020
Lactobacillus spp. count	MRS	30°C	3-5 days	Jokovic et al., 2008

TSA: Tryptic Soy Agar, NaCl: Sodium Chloride, MRS: DeMan, Rogosa ve Sharpe Agar

Bacteria were defined as heterotrophic, halophilic, and lactic acid bacteria groups, according to the media in which they grow. Forty-one isolates were identified as *Pseudomonas* spp., 40 were identified as *Staphylococcus* spp., 36 were identified as *Carnobacterium* spp., ten identified as *Vibrio* spp., and five identified as *Lactobacillus* spp. by standard microbiological procedures. These procedures included analyses of colony type, color, morphology, gram +/- characteristics, oxidase, catalase, H_2S, indole, oxidation/fermentation, arginine dihydrolase, lysine decarboxylase, β galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate, H2S, simmons citrate, nitrate reduction, sensitivity to: O/129, tolerance to salinity (NaCl), growth at different temperature ranges, pH ranges, acid production from various carbohydrates like mannitol, inulin, rhamnose, dulcitol, salicin, sorbitol, trehalose, lactose, sucrose, maltose, galactose, xylose, cellobiose, and raffinose In the next step, 16S rDNA gene sequencing analysis was performed to precisely determine the bacterial species resistant to three or more antibiotics detected due to the antibiogram test. DNA isolation was carried out using the EurX GeneMATRIX Bacterial & Yeast DNA Isolation Kit (Poland). The PCR reaction mixture (35 µL) contained 1x PCR buffer, 1.5 mM MgCl2, 0.2 mM dNTPs, 100 ng of template DNA, 0.3 µM of each primer, and nuclease-free water. PCR amplification was performed in a thermal cycler (Kyratec, Geumcheon-gu, Seoul, Korea) under the following conditions: an initial denaturation at 95°C for 5 minutes, followed by 30 cycles of 95°C for 45 seconds (denaturation), 57°C for 45 seconds (annealing), and

72°C for 60 seconds (extension), with a final extension at 72°C for 5 minutes. A 10 µL aliquot of the PCR product was loaded onto a 1.5% agarose gel in 1x TAE buffer containing ethidium bromide, and electrophoresis was conducted for 90 minutes at 100 volts. The PCR products were subsequently purified and sequenced by BM Labosis (Ankara, Türkiye) using the universal primers 27F (5′ AGAGTTTGATCMTGGCTCAG 3′) and 1492R (5′ TACGGYTACCTTGTTACGACTT 3′) (Lane, 1991). Sequence editing was performed using BioEdit software (BioEdit v7.0.0). The obtained 16S rRNA gene sequences were compared against the GenBank bacterial sequence database using the BLASTN tool available at http://blast.ncbi.nlm.nih.gov/.

Determination of antibiotic resistance

Kirby-Bauer's disc diffusion method (Bauer et al., 1966) was used to determine the antibiotic resistance of bacteria. Bacterial isolates were grown in Muller Hinton (MH) (1.5% saline and 10% saline) and MRS media. Afterward, they were transferred to solid media of the same growth medium. By choosing the colonies that show the best growth in the growth medium, density was adjusted to 0.5 McF in the broth medium. The transfer was made with a sterile cotton swab from the liquid broth medium with an adjusted density (0.5 McF) on the appropriate solid growth medium. Various antibiotic discs amoxicillin / clavulanic acid (30 µg), ampicillin (AMP) (10 µg), cefotaxime (30 µg), ceftriaxone (30 µg), cephalothin (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), clindamycin (10 µg), doxycycline hydrochlorıde (30 µg), erythromycin (15 µg), gentamicin (10 µg), imipenem (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), oxacillin (5 µg), streptomycin (10 µg), sulfamethoxazole/trimethoprim (25 µg), tetracycline (30 µg) and vancomycin (30 µg)] were placed in the medium at regular intervals using sterile forceps and left to incubate at the appropriate temperature and time (Table 1). The analyses were conducted with three repetitions. Zone diameters formed after incubation were measured. Afterward, the resistance or susceptibility of bacteria to antibiotics was evaluated according to the Clinical and Laboratory Standards Institute standards (CLSI, 2017), which provide interpretive criteria for classifying bacterial isolates as resistant, intermediate, or susceptible based on zone diameter measurements.

Identification of resistance genes of bacterial isolates

As a result of the antibiogram test, the presence of antibiotic-resistance genes in bacteria resistant to three or more antibiotics was investigated using a method previously applied in our laboratory (Kahraman Yilmaz and Berik 2024), briefly described below. Twenty-two antibiotic-resistant bacterial strains were screened for the presence of antibiotic resistance genes using uniplex PCR. The PCR reaction mixture (35 µL) consisted of 1x PCR buffer (Solis Biodyne), 2 mM $MgCl_2$, 0.2 mM dNTP mix, 0.5 µM of each forward and reverse primer, 2 U Taq polymerase, the DNA template, and PCR-grade distilled water. The primer sequences used for amplification are listed in Table 2, while the PCR conditions for groups A, B, and C are detailed in the table footnote.

The PCR products were analyzed on a 1.0% agarose gel prepared in 1x TAE buffer. Electrophoresis was performed at 100 volts for 60 minutes, and the bands were visualized under UV light using ethidium bromide staining. The sizes of the amplicons were determined using 50 bp and 100 bp DNA size markers (Siripornmongcolchai et al., 2002).

Table 2. List of primers for detection of antimicrobial resistance genes.

Targeted gene		Sequence (5′-3′)		References
β-lactamases	bla_{TEM***}	F	CATTTCCGTGTCGCCCTTATTC	Dallenne et al., 2010
		R	CGTTCATCCATAGTTGCCTGAC	
	$bla_{S}Hv***$	F	AGCCGCTTGAGCAAATTAAAC	
		R	ATCCCGCAGATAAATCACCAC	
	$bla_{C}rx\text{-}M**$	F	CGCTTTGCGATGTGCAG	Paterson et al., 2003
		R	ACCGCGATATCGTTGGT	
	*blaZ***	F	CAAAGATGATATAGTTGCTTATTCTCC	Kaase et al., 2008
		R	TGCTTGACCACTTTTATCAGC	
	*mecA**	F	GTGAAGATATACCAAGTGATT	Alfatemi et al., 2014
		R	ATGCGCTATAGATTGAAAGGAT	
	$bla_{IM}p*$	F	GAATAGAGTGGATTAATTCTC	Henriques et al., 2006
		R	GGTTTAAYAAAACAACCACC	
Tetracycline	*tetA****	F	GTAATTCTGAGCACTGTCGC	Sengeløv et al., 2003
		R	CTGCCTGGACAACATTGCTT	
	*tetB***	F	CTCAGTATTCCAAGCCTTTG	Sunde & Sørum, 2001
		R	CTAAGCACTTGTCTCCTGTT	
	*tetE****	F	GTGATGATGGCACTGGTCAT	Sengeløv et al., 2003
		R	CTCTGCTGTACATCGCTCTT	
	*tetK****	F	TATTTTGGCTTTGTATTCTTTCAT	Trzcinski et al., 2000
		R	GCTATACCTGTTCCCTCTGATAA	
	*tetM**	F	ACAGAAAGCTTATTATATAAC	Aminov et al. 2001
		R	TGGCGTGTCTATGATGTTCAC	

Table 2. Continue.

Targeted gene		**Sequence (5′-3′)**		**References**
Chloramphenicol	*Cat A***	R	GGATATGAAATTTATCCCTC	Aarestrup, 2000
		F	CAATCATCTACCCTATGAAT	
	*Cat B***	R	TGAACACCTGGAACCGCAGAG	Xia et al., 2013
		F	GCCATAGTAAACACCGGAGCA	
Plasmid-mediated quinolone resistance	*qnrA***	F	AGAGGATTTCTCACGCCAGG	Cattoir et al., 2007
		R	TGCCAGGCACAGATCTTGAC	
	*qnrB***	F	GATCGTGAAAGCCAGAAAGG	Katalin, 2000
		R	ACGATGCCTGGTAGTTGTCC	
	*qnrS***	F	GCAAGTTCATTGAACAGGGT	Cattoir et al., 2007
		R	TCTAAACCGTCGAGTTCGGCG	
Aminoglycoside resistance	*strA-strB***	F	TATCTGCGATTGGACCCTCTG	Sunde & Sørum, 2001
		R	CATTGCTCATCATTTGATCGGCT	
	*aphAI-IAB***	F	AAACGTCTTGCTCGAGGC	Frana et al., 2001
		R	CAAACCGTTATTCATTCGTGA	
	*aac(3)-IIa***	F	ATGGGCATCATTCGCACA	Dai et al., 2010
		R	TCTCGGCTTGAACGAATTGT	
	*aac(6′)-Ib***	F	TTGCGATGCTCTATGAGTGGCTA	Katalin, 2000
		R	CTCGAATGCCTGGCGTGTTT	
Vancomycin	*VanA***	F	GTACAATGCGGCCGTTA	Dutka-Malen et al., 1995
		R	GGGAAAACGACAATTGC	
	*VanB***	F	GTGCTGCGAGATACCACAGA	Ramos-Trujillo et al., 2003
		R	CGAACACCATGCAACATTTC	
Folate pathway inhibitors	*dfrD***	F	CCCTGCTATTAAAGCACC	Dale et al., 1995
		R	CATGACCAGATAACTC	
	*dfrK**	F	CAAGAGATAAGGGGTTCAGC	Argudín et al., 2011
		R	ACAGATACTTCGTTCCACTC	
	*dfrG***	F	TGCTGCGATGGATAAGAA	
		R	TGGGCAAATACCTCATTCC	
	*dfrA**	F	CACTTGTAATGGCACGGAAA	
		R	CGAATGTGTATGGTGGAAAG	
Macrolides	*ermA***	F	GTTCAAGAACAATCAATACAGAG	Lina et al., 1999
		R	GGATCAGGAAAAGGACATTTTAC	
	*ermB***	F	CCGTTTACGAAATTGGAACAGGTAAAGGGC	
		R	GAATCGAGACTTGAGTGTGC	
	*ermC***	F	GCTAATATTGTTTAAATCGTCAATTCC	
		R	GGATCAGGAAAAGGACATTTTAC	
Lincosamides	*msrA****	F	GGCACAATAAGAGTGTTTAAAGG	
		R	AAGTTATATCATGAATAGATTGTCCTGTT	
	*msrB***	F	TATGATATCCATAATAATTATCCAATC	
		R	AAGTTATATCATGAATAGATTGTCCTGTT	
	*lnuA***	F	GGTGGCTGGGGGGTAGATGTATTAACTGG	
		R	GCTTCTTTTGAAATACATGGTATTTTTCGA	
	*lnuB***	F	CCTACCTATTGTTTGTGGAA	Bozdogan et al., 1999
		R	ATAACGTTACTCTCCTATTC	

*PCR conditions group A: initial denaturation at 95 °C for 5 min, followed by 35 cycle consisting of denaturation at 95 °C for 40 s, 40 s annealing at 56 °C, 30 s extension at 72 °C, followed by a final extension step at 72 °C for 5 min.

**PCR conditions group B: initial denaturation at 95 °C for 5 min, followed by 35 cycle consisting of denaturation at 95 °C for 40 s, 40 s annealing at 56 °C, 45 s extension at 72 °C, followed by a final extension step at 72 °C for 5 min.

***PCR conditions group C: initial denaturation at 95 °C for 5 min, followed by 35 cycle consisting of denaturation at 95 °C for 40 s, 40 s annealing at 56 °C, 60 s extension at 72 °C, followed by a final extension step at 72 °C for 5 min.

RESULTS AND DISCUSSIONS

Detection of antibiotic-resistant bacteria in seafood is a major concern due to its implications for food safety and public health. In this study, multiple antibiotic-resistant bacteria, including *Pseudomonas fluorescens, Staphylococcus haemolyticus, Carnobacterium maltaromaticum* and *Carnobacterium mobile*, were identified in lakerda samples. The presence of resistance genes such as blaTEM, mecA and qnrB in these isolates highlights the potential of these bacteria to contribute to the spread of antimicrobial resistance. Such findings highlight the importance of monitoring ready-to-eat seafood, as they may serve as reservoirs for resistant bacteria and increase the risk of transmission of resistance genes to human pathogens. This issue is particularly critical for public health as it may lead to treatment failures and limited therapeutic options in bacterial infections.

Bacterial isolations, identifications, and antibiotic resistance

In this study, 132 bacteria were isolated from lakerda samples. Among these bacteria, 59.09% did not resist any of the 19 antibiotics tested, while 40.91% resisted at least one antibiotic. Among the bacterial isolates, 27.27% were resistant to three or more antibiotics (Figure 1). In addition, 40.74% of the isolates from commercial lakerda and 59.26% from fisherman-prepared lakerda exhibited resistance to at least one antibiotic. Among the bacterial isolates, resistance to antibiotics varied, with the highest resistance observed for NA (11.3%) and SXT (10.8%). This was followed by OX and CTX (both 7.9%), DA and S (both 7.4%), and CRO (6.4%). Additionally, resistance rates for other antibiotics were as follows: KF (5.9%), K (5.4%), E (4.9%), CN and VA (both 4.4%), AMP (3.9%), CIP (3.4%), AMC (3.0%), C (2.5%), and DO, TE, and IPM (each 1.0%).

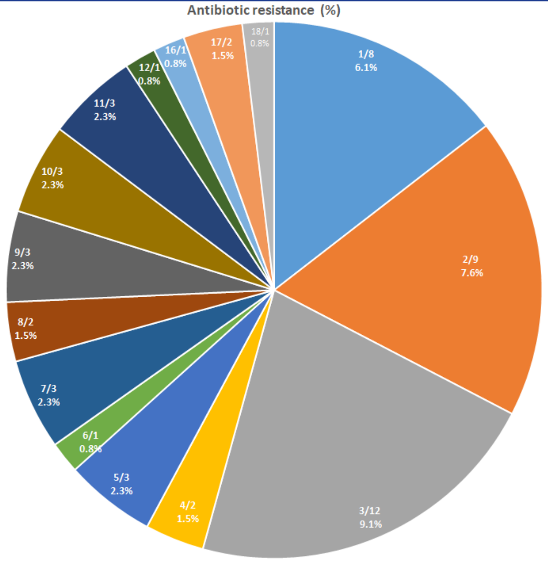

Figure 1. Antibiotic resistance rates (%) of 54 strains isolated from lakerda samples determined to be resistant to at least one antibiotic. Numerator (e.g., 1, 2, 3, etc.): Indicates the number of antibiotics to which the bacteria are resistant. Denominator (e.g., 8, 9, 12, etc.): Represents the number of bacterial isolates showing resistance to that specific number of antibiotics. For example, 1/8 means that 8 bacterial isolates were resistant to one antibiotic.

Among the isolated bacteria, 14 bacterial isolates with high similarity rates (≥99.1 to 100%) with gene bank records and definitive species identification were used in the study (Table 3).

Table 3. Bacterial isolate identification results.

Bacterial isolate	Accession numbers and Sequence result (similarity with 16S rRNA)
Pseudomonas fluorescens	AB204715.1 (99.3%)
Staphylococcus pasteuri	MF429378.1 (99.6%)
Staphylococcus haemolyticus	MT539735.1 (99.1%)
Staphylococcus equorum	MN758799.1 (99.1%)
Carnobacterium maltaromaticum isolate No. 1	MT631976.1 (99.9%)
Carnobacterium maltaromaticum isolate No. 2	MT631976.1 (99.9%)
Carnobacterium maltaromaticum isolate No. 3	MH119758.1 (100%)
Carnobacterium maltaromaticum isolate No. 4	MH119758.1 (100%)
Carnobacterium maltaromaticum isolate No. 5	MH119758.1 (100%)
Carnobacterium mobile	LT223645.1 (99.7%)
Vibrio hibernica isolate No. 1	MN796086.1 (99.4%) and NR_180997.1 (99.4%)
Vibrio hibernica isolate No. 2	MN796086.1 (99.4%) and NR_180997.1 (99.4%)
Vibrio rumoiensis isolate No. 1	KC534429.1 (99.8%)
Vibrio rumoiensis isolate No. 2	KC534429.1 (99.7%)

In this study, among the bacteria showing phenotypically multi antibiotic resistance in lakerda samples were *Pseudomonas fluorescens*, *Staphylococcus haemolyticus*, *Staphylococcus equorum*, *Carnobacterium maltaromaticum*, *Carnobacterium mobile* and *Vibrio hibernica* isolated (Table 4). In addition, antibiotic resistance genes in two more bacteria (*Vibrio rumoiensis* and *Staphylococcus pasteuri*) that did not have multiple antibiotic resistance was investigated. *V. rumoiensis* was chosen because it is a strong catalase producer. Detecting the presence of *V. rumoiensis* in Lakerda samples is important as it indicates that H_2O_2 may have been used in fillet bleaching. The presence of *S. pasture* in high-salt fermented various seafood dishes was reported, but it was not isolated in lakerda products before.

P. fluorescens is a regular contaminant of ready-to-eat foods and has been previously isolated from milk, dairy products, fish, chicken, beef, and vegetables (Kumar et al., 2019). In this study, it was determined that *P. fluorescens* isolate was resistant to 13 different antibiotics and carried genes associated with Extended Spectrum β-lactamases (*blaTEM*, *blaZ*), plasmid-mediated quinolone (*qnrB*, *qnrS*) and lincosamides (*msrA*) groups. The resistance of *Pseudomonas* spp. to β-lactam antibiotics is mainly attributed to Extended Spectrum β-lactamases (ESBLs) such as penicillin (1st, 2nd, and 3rd generations) and cephalosporins (cefotaxime, etc.) (Algammal et al., 2020). It was found that *P. fluorescens* isolated from lakerda carried resistance genes associat-

Table 4. Phenotypic and genotypic antibiotic resistance profiles of bacteria detected in lakerda samples.

Code	Bacteria	N*	Phenotype	Genotype
A	*Pseudomonas fluorescens*	1	OX, NA, VA, E, DA, C, KF, CRO, TE, CTX, AMC, SXT, AMP	*blaTEM, qnrB, qnrS, blaZ, msrA*
B	*Staphylococcus pasteuri*	1	K, S, CN	*mecA*
B	*Staphylococcus haemolyticus*	1	K, S, OX, CIP, NA, VA, E, DA, DO, IPM, C, KF, CRO, TE, CTX, AMC, SXT	*blaTEM, tetK, dfrD, blaZ,msrA, msrB, mecA*
A	*Staphylococcus equorum*	1	K, S, NA	*mecA*
C	*Carnobacterium maltaromaticum*	2	K, S, OX, CN, CIP, KF, CRO, NA, DA, CTX, AMP	*BlaTEM, mecA*
D	*Carnobacterium maltaromaticum*	3	K, S, OX, CN, NA, E, DA, KF, CRO, CTX, AMP	*blaTEM, qnrA, qnrB, qnrS, strA-strB, aphAI-IAB, mecA*
E	*Carnobacterium mobile*	1	K, S, OX, CIP, NA, VA, E, DO, DA, IPM, C, KF, CRO, TE, CTX, AMC, SXT, AMP	*blaTEM, blaZ, msrA, dfrD, mecA*
F	*Vibrio hibernica*	2	OX, KF, VA	*blaTEM, blaZ, mecA, VanA*
F	*Vibrio rumoiensis*	2	K, S, VA	-

*N: number of bacterial isolates

A, B and E: Fishermen samples

C, D and F: Fish market samples

AMC:AMOXYCILLIN / CLAVULANIC ACID 30 μG, AMP: AMPICILIN (AMP) (10 μg), E:ERYTHROMYCIN 15 μG, DA:CLINDAMYCIN 10 μG, VA:VANCOMYCIN 30 μG, OX:OXACILIN 5 μG, TE:TETRACYCLINE 30 μG, DO:DOXYCYCLINE HYDROCHLORIDE 30 μG, C:CHLORAMPHENICOL 30 μG, S:STREPTOMYCIN 10 μG, CN: GENTAMICIN 10 μG, K:KANAMYCIN 30 μG, NA:NALIDIXIC ACID 30 μG, CIP: CIPROFLOXACIN 5 μG, IMP: IMIPENEM 10 μG, SXT: SULPHAMETHOX. / TRIMETHOPRIM25 μG, CTX: CEFOTAXIME 30 μG, KF: CEPHALOTHIN 30 μG, CRO: CEFTRIAXONE 30 μG

ed with ESBLs (*blaTEM, blaZ*), plasmid-mediated quinolone (*qnrB*, *qnrS*), lincosamides (*msrA*) groups and methicillin/oxacillin resistance (*mecA*). Previous studies reported that *P. fluorescens* carried *blaTEM*gene in airborne isolates (Wang et al., 2022) and *qnrS* gene isolated from freshwater fish farms (Sherif et al., 2021). However, no study was found regarding *blaZ* and *msrA* genes in *P. fluorescens*.

A total of 222 isolates of *Pseudomonas* sp. were isolated in samples taken from a salmon processing company in Norway from surfaces (drain slaughter and drain filleting department) with or without food (fillet, skin, and gills, etc.) contact and 86% of them showed multi-antibiotic resistance (Thomassen et al., 2022). While these isolates are not phenotypically resistant to amikacin or tobramycin, it has been reported that the isolates are resistant to ampicillin, amoxicillin, oxolinic acid, florfenicol, cephalosporins, cefotaxime, ceftriaxone, and ciprofloxacin and genotypically carry the *adeF*, *soxR*, and *AbaQ* genes. Studies on antibiotic resistance of *P. fluorescens* isolated from salty food products are limited (Rodrigues et al., 2003). Rodrigues et al. (2003) reported that *Staphylococcus* species from gram-positive bacteria and *P. fluorescens* from gram-negative bacteria isolated from salted cod produced by different methods were intense. It was reported that *Pseudomonas* sp. isolated from sea bass (*Dicentrarchus labrax*) and shrimp (*Philocheras trispinosus*) were resistant to ampicillin, oxytetracycline, amoxicillin-clavulanic acid, trimethoprim/sulfamethoxazole, florfenicol, sulfamethoxazole and erythromycin (Güngör et al., 2021).

Bacteria in the *Staphylococcus* genus are pathogenic in mammals and many other living species. In this study, among three different *Staphylococcus* species, *S. haemolyticus* can cause opportunistic infections in immunosuppressed patients, especially hospitalized patients and those with medical implants. It is one of the coagulase-negative staphylococci that live commensal in the skin (Eltwisy et al., 2020).

It was found that *S. haemolyticus* isolated from lakerda was resistant to 17 different antibiotics and carried genes associated with ESBLs (*blaTEM, blaZ*), lincosamides (*msrA, msrB*), tetracycline (*tetK*), folate pathway inhibitors (*dfrD*) and methicillin/oxacillin resistance (*mecA*). Similarly, Regecová et al. (2014) reported that *S. haemolyticus* isolated from frozen Atlantic herring meat resisted erythromycin, oxacillin, and ampicillin antibiotics. *S. haemolyticus* isolated from fish meat showed resistance to ampicillin, oxacillin, tetracycline, and gentamicin (Hammad et al., 2012). Chajęcka-Wierzchowska et al. (2023) reported that *S. heamolyticus* isolated from fish tartar from ready-to-eat foods they bought from bars and restaurants were phenotypically resistant to gentamicin, clindamycin, erythromycin, cefoxitin, fusidic acid, norfloxacin, penicillin, tetracycline, and quinupristin/dalfopristin. They genotypically carried *aac(6')-Ie-aph(2'')-Ia*, *blaZ*, *erm*A, *erm*B, *mec*A, *msr*(A/B), and *tet*K genes (Chajęcka-Wierzchowska et al., 2023).

This study determined that while *S. pasteuri* isolated from lakerda samples prepared by fishermen was resistant to K, S, and CN antibiotics, it carried the methicillin/oxacillin resistance gene *mecA*. *S. pasteuri* species isolated from jeotgal, a Korean food and a high-salt fermented dish [20–30% (w/w) salt] that can be made with a variety of seafood (fish, shrimp, oysters, scallops and caviar), was found to be resistant to linezolid, penicillin, and trimethoprim antibiotics and carried the *dfrA* gene associated with trimethoprim resistance (Lee & Jeong, 2015). Similarly, *S. pasteu-*

ri isolated from ready-to-eat foods (e.g., burgers, cheeses, juices, sushi, salads, sandwiches, meat, and fish tartare) in bars and restaurants were found to be resistant to gentamicin, clindamycin, erythromycin, cefoxitin, fusidic acid, penicillin, quinupristin/dalfopristin and rifampicin and carried *bla*Z, *erm*A, *erm*B, *mec*A, *msr*(A/B), *tet*K, and *tet*M genes (Chajęcka-Wierzchowska et al., 2023).

S. equorum is one of the coagulase-negative staphylococci isolated from fermented foods, bacterial surface-ripened cheeses, cattle, goats, horses, and sheep (Becker et al., 2014). No staphylococcal food poisoning via fermented foods has been associated with S. *equorum*, and no evidence of its pathogenicity has been reported. In this study, *S. equorum* isolated from lakerda samples showed resistance to K, S, and NA antibiotics carrying the *mecA* gene. *S. equorum* isolated from jeotgal are resistant to penicillin G, erythromycin, trimethoprim, lincomycin, and chloramphenicol; it was reported that they carry *lnuA* and *pbp* resistance genes (Jeong et al., 2014).

Carnobacterium is a lactic acid bacteria found in the natural environment and foods, and its positive and negative effects are still the subject of research (Leisner et al., 2007). It was previously isolated from aquatic products such as salted lumpfish, cold-smoked salmon, gravad rainbow trout, brine shrimp, seafood salad, cooked modified atmosphere packaging shrimp, and modified atmosphere packaging rough head grenadier (Françoise, 2010). *Carnobacterium* can be toxic to sensitive individuals because it can produce tyramine in food (Leisner et al., 2007). For this reason, it is crucial for public health to monitor *Carnobacterium* species in food and to investigate the antibiotic resistance of strains.

This study isolated three different *Carnobacterium* isolates with multiple antibiotic resistance from lakerda samples. One of the *Carnobacterium maltaromaticum* isolated from two other fish markets was found to be resistant to 11 different antibiotics. In addition, it was observed that one carried the *blaTEM* and *mecA* resistance genes, while the other had the *blaTEM*, *qnrA*, *qnrB*, *qnrS*, *strA-strB*, *aphAI-IAB,* and *mecA* resistance genes. In lakerda samples taken from fish markets, *Carnobacterium mobile* species were isolated and showed resistance to 18 antibiotics. It was found to carry *blaTEM*, *blaZ, msrA, dfrD, and mecA* resistance genes. Although *Carnobacterium* was previously isolated from 20-30% salty Geotgal product, its species could not be defined precisely, and antibiotic resistance information was not reported (Guan et al., 2011). A previous study reported that *Carnobacterium*isolated from food carried the *tetM*and *tetS* genes associated with tetracycline resistance (Li & Wang, 2010).

Vibrio species are among the bacterial species frequently isolated in aquatic ecosystems and food samples (Zeidler et al., 2024).

In this study, two different *Vibrio* species, *Vibrio rumoiensis* and *Vibrio hibernica* were isolated from lakerda samples taken from fish markets. Yumoto et al. (1999) isolated *V. rumoiensis* as a potent catalase producer from the drainage pond of a fish processing plant using H_2O_2 as a bleaching and microbial agent. The presence of this species in the lakerda samples produced in the facility suggests that H_2O_2 may have been used in the fillet bleaching. In addition, histamine-producing *V. rumoiensis* was isolated from the Chinese salted fish pickled overnight product (Tao et al., 2022).

In this study, *V. rumoiensis* isolated from lakerda obtained from the fish market showed resistance to K, S, and VA antibiotics. All of the *Vibrio* species isolated from water and sediment samples near the shrimp farm were resistant to at least one antibiotic, and the presence of *V. rumoiensis* was reported among the sediment isolates (dos Santos Rocha et al., 2016). It was reported that the marine sediment isolate *V. rumoiensis* is resistant to ampicillin, oxytetracycline, and penicillin (Rocha, 2011).

Woods et al. (2020) propose that *V. hibernica* is a member of the rumoiensis clade. It is an essential feature of an industrial food processing bacterium, and it was isolated in fermented foods and Greek table olives (Woods et al., 2020; Mougiou et al., 2023). This study observed that *V. hibernica* isolated from lakerda obtained from the fish market was resistant to OX, KF and VA antibiotics and carried *blaTEM*, *blaZ, mecA,* and *VanA* resistance genes. However, to our knowledge, the resistance genes we detected for the lakerda isolate *V. hibernica* have yet to be reported in this bacterial species before.

CONCLUSION

In this study, eight different bacterial isolates showing multiple antibiotic resistance were identified in prepared lakerda samples. The detected bacteria include *Pseudomonas fluorescens, Staphylococcus haemolyticus, Staphylococcus pasteuri, Staphylococcus equorum, Carnobacterium maltaromaticum, Carnobacterium mobile*, *Vibrio hibernica*, and *Vibrio rumoiensis*. Among the identified resistance genes, blaTEM and mecA were the most common and were found in almost all isolates, followed by blaZ and qnrB. The widespread presence of these genes highlights the significant risk posed by beta-lactam and methicillin/oxacillin resistance in bacteria associated with salted seafood. To minimize these risks, strict hygiene protocols should be implemented throughout the lakerda production and storage processes, especially in traditional preparation methods. Public health officials need to ensure hygiene conditions in the preparation of lakerda, determine the ways of transmission, and take precautions to raise awareness among producers and consumers.

Conflict of interest: There is no conflict of interest.

Ethics Committee Approval: The authors declare that this study did not include any experiments with human or animal subjects.

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Research Article

Effect of Phosmet Toxicity on Some Physiological Traits in Duckweed (*Lemna gibba* L.)

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ABSTRACT

Pesticides, one of the chemicals that adversely affect the environment and human health, can have effects on non-target organisms due to their chemical properties and wide range of use. Therefore, in this study, the toxic effects of phosmet insecticides on *Lemna gibba*, an aquatic macrophyte, were determined as a non-target organism. The study was carried out in a climate cabinet under controlled conditions. It was determined that the photosynthetic pigments and total carbohydrate content of the macrophyte decreased with increasing phosmet concentration. Similarly, decrease in total phenolic contents were found. A significant and positive correlation between non-protein sulfhydryl groups (NP-SH) and H_2O_2 contents may indicate their role in antioxidant defense mechanism. Besides, increases in malondialdehyde (MDA) and H_2O_2 contents showed that phosmet toxicity caused oxidative stress in *L. gibba* tissues.

Keywords: *Lemna gibba*, phosmet, toxicity, oxidative stress.

INTRODUCTION

Pesticides are chemical substances that are used to control pests. Their persistent nature, poisonous qualities, bioaccumulation, lipophilicity, and detrimental effects on the environment and human health make them extremely concerning. Pesticide residues contaminate the land and water, build up in plants, make their way up the food chain, and finally end up in human diets and water sources. As a result, they negatively impact non-target species (Barcelo and Hennion, 1997; Taylor et al., 2003).

Different standards have been used to classify pesticides. Mechanism of action and/or mechanism of entrance, method of controlling or killing the target organism, chemical composition, and pesticide properties are the most often used classification criteria. The main classification of insecticides, herbicides, and fungicides is based on the target organism group (Drum, 1980; Hassaan and El Nemr, 2020).

Pesticide poisoning has a negative impact on plants' growth and development because of its impacts on metabolism. According to Sharma et al. (2019), their toxicity results in a drop in photosynthetic pigments and a decrease in photosynthetic efficiency. Reactive oxygen species are created when oxidative stress is triggered by pesticide damage. Plants have both enzymatic and non-enzymatic antioxidant defense systems that work to lessen the harmful consequences of oxidative stress (D'Souza, 2017; Sharma et al., 2019).

Phosmet, non-systemic and broad-spectrum organophosphate, is an anti-cholinesterase chemical used to control aphids, suckers, moths and sucker on plants and animals (FAO, 2019). The runoff and spray drift are main routes for it to enter water bodies and the reported maximum phosmet concentrations in ground- and surface water were 0.20 and 0.63 µg/L (United States Environmental Protection Agency (US EPA), 2010).

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Limited data on other organophosphorus insecticides indicate that they may have the potential for toxic effects on aquatic plants. However, these effects vary depending on the chemical substance and species tested. On the other hand, it is not known whether phosmet will have similar impacts on non-target plants (United States Environmental Protection Agency (US EPA), 2010).

The present study was performed to evaluate the toxic effects of phosmet on (i) photosynthetic pigments (*chlorophyll-a*, *chlorophyll-b* and carotenoid), (ii) total carbohydrates, (iii) phenolic compounds and potential to cause oxidative stress by occupying biomarkers of non-protein sulfhydryl groups (NP-SH), hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) in an aquatic free-floating macrophyte, *Lemna gibba*. *L. gibba* was chosen as experimental model organism because of its advantages of ease of culture, quick development, and tiny size (Park et al., 2021).

MATERIALS AND METHODS

L. gibba was collected from water bodies in Gaziantep province (Türkiye). The macrophytes brought to the laboratory were acclimatized in a controlled climate cabinet (light level 120 μE $m^2.s^{-1}$, temperature 23±1 °C, Snijders Scientific, Netherlands) for two weeks in containers containing 10% nutrient solution (Dogan et al., 2021). After the macrophytes were acclimated to the experimental conditions, they were randomly divided into four groups. Stock phosmet solution was prepared by dissolving in acetone. The final concentration of acetone in the assay was 0.01% (v/v). Group I containing pesticide-free water with 0.01% acetone was used as control. Group II, III and IV were exposed to 0, 0,1, 10 and 100 ppm phosmet for 96 hours. 96 hours test duration was performed due to rapid hydrolysis of phosmet in aqueous environment with with half-lives of 13 days at pH 4.5, 12 hours at pH 7 and 4 hours at pH 8.3 (Tomlin, 2004). The applications were carried out in glass beakers containing 10% nutrient solution at concentrations of 200 mL. Deionized water used for preparing the solutions. At the end of treatment, fresh *L. gibba* samples were harvested and immediately stored at -80 °C until analysis.

To determine photosynthetic pigment contents, duckweed fronds were homogenized in 80% acetone. Following readings in a UV/VIS spectrophotometer, the contents of photosynthetic pigment (*chlorophyll-a, chlorophyll-b*, and carotenoid) were computed in accordance with Lichtenthaler and Wellburn (1985). The anthron method was used to determine the total soluble carbohydrate content of the macrophyte (Plummer, 1998). Glucose was used as standard in carbohydrate calculations. Non-protein sulfhydryl groups (NP-SH) were made according to Ellman's method (Ellman, 1959). Reduced glutathione (GSH) standard was used in NP-SH calculations. Total phenolic content was determined using Folin-Ciocalteu reagent (Ratkevicius et al., 2003). The curve of the gallic acid standard was used for total phenolic calculations. The lipid peroxidation level was determined by detecting the amount of malondialdehyde (MDA) using the method proposed by Zhou (2001). The H_2O_2 content was determined according to Sergiev et al. (1997). All chemicals used in the analyzes were of analytical grade.

All analyzes were carried out with three replicates. SPSS 22 was used in statistical analysis of the data. Comparison of means was performed using the least significant difference (LSD) test at $p<0.05$. The Pearson correlation was used to determine the relationship between the data obtained.

RESULTS AND DISCUSSIONS

It was determined that the photosynthetic pigment content of macrophyte leaves decreased with increasing phosmet concentration. *Chlorophyll-a* contents reduced by 4.0% ($p>0.05$), 16.0% ($p<0.05$) and 22.9% ($p<0.05$) under the effect of 1, 10 and 100 ppm of phosmet, respectively (Figure 1A). *Chlorophyll-b* (Figure 1B) and carotenoid (Figure 1C) contents decreased by 14.94% ($p>0.05$) and 33.67% ($p<0.05$), respectively. Similar to these findings, adverse effects of on photosynthesis and photosynthetic pigment contents in plants have been stated (Salem, 2016; Iwaniuk and Lozowicka, 2022). This affect was attributed to inhibition of chlorophyll biosynthesis by excess production of reactive oxygen species (ROS) in addition to the direct effect of pesticides (Aarti et al., 2006). Negative relationships between photosynthetic pigments (*Chl-a*, *Chl-b* and carotenoid) and hydrogen peroxide were determined (Table 1). The findings may be explained by inhibition of pigment synthesis by the phosmet and/or by accelerated degradation of pigments due to ROS generation induced by the phosmet (Mostafa and Helling, 2002).

It has been reported that pesticide toxicity causes metabolic disorders in plants (Sharples et al., 1997). Phosmet applications resulted in decrease in total carbohydrate content reaching 55.95% ($p<0.05$) (Figure 1D). Similar result was reported dimethoate, organophosphorus insecticide, applied mung plant (Seth et al., 2014). Significant decrease in total carbohydrate content *Vitis vinifera* following flazasulfuron, herbicide, application was also stated (Magne et al., 2006). Kumar (2012) also declared comparable findings in 2,4-Dichlorophenoxy acetic acid and isoproturon treated wheat (*Triticum aestivum* L.) and suggested that carbohydrate depleting affect may be involved in the toxicity of these two herbicides in addition to their main adverse effects of being synthetic auxin and acetolactate synthase inhibitor, respectively. As for phosmet, ROS elicited oxidative damage to carbohydrates may be suggested as underlying mechanisms of observed affect as supported with significant and negative correlation between total carbohydrate and hydrogen peroxide contents (Table 1).

Plant phenolic compounds, called secondary metabolites, have roles in environmental stress (Dogan and Gultekin, 2017; Cinar and Dogan, 2020). Phosmet exposure caused dose-dependant reduction in the content of total phenolic compound reaching 57.1% ($p<0.05$) (Figure 2A). Lin et al. (2022) stated decrease in phenolic acids in peppermint (*Mentha piperita* L.) following application of five insecticides (imidacloprid, pyriproxyfen, acetamiprid, chlorantraniliprole, and chlorfenapyr.) and suggested it as an adaptive response causing generation of lignin as permeability barrier to prevent absorption of insecticides.

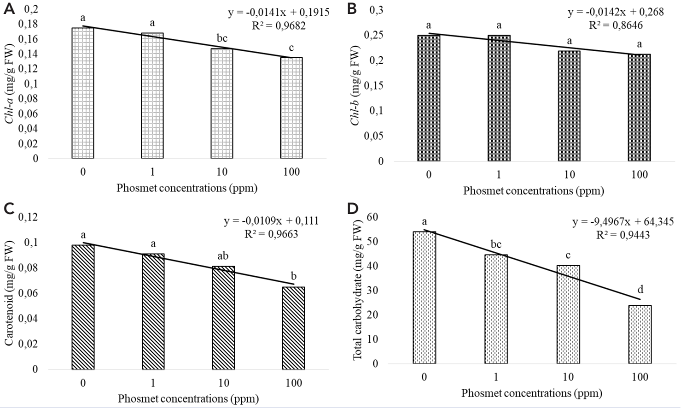

Figure 1. Photosynthetic pigments (A-C) and total carbohydrate (D) contents of *L. gibba* after phosmet applicaitons and their statistical evaluations. Means with different letters are significantly different from one another according to LSD test ($p<0.05$).

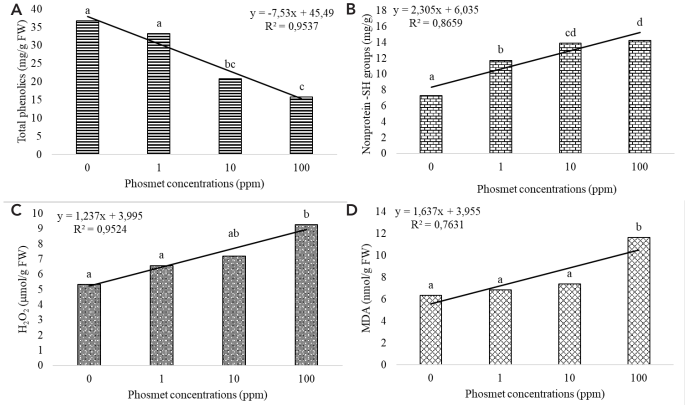

Figure 2. Total phenolic (A), NP-SH (B), H_2O_2 (C) and MDA (D) contents of *L. gibba* after phosmet applicaitons and their statistical evaluations. Means with different letters are significantly different from one another according to LSD test ($p<0.05$).

The non-protein sulfhydryl groups (NP-SH) showed an increasing tendency with the percentage changes of 60.8%, 90.1% and 95.5% at 1, 10 and 100 ppm concentrations ($p<0.05$), respectively (Figure 2B). Glutathione (GSH), the greater part of the non-protein sulfhydryl groups playing vital role in detoxification of xenobiotics, including pesticides, in plants (Yu et al., 2022). Mitton et al. (2016) determined increase in levels of NP-SH groups in alfalfa roots and soybean leaves following organochlorine pesticide dichlorodiphenyltrichloroethane (DDT) exposure. This result was referred as a general mechanism to maintain redox status via increase in low molecular weight thiols like glutathione by authors. In the present study, a significant and positive relationship between NP-SH and H_2O_2 contents were also determined after phosmet applications (Table 1). It manifests induction of antioxidant defense system as a response to phosmet triggered oxidative stress.

Pesticide induced oxidative stress either by direct action of chemical or by increase in the production of ROS have been reported (D'Souza, 2017). Phosmet applications resulted in increase in H_2O_2 level by 23.5%, 34.9% ($p>0.05$) and 73.5% ($p<0.05$) following 1, 10 and 100 mg/L concentrations, respectively (Figure 2C). H_2O_2, a non-radical, is the two-electron reduction product of oxygen and involved in oxidative degradation of lipids which is known as lipid peroxidation. 82.99% increase in MDA content was elicited by phosmet (Figure 2D; $p<0.05$) and correlation analysis showed that H_2O_2 and MDA contents were positively and significantly correlated (Table 1). Similarly, Dubey et al. (2015) stated rise in H_2O_2 and lipid peroxidation in barley (*Hordeum vulgare* L.) following methyl parathion (insecticide) and hexaconazole (fungicide) exposure. Nohatto et al. (2016) reported occurrence of oxidative stress manifested by increased H_2O_2 and lipid peroxidation levels in rice plants exposed to three different herbicides namely bentazon, penoxsulam and cyhalofop-butyl.

CONCLUSIONS

The determined decrease in the contents of photosynthetic pigments and total carbohydrate shows the involvement of disruption of photosynthesis and energy metabolism in the toxicity of phosmet. The elevation in occupied biomarkers of H_2O_2, lipid peroxidation and NP-SH clearly indicates phosmet elicited oxidative stress in duckweed.

Table 1. Correlation coefficients of the parameters obtained after phosmet applications.

	Chl-a	Chl-b	Car	TC	TP	NP-SH	H_2O_2	MDA
Chl-a	1							
Chl-b	0.757**	1						
Car	0.746**	0.885**	1					
TC	0.900**	0.566	0.693*	1				
TP	0.954**	0.782**	0.731**	0.843**	1			
NP-SH	-0.807**	-0.541	-0.607*	-0.798**	-0.858**	1		
H_2O_2	-0.673*	-0.307	-0.432	-0.848**	-0.628*	0.627*	1	
MDA	-0.820**	-0.631*	-0.684*	-0.888**	-0.759**	0.581*	0.765**	1

Chl-a: Chlorophyll-a, Chl-b: Chlorophyll-b, Car: Carotenoid, TC: Total carbohydrate, TP: Total phenolics, NP-SH: Nonprotein sulphydril groups, H_2O_2: Hydrogen peroxide, MDA: Malondialdehyde

*: Correlation is significant at the 0.05 level (2-tailed)

**: Correlation is significant at the 0.01 level (2-tailed)

Conflict of Interest: The authors declare that they have no competing interests.

Ethics Committee Approval: Ethics approval was not required for this study.

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Research Article

Mathematical Modelling of the Population of *Potamon potamios* Olivier 1804 in Terms of Climatic Factors

Tuğrul Öntürk[1]

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ABSTRACT

The amount of the *Potamon potamios* Olivier 1804 population has many positive/negative effects on other aquatic life and is essential for aquatic biodiversity. It is therefore important to determine the population of *Potamon potamios* Olivier 1804. The population density of *Potamon potamios* Olivier 1804 is affected by temperature, precipitation, amount of water, and many other climatic factors. In this sense, mathematical model based on a fuzzy inference system was made to determine the population dynamics of this creature considering the climatic data in this study. In addition, correlation analysis has shown that this model can predict the population of this creature with 91% accuracy in untested climatic conditions.

Keywords: Population Variations, Fuzzy Logic Modelling, Fuzzy Inference System, Freshwater crabs, *Potamon potamios*

INTRODUCTION

Crabs are found in oceans all over the world, as well as in many freshwater and terrestrial species. There are species with a leg length of several metres (*Maja kaempferi* Temminck, 1836) with known lengths of less than a few (*Pinnotheres pisum* (Linnaeus, 1767)) (Brandis, 2001). Freshwater crabs live in many habitats, from the cold waters at the top of the Andes to rivers and lakes in the tropics, swamps and rainforest floors, and even arid areas. Some freshwater crab species are fully aquatic, while others are semi-terrestrial. A few species have adapted to living in trees and breathing air (Sternberg et al, 1999). Since they have legs, they are in the Brachyura infrateam of the Decapoda (Ten-legged) team. Although they have legs , the first pair of legs close to the head is used for hunting and catching. They do not have walking functions. The remaining pairs of legs perform the walking work (Brandis et al, 2000). Crabs are both a high-protein food source and play a role in the nutrition of some living organisms (Buck et al, 2003). However, their superior hunting and olfactory abilities, negatively dominate the fast-reproducing protozoa, plankton and fish populations (Giller and Malmqvist 2000). Temperature is one of the most important factors in the distribution of freshwater crabs. It has been reported that there are species of Potamon in the Middle East and species in Europe (Brandis et al, 2000; Brandis, 2001; Giller and Malmqvist, 2000. Regarding population density, the *Potamon potamios* Olivier species is mainly found in the Greek islands and the island of Cyprus to the southern coasts of Turkey. It shows less population density in the parts of the countries up to northeast Egypt close to the Mediterranean (Brandis et al. 2000). The regions where the species is most widely distributed in Turkey are the coastal and interior parts of the Mediterranean (Brandis et al, 2000; Özbek and Ustaoglu, 2005; Özbek and Ustaoglu, 2006; Öntürk, 2018).

The amount of *the P. potamios* population has many positive/negative effects on other aquat-

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ic creatures and is essential for aquatic biodiversity. Therefore, it is essential to determine the population of *P. potamios*. The population density of *P. potamios* is affected by temperature, precipitation, amount of water, and many other climatic factors (Kennish, 1996). The main motivation of this study is to determine how the population dynamics of this creature are affected by climatic data. In this sense, the climate data were measured monthly for years between and the number of individuals identified in this period was recorded. In this study, considering data obtained from field studies, mathematical model based on the fuzzy inference system was constructed to determine the population change. Moreover, it has been shown in the correlation analysis that the untested data can be estimated with accuracy thanks to this mathematical model.

Materials and Methods

Data collection

The inputs in this study are the real data obtained from the 11 years of work conducted by Phd. Tuğrul Öntürk between and in Gökçekaya Dam Lake (Figure 1). In this study, *P. potamios* individuals were collected monthly, and the total number of individuals was determined. The samples collected during this process were marked with a water-resistant pen to prevent recounting and released back into the living spaces.

Figure 1. General view of the Gökçekaya Dam and its surroundings.

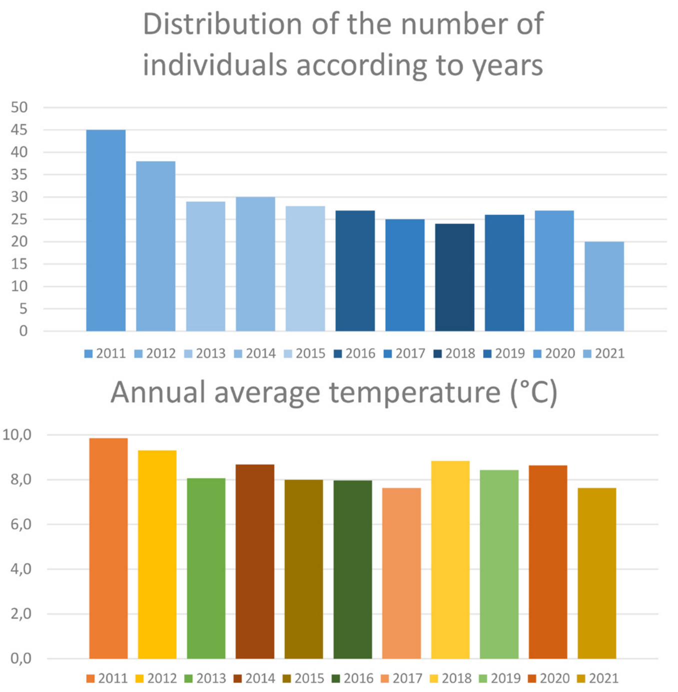


Figure 2. Number of individuals and temperature averages by years.

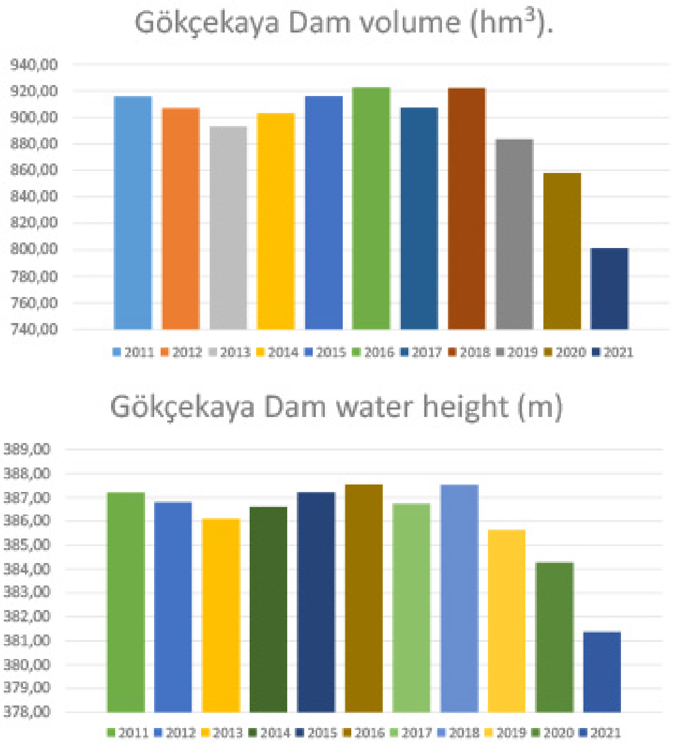


Figure 3. The volume of water collected in the dam and the height of the water by years.

In addition to the total number of individuals, the annual average temperature (Figure 2), dam volume , dam water height (Figure 3), annual average wind speed , annual average rainfall (Figure 4) were measured regularly.

Fuzzy logic approach

It is impossible to precisely define many problems, situations or events encountered due to the uncertainties they encompass. Because real life has an extremely complex and chaotic structure. It is very difficult to define everything we experience in our daily life with " 0 " and "1", to classify them only between two extremes like black and white, and to examine them under mathematical precision. Because there are also uncertainties in our lives that do not have sharp boundaries. Therefore, the necessity of having exact values about a phenomenon in mathematics has caused difficulties in practice, and for centuries, scientists have developed methods to find a solution. Mathematical precision was not enough despite the limitlessness and complexity of the universe. Although it is possible to explain concepts such as

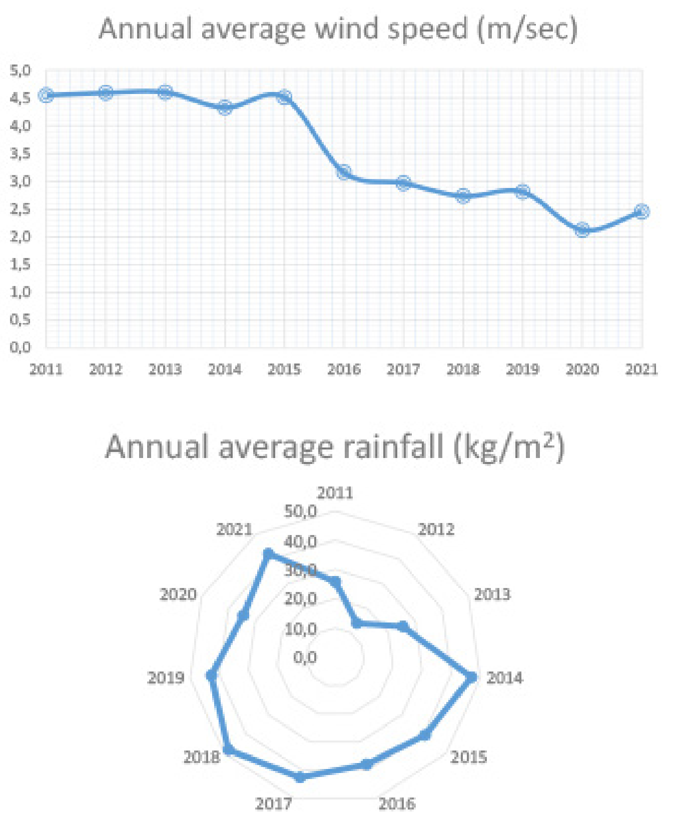


Figure 4. Average wind speeds and precipitation per square metre in kilogrammes in the study area by the year.

black-white, right-wrong, and good-bad with mathematical thinking, it is not possible to represent the entire universe with this binary logic alone. Based on this, the fuzzy set theory was introduced to the literature by Azeri origin Lütfi Aliasker Zade in 1965 with the study "L.A. Zadeh, Fuzzy sets. Information and Control 8(3):338–353." With this study, a new perspective was given to classical logic, which came from Aristotle, and machines learned the sentences produced by humans. Thus, the two-valued Aristotelian logic consisting of " 0 " and " 1 " has been generalised by evolving into a technology-supported logic. The fuzzy set generalises the two-choice membership status of "yes-no" to the concept of partial membership. In this case, the value of " 1 " indicates full membership to the set, the value of " 0 " indicates not being a member of the set, while values between " 0 " and " 1 " represent the concept of relative (partial) membership. Accordingly, an arbitrary element in a fuzzy set may belong to different fuzzy sets according to its degree of membership. The traditional set approach uses characteristic functions while expressing the sets. A traditional set with the help of characteristic functions is defined by

$$\begin{aligned} \chi_A \quad &: \quad U \quad \rightarrow \quad \{0,1\} \\ x \quad &\mapsto \quad \begin{cases} 1 \;, & x \in A \\ 0 \;, & x \notin A' \end{cases} \end{aligned}$$

where U is the universal set and $A \subseteq U$. In the fuzzy set approach, membership transitions of the elements in a universal set, fuzzy sets, occur gradually. If an element is included in any cluster, the element belonging to that cluster is graded. With this rating, an ambiguous situation occurs at the fuzzy set boundaries. For this reason, a function to measure the uncertainty in the case of an element belonging to a set is defined. Such functions are called "Membership Function", and the set formed by this function is called "Fuzzy Set". A fuzzy set A can be mathematically expressed as

Let *U* be a universal set. The fuzzy set *A* in *U* is characterised by its membership function $\mu_A\colon U$ and is defined as a set of ordered pairs;

$$A = \{(x, \mu_A(x)) : x \in U\}.$$

As the membership function, μ_A maps each element of *U* to a membership grade between and , the value of $\mu_A(x)$ is interpreted as the degree to which a point $x \in U$ belongs to the fuzzy set *A*.

In fuzzy sets, an element of a set that "somewhat belongs or does not belong to a set" is expressed as. As with traditional sets, the all-or-nothing logic is not true for sets. In the fuzzy system, the data to be used as input should be fuzzified, that is, these data should be converted to values between. The goal of this process is to express the data in question with verbal expressions. In the fuzzification process, the fixed logic calculations are determined first. Then, each point in these sets is expressed with a membership function. There is no limit to what form and how many membership functions can be. The number of membership volumes varies according to the knowledge and experience of the specialist. The most commonly used membership function types are trapezoidal, Gaussian, and triangular.

Triangle membership function

The triangle membership function is used to specify the input and output variables in fuzzy logic-based systems. This membership function is determined by three variable real numbers such as *p*, *q* and *r* and its equation is as follows;

$$\mu(x; p, q, r) = \max\left\{\min\left\{\frac{x-p}{q-p}, \frac{r-x}{r-q}\right\}, 0\right\}$$

Trapezoidal membership function

The trapezoidal membership function is used to define the input and output variables, similar to the triangular membership function, and is defined as follows;

$$\mu(x; p, q, r) = \max\left\{\min\left\{\frac{x-p}{q-p}, 1, \frac{r-x}{r-q}\right\}, 0\right\},$$

where *p*, *q*, *r* and *s* are real numbers;

Implementation of the fuzzy inference system

The fact that it is close to human logic and easy to create has made the Mamdani-type fuzzy inference system very popular in natural sciences such as physics, chemistry and biology recently (Ermiş et al, 2022; Putti, 2017; Şahiner, 2015; Şahiner, 2016; Şahiner, 2018; Şahiner, 2022; Şahiner, 2023). In this study, we used the Mamdani-type fuzzy inference system to guide the fuzzy logic models that are planned to be used in future biology studies.

Determine and predict the *P. potamios* crab population according to various climatic conditions on a personal computer with Intel(R) Core(TM) i7 (2.81 GHz) configuration on the MATLAB R2015a platform, we constructed mathematical model using the Mamdani-type fuzzy inference system, the steps of which are given below. Our Mamdani inference mechanism has six inputs and one output value. The input variables are months, annual average temperature (°C), dam volume (hm^3), altitude (dam water height (*m*)), annual average wind speed (*m/sec*) and annual average precipitation (kg/m^2). The output variable is the population of *P. potamios* crab.

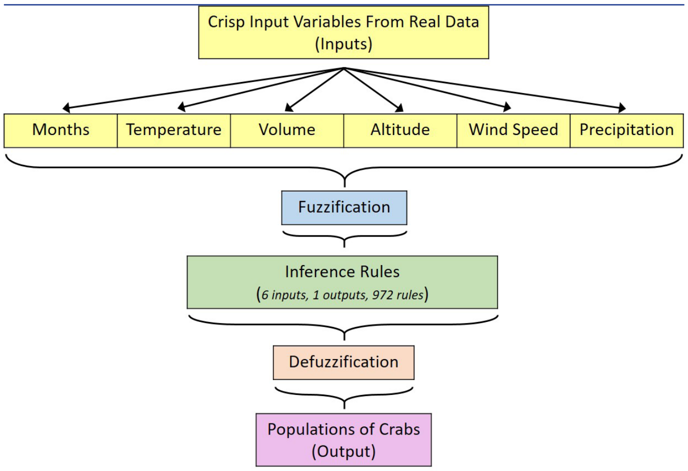


Figure 5. General Structure of the Mamdani Fuzzy Inference System.

Fuzzification

In this step, we determined the membership degrees by converting the input and output values to values between with the help of the membership functions. Thus, by the fuzzification of our input and output variables through membership functions, we have turned precise numerical values into linguistic variables. As a result, based on our experience and expertise, we have made our output data interpretable according to these verbal evaluations. In Figure 6, months are labelled as follows; *0 = February, 2 = March, 3 = April, 4 = May, 5 = June, 6 = July, 7 = August, 8 = September, 9 = October, 10 = November, 11 = December.* Also, t1, t2, and t3 represent low, medium, and high temperatures, respectively, in Figure 7. Similarly, linguistic labelling of low, medium and high is used in Figures 8, 9, 10, 11 and 12.

Inference rules

In the previous step, we the input variables of our model into fuzzy input. In this step, we created fuzzy rules using logical operations such as "if-then", "and", "or", "not" by taking expert opinions. Then, we obtained fuzzy outputs by processing fuzzy inputs according to these rules. Briefly, we transformed verbal/linguistic input and output variables into mathematical form. For this, we defined the rules as much as the multiplication of the membership function numbers we defined for each input value while determining the rules. That is, since there are input variables in our model and 4,3,3,3,3,3 defined membership functions

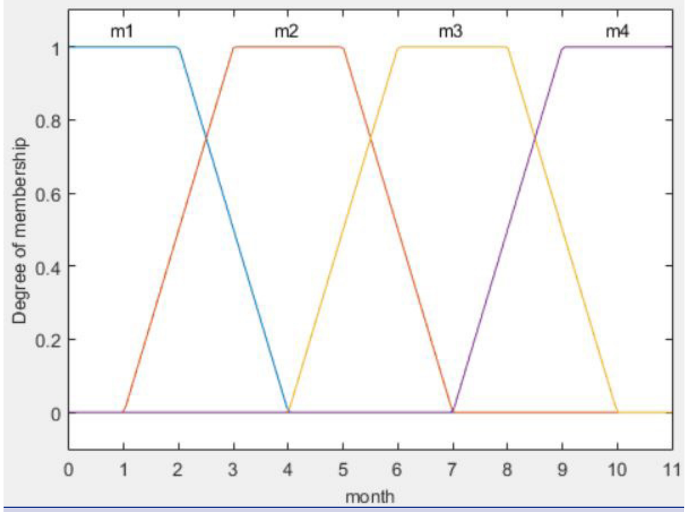


Figure 6. Membership functions for months.

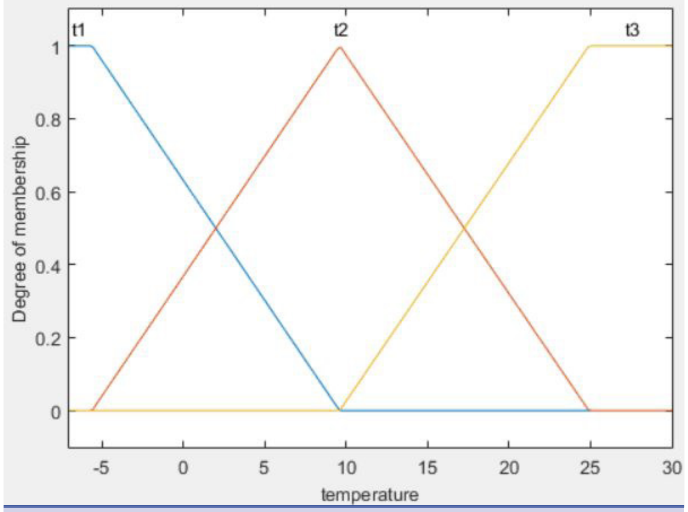


Figure 7. Membership functions for the temperatures.

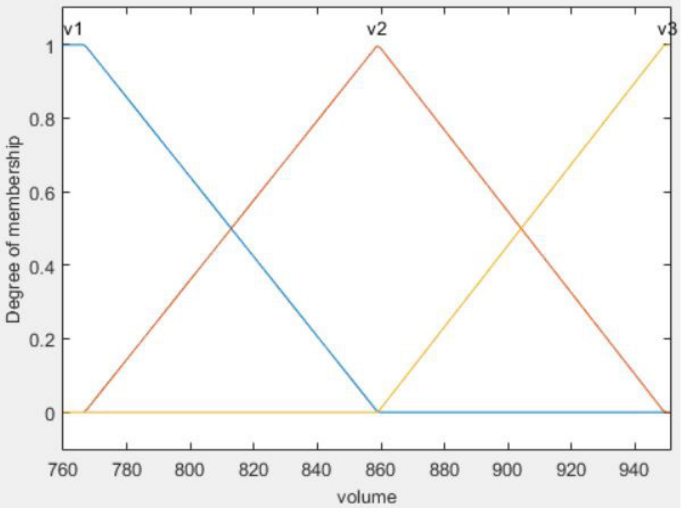


Figure 8. Membership functions for volumes.

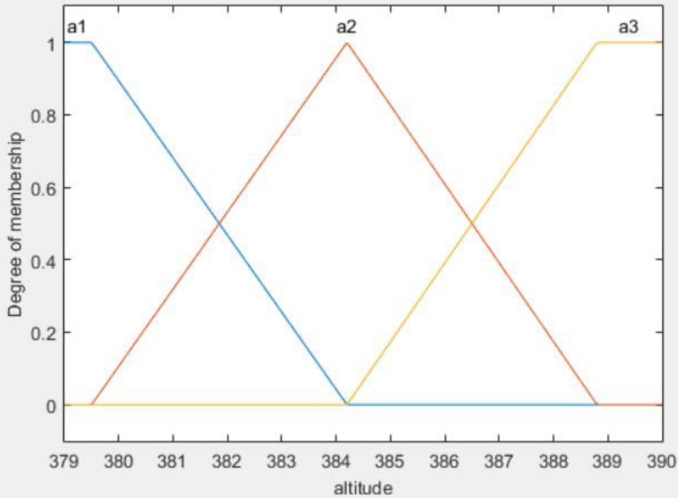


Figure 9. Membership functions for altitudes.

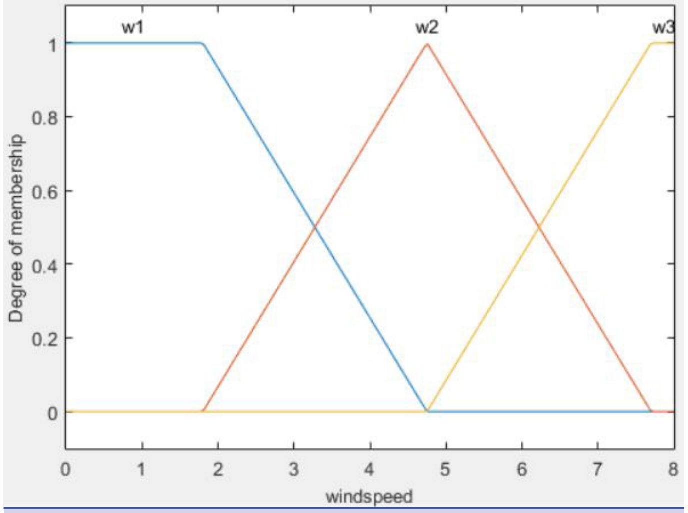


Figure 10. Membership functions for the wind speeds.

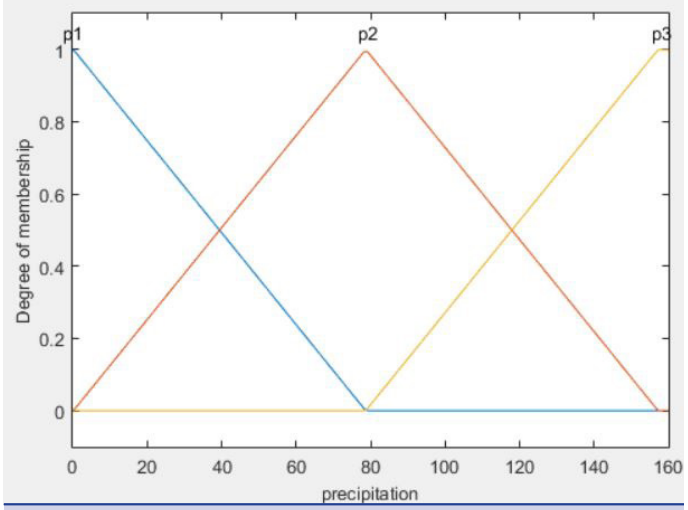


Figure 11. Membership functions for precipitation.

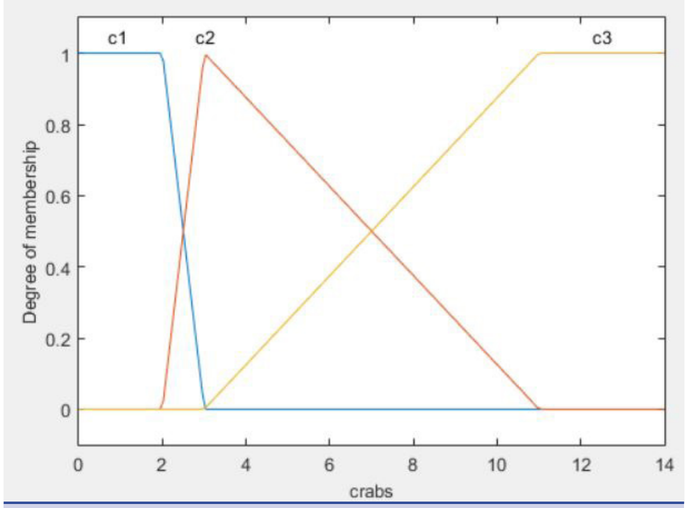


Figure 12. Membership functions for a population of crabs.

for each input variable, we have defined 4×3×3×3×3×3=972 rules. Some of these rules are as follows;

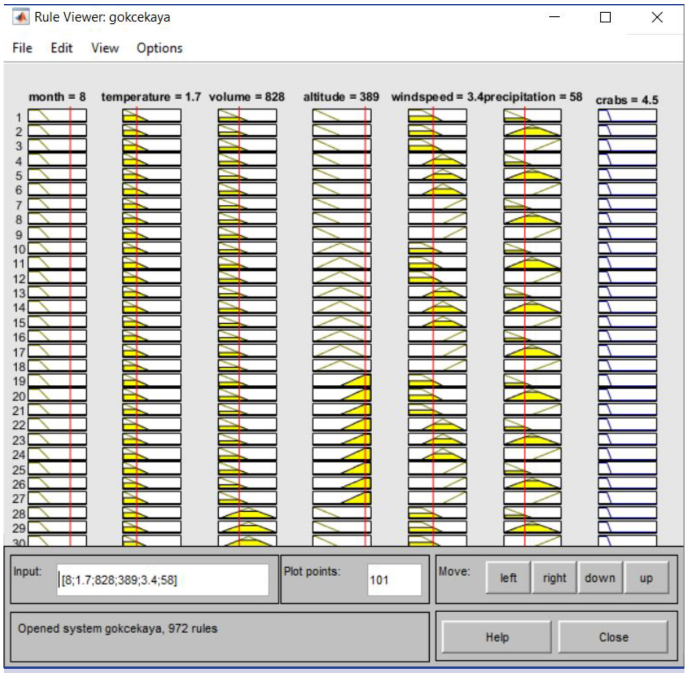
- If month is m1, temperature is t2, volume is v3, altitude is a1, wind speed is w2, and precipitation is p2, then population is c1,
- If the month is m4, temperature is t3, volume is v2, altitude is a3, wind speed is w1, and precipitation is p3, then population is c3,
- If the month is m4, temperature is t1, volume is v1, altitude is a2, wind speed is w3, and precipitation is p1, then the population is c2.

Defuzzification

In the previous step, after we input values to fuzzy numbers under the membership functions, we fuzzy output values under the rules we defined. However, since these values are the values of the fuzzy set, they must be turned into crisp values to be processed. In this sense, in this step where we reversed the fuzzification process, the crisp values obtained by the fuzzification process were made comparable with the classical systems.

RESULTS AND DISCUSSION

In this study, real data have been converted into fuzzy values under the rules created with linguistic values, and then a fuzzy inference system has been built, in which crisp values can be obtained again by fuzzification of these fuzzy values. Our model results are crisp numbers from the cluster [0,14], the population scale of *P. potamios*. Estimation of the number of P Potamios populations is accomplished by outputs from the "if...then" rule base, where input values are entered. For example, the input variables month=8, annual average temperature=1.7, dam volume=828, altitude (dam water height)=389, dam water height=3.4, and annual average precipitation=58 are not observed in the real data. If one enters these input data into our fuzzy inference system as [8,1.7,828,389,3.4,58], it is obtained that the output value is 4.5, which is *the P. potamios* population number (see Figure 13). Accordingly, it can be easily obtained that the number of populations in these input variables should be at least 4 and at most 5.



Input: [8;1.7;828;389;3.4;58]

Plot points: 101

Opened system gokcekaya, 972 rules

Figure 13. Rule-based fuzzy inference system.

Thus, based on the climatic and topographic characteristics of the geographical region where this dam is located, a mathematical model was created to determine the population number of *P. potamios* using a fuzzy logic approach. Thanks to this model, the number of *P. potamios* populations in the Gökçekaya Dam can be estimated by entering the previously unobserved and unmeasured climatic and geographical data into the rule base of our fuzzy logic model. The rule base we have constructed in the Mamdani inference system method has been created by using all of the 132 dam data obtained from the study carried out by Phd. Tuğrul Öntürk between 2011-2021 on Gökçekaya Dam (in Turkey) and benefiting from the experience and opinions of the experts. Regression analysis is used to measure the relationship between two or more quantitative variables. Regression analysis was performed to demonstrate the success and accuracy of the created model. The results obtained in the model were compared with the real data, and it was shown that the results in this model were 91% compatible (see Figure 14).

As a result, this mathematical model, which we created using the Fuzzy Logic Toolbox of the MATLAB platform, can determine the *P. potamios* population number with great accuracy. Therefore, our study may guide future academic studies on *P. potamios* population dynamics in this area.

CONCLUSION

The mathematical model we have created will not only predict the number of *P. potamios* population with great accuracy under future climatic changes but will also enable the effects of the relationships between input parameters on the dynamics of the *P. potamios* population to be interpreted quite simply, similar to the comments below.

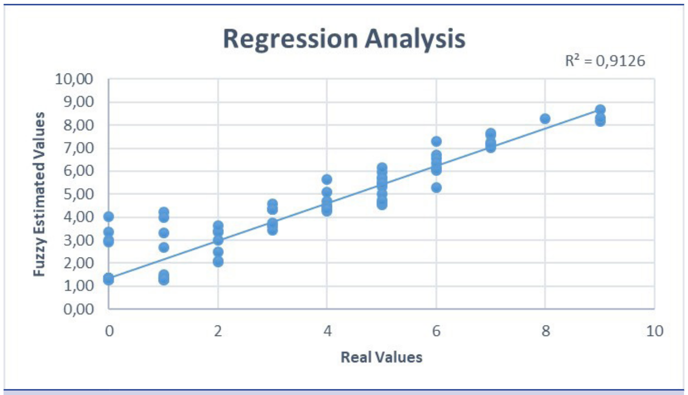


Figure 14. Regression analysis.

During the data analysis, the number of individuals in June was ignored in all years. Because of the climate zone in which the research area is located, the mating and breeding period may start at the end of May. The hatched individuals of the *P. potamios* species are carried and fed by the female in the abdomen for a while until they pass the larval stage. This is seen in many crab species.

Therefore, female individuals carrying eggs and larvae in June generally do not leave their nests for protection. They only feed in a close environment without leaving the nest to meet their nutritional needs. Accordingly, the June were ignored as they showed variability compared to other summer months.

Our findings have shown us that; The height of the air temperature, the height of the water level, the excess amount of precipitation and the wind speed have a direct positive effect on the population density.

For crustaceans such as shrimp and crabs, the water temperature is an important survival-related environmental factor that not only directly influences their metabolism, growth, moulting, and survival but also affects other environmental parameters (e.g., dissolved oxygen).

Therefore, the temperature has become an essential factor that restricts shrimp and crab culture (Ren et al, 2021).

As the air temperature increases, the water temperature also increases. Since this affects the living conditions of *P. potamios* positively, it will start the spawning and mating period and increase the number of individuals. However, depending on the increase in temperature, there will be an increase in the amount of food, as the number of plankton and other monocellular invertebrates in the water body will also increase. More food means more individuals survive (see Figure 15).

The amount of precipitation is important to maintain the water level at a certain height. Depending on the sufficient rainfall, the dam water level will remain at a sufficient level. Thus, hiding in

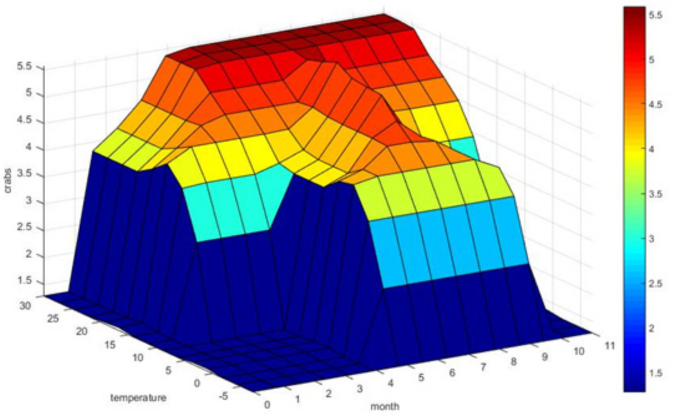


Figure 15. The population of crabs depends on the months and temperature.

the nest, which is also seen in *P. potamios* and many crab species, will continue. As the water level decreases, the nests will remain on the surface and in the dry area, which can cause the death of the individual and the offspring (see Figure 16). Because both the individuals emerging from the larva and the female and male individuals provide of their nutritional needs from the water. What is more important than all these is that the individuals that hatch from the eggs can only survive in water until they become adults. Systems to meet the oxygen needs in the external environment have not been developed (see Figure 16). Therefore, they cannot live in dry and arid areas (Buck et al, 2003).

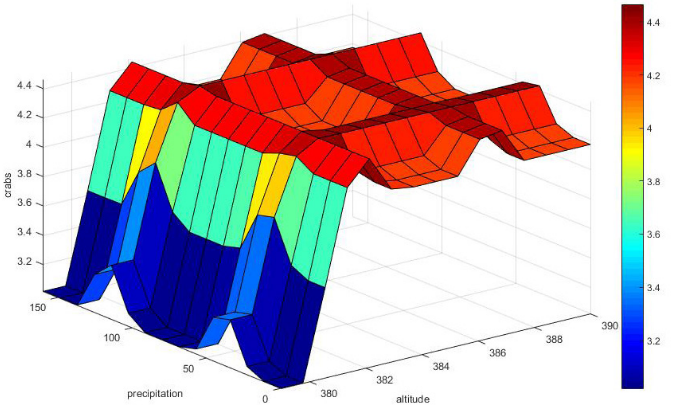


Figure 16. The population of crabs depends on the rainfall and water level.

Crabs feed on fish eggs or newly hatched fish fry. Therefore, with this study, the population density can be determined based on climatic data. Thus, the negative impact on the fish population can be kept in balance and the healthy growth of the fish population can be ensured by taking the necessary precautions in the regions where fishing is the livelihood.

In addition to all environmental variables, we think that the structure of the mountains around the research area, the forested area, and height are effective on temperature and rainfall. In general, due to the height of the Gökçekaya Dam and the southern part of the southern part, it takes snow and rainfall above seasonal norms in winter. This maintains the amount of water in the dam lake. Consequently, the crab population is positively affected. The height map of the Gökçekaya Dam and its surroundings is given in Figure 17.

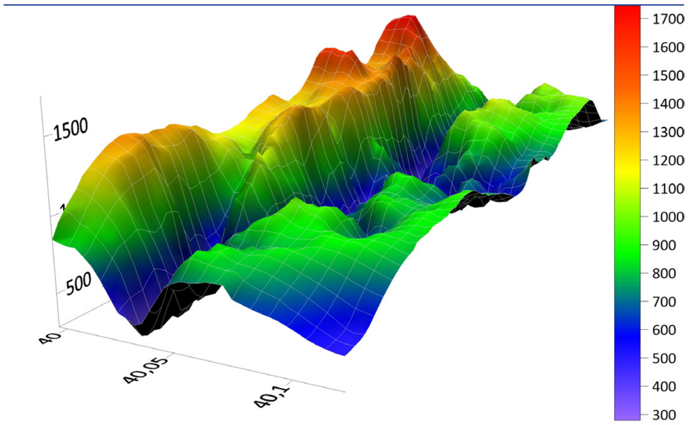


Figure 17. The height map of the Gökçekaya Dam and its surroundings.

The population dynamics of Potamon potamios can be accurately predicted using a mathematical model based on fuzzy logic, and a prediction accuracy of over 90% is achieved under various climatic conditions. Thus, the population dynamics of Potamon potamios can be accurately predicted with the climate data to be obtained in the coming years.

This model not only predicts the P. potamios population with high accuracy under varying climatic conditions but also provides a framework for studying the impact of climate change on similar aquatic species.

Declarations: -

Ethical Approval: Not applicable because this article does not contain any studies with human or animal subjects

Conflict of Interest: The author declare that they have no competing interests.

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Short Communication

First Record of Goldblotch Grouper, *Epinephelus costae* (Steindachner, 1878) (Family: Serranidae) from the Sea of Marmara

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ABSTRACT

Two specimens of *Epinephelus costae* (Steindachner, 1878) have been observed in the rocky littoral zone of the northeastern Sea of Marmara in the early days of October 2024. *E. costae* is recorded for the first time from the Sea of Marmara. The present recording also emphasises that the northward migration of thermophilic Mediterranean species is gradually reaching higher latitudes.

Keywords: *Epinephelus,* coastal, photoidentification, inventory, Türkiye

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INTRODUCTION

The serranid genus *Epinephelus* Bloch, 1793, or commonly known as groupers, is represented by 89 species globally (Parenti & Randall, 2020), of which 10 of them are also occurring in the Mediterranean Sea (Kovačić et al., 2021). One of the Mediterranean species of the genus is *Epinephelus costae* (Steindachner, 1878), of which distribution range also extends from eastern Atlantic from Portugal to Angola including Canary Islands and Cape Verde Islands (Parenti & Randall, 2020; Kovačić et al., 2021). It's maximum standard length can reach to 140 cm and being a protogynous hermaphrodite territorial fish, *E. costae* inhabits mixed bottoms of sea grass meadows and mud between the depths from 10 to 300 m (Heemstra & Randall, 1993; Louisy et al., 2007; Zaidi et al. 2017). It feeds on crustaceans, cephalopods and teleost fishes (Froese & Pauly, 2024). *E. costae* is of some importance to the fisheries of the Mediterranean and west coast of Africa (Heemstra & Randall, 1993) and mainly captured by handliners, longliners, netters and harpooners. It's body colouration can vary depending on the size (Heemstra & Randall, 1993).

Although *E. costae* is an abundant serranid fish along the Turkish Mediterranean coast and rarely occurs in the Aegean Sea (Bilecenoğlu, 2024), and has not been reported neither from the Sea of Marmara nor from the Black Sea (Artüz & Fricke, 2019; Parenti & Randall, 2020). In the present short communication we report on the first record of *E. costae* from the Sea of Marmara.

MATERIAL AND METHODS

The present specimens of *E. costae* have been reported from the northeastern part of the Sea of Marmara, which defined as geographical subarea (GSA) 28 of the Mediterranean (GFCM, 2018; Figure 1). The nature of sampling of the present study, which was performed either by means of opportunistic photographic record or collaboration with recreational handliners, is a typical example of opportunistic study of marine fauna, which is not the direct result of a scientific field survey (Hiddink et al., 2023). Photographs of the present samples were emailed to the authors for species identification, which are now archived by the first author. Species identification of the present specimens follows Heemstra and Randall (1993) and taxonomic nomenclature follows Parenti and Randall (2020).

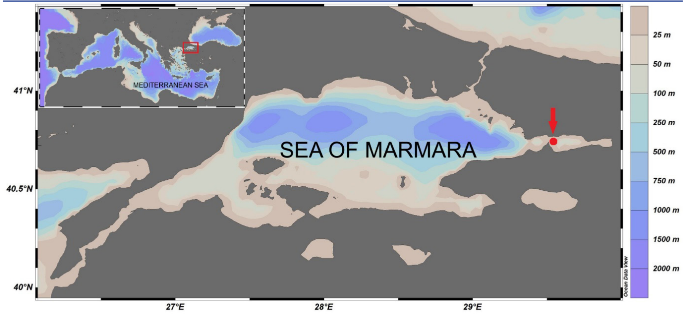

Figure 1. Red rectangle on the map in the small panel depicts the location of the Sea of Marmara (GSA 28) in the Mediterranean basin. Red arrow and solid circle depict the approximate locality of capture of present specimens of *E. costae* in the Sea of Marmara.

RESULTS AND DISCUSSION

On 2 October 2024 a recreational spearfisherman was recorded a short video of specimen 1 of *E. costae* (Figure 2) among rocky substratum densely covered with *Mytilus galloprovincialis* and unidentifed sea urchins, off Dilovası coast at the depths ranging from 10 to 12 m (40°46′1.48″N; 29°33′59.50″E). A few days later specimen 2 (Figure 2) was caught by a recreational handliner almost at the same locality (40.761944°N; 29.516667°E). Based on the descriptive characters (caudal fin truncate to concave; no dark spots or dark bars on body; juveniles with 3 to 5 dark longitudinal stripes on dorsal part of body) given in Heemstra and Randall (1993) both specimens identified as *E. costae.*

In the most recent ichthyological inventory of the Sea of Marmara (Artüz & Fricke, 2019), *E. costae* has not been reported from the region. Therefore, we considered the occurrence of these specimens in the area as the first record of *E. costae* in the Sea of Marmara. According to Artüz and Fricke (2019) the contemporary fish fauna of the Sea of Marmara included 263 species of teleost fishes representing 78 families, and the following 7 species of the family Serranidae have been reported from the region to date: *Anthias anthias* (Linnaeus, 1758), *E. aeneus* (Geoffroy Saint-Hilaire, 1817), *E. fasciatus* (Forsskål, 1775), *E. marginatus* (Lowe, 1834), *Serranus cabrilla* (Linnaeus, 1758), *S. hepatus* (Linnaeus, 1758) and *S. scriba* (Linnaeus, 1758). Thus with the first record of *E. costae* the number of serranids in the Sea of Marmara is apparently increased to 8.

In a previous study on the dispersal of marine teleosts in the Mediterranean Sea, Azzurro et al. (2011) reported that thermophilic fish species have been recorded in the northern Mediterranean with an increasing tendecy, supporting the impact of global warming on their northerly distribution. According to Azzurro et al. (2011) the congeneric *E. marginatus* is one of the Mediterranean fish species of which contributing the northerly distributed thermophilic taxa at a rate of 16.26% in terms of abundance. Based on available data we suggest that northerly dispersal of *E. costae* across the Sea of Marmara may due to global warming, and the occurrence of the congeneric *E. marginatus* in the Black Sea (Yağlıoğlu & Turan, 2021) and several non-native fish species in the Sea of Marmara (Karakulak et al., 2020) support this suggestion.

Last but not least, the sampling methodology of the present study is one of the many examples of surveying regional fish fauna of the Sea of Marmara based on photographic documentation (e.g. Bilecenoğlu, 2019; Kabasakal & Türetken, 2021; Kabasakal et al., 2024). According to Kovačić et al. (2020), in the absence of physical material (captured or stored specimen), photographic documentation of a fish species that allows researchers to positively identify the species is acceptable as confirmed photographic evidence. Therefore, it is also worth mentioning the importance of collaborating with recreational spearfishermen, anglers, handliners, etc. as citizen scientists and encouraging them to take the photo or video footage that may be useful to fill the gaps in local ichthyological inventories.

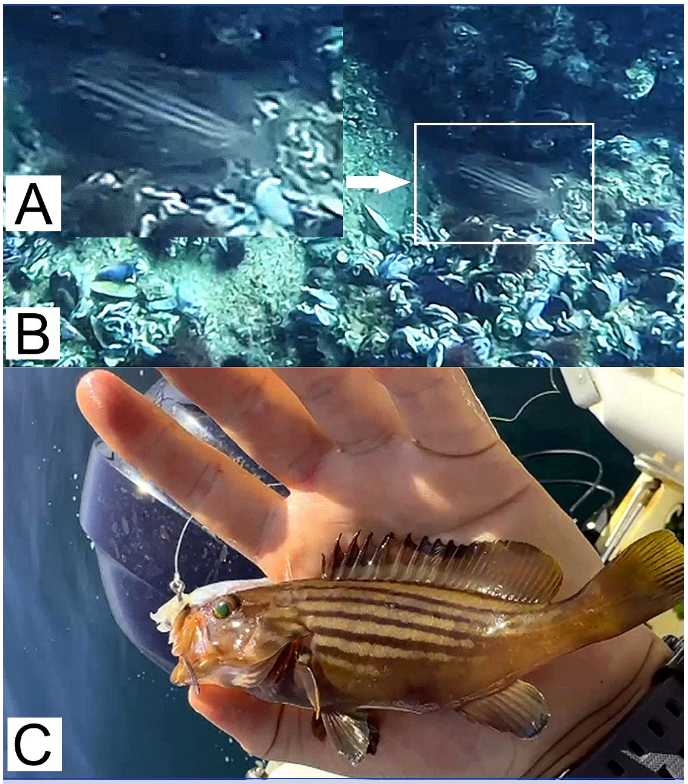

Figure 2. A close up cropped frame of the specimen 1 of *E. costae* (a) captured from the video footage depicting the same specimen in white rectangle, sheltering between the rocks densely covered with Mediterranean mussel, *Mytilus galloprovincialis*, and unidentified sea urchins (b), and specimen 2 (c).

CONCLUSIONS

This study not only records a species for the first time in the Sea of Marmara, but also emphasises that the northward migration of thermophilic Mediterranean species is gradually reaching higher latitudes. It is well known that the environmental conditions in the Sea of Marmara are gradually deteriorating. Serranidae species are mostly benthic, living in the littoral or sublittoral zone, and are particularly solitary as adults (Tortonese, 1986), and it is therefore, the ongoing coastal degradation in the Marmara region is likely to disrupt the territories of *Epinephelus* species.

Acknowledgments: We thank to recreational spearfisherman Mr. Volkan Topçu and the recreational handliner Mr. Savaş Dursun, for sending us the video footages of present specimens of *E. costae*.

Conflict of Interest: The author declares that there is no conflict of interest.

Ethics Committee Approval: Since no experimental animal was used in the submitted study, approval of the ethics committee is not required.

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Short Communication

First Record of Half-smooth Golden Pufferfish *Lagocephalus spadiceus* (Richardson, 1845) in the Black Sea

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ABSTRACT

This study presents the first confirmed record of the half-smooth golden pufferfish (*Lagocephalus spadiceus*) in the Black Sea, marking a significant extension of its range. A male specimen of *L. spadiceus* was captured as bycatch off the coast of Ayancık, Sinop, at a depth of 17 meters using a bonito drift gillnet with a mesh size of 32 mm. The specimen was 15.6 cm in total length and weighed 53.0 g. Aside from the known possible spread pathways of invasive species, this species, which is already known to exist in the Sea of Marmara, is thought to have entered the Black Sea through direct invasion. The warming temperatures of the Black Sea, influenced by global climate change, have created suitable conditions for the spread of non-native species, and the Sea of Marmara is likely to have acted as a biological corridor. The spread of this species into the Black Sea indicates potential challenges ahead for local ecosystems, as its aggressive invasive behaviour and competitive characteristics could disrupt native food webs and pose risks to regional fisheries. This finding underscores the need for continuous monitoring and management efforts to address the potential ecological impacts of invasive species in the Black Sea.

Keywords: Range expansion, new record, Tetraodontidae, invasive species, Black Sea

INTRODUCTION

Pufferfish (family Tetraodontidae) are represented by 28 genera and 206 species inhabiting tropical and subtropical coastal waters around the world (Froese & Pauly, 2024). The half-smooth golden pufferfish (*Lagocephalus spadiceus* Richardson, 1845), native to the Indo-Pacific region, is one of the most abundant non-indigenous pufferfish species in the Eastern Mediterranean (Tuncer, Cihangir, & Bilecenoğlu, 2008). The species inhabited in a range of Mediterranean Sea habitats, often favouring sandy or soft substrates at depths of 3 to 200 meters but primarily inhabiting shallow coastal waters less than 50 meters deep (Matsuura et al., 2014; Tuncer et al., 2008*). L. spadiceus* is a generalist predator, feeding on a variety of marine organisms, including fish, molluscs, and crustaceans (Xu et al., 2024). The population of pufferfish, which has been established in the Mediterranean Sea for many years, is steadily increasing (Aydın, 2011). Pufferfish pose a risk to human health due to their toxic content (Aydın, Erkan, & Dal, 2017).

This species is one of the earliest lessepsian migrants enter the Mediterranean Sea through the Suez Canal (Kosswig, 1950). It is more common in the Mediterranean Sea (Başusta, Başusta, & Özer, 2013). Its aggressive expansion has resulted in a geographical range extension, reaching the northernmost part of the Mediterranean basin, including the Sea of Marmara, in addition to its known distribution along the Levant and Aegean coasts (Tuncer et al., 2008). This document reports the first confirmed record of *L. spadiceus* in the Black Sea, signalling a concerning geographical expansion of the species beyond its natural habitat.

MATERIALS AND METHODS

A specimen of *L. spadiceus* was caught as bycatch during commercial fishing operations on September 18, 2024, off the coast of Türkiye in the Black Sea (Ayancık, Sinop; 41.94694° N, 34.66190° E) (Figure 1A). The sampling location falls within Geographic Sub-Area (GSA) 29, as defined by the General Fisheries Commission for the Mediterranean (GFCM). The specimen was captured using a bonito drift gillnet with a mesh size of 32 mm at a depth of 17 meters. The taxonomic identification of the specimen was conducted using a fish taxonomic key (Fischer, Schneider, & Bauchot, 1987). Identification was further verified based on FishBase (Froese & Pauly, 2024). Fourteen traditional variables were measured on separately sample with an electronic caliper to the nearest mm (Farrag, Soliman, Akel, Elhaweet, & Moustafa, 2015; Habib, Neogi, Oh, Lee, & Kim, 2019). Body weight was recorded using an electronic scale with a precision of 0.01 g. The colour and shape of the gonads were used as primary indicators for sex determination (Rajendiran et al., 2021).

RESULTS AND DISCUSSION

A *L. spadiceus* specimen recorded in this study measured 15.6 cm in total length and weighed 53.0 g (Figure 1B). Fourteen traditional morphological measures are presented in Table 1. Macroscopic examination of the gonads revealed that the specimen was male, as indicated by the flat, white-creamy, soft texture of the gonadal tissue.

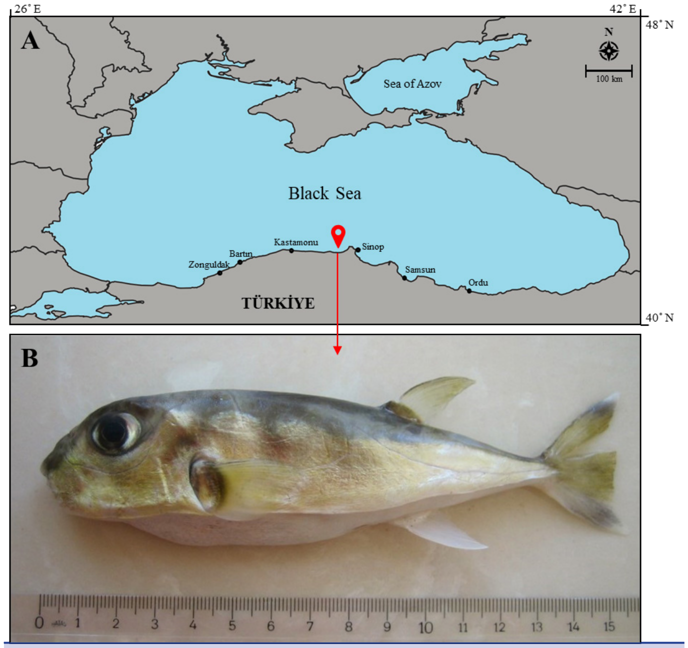

Figure 1. Sampling point (A) and first specimen of *Lagocephalus spadiceus* (B) caught in the Black Sea.

Table 1. Description and measurements of morphological characters of *Lagocephalus spadiceus* sampled in the Black Sea

Abbreviations	Character	Description	Measurement (cm)
TL	Total length	The longest distance between the tip of the snout and the end of the tail	15.6
SL	Standard length	Distance from the tip of the nose to the middle part of the end of the vertebral column	13.5
HL	Head length	Distance from the tip of snout to the upper posterior to gill cover	3.87
ED	Eye diameter	The maximum diameter of the eye with parallel to the longitudinal axis of the body	1.31
PsOL	Post-orbital length	Distance from the end of the orbit to the posterior margin of the operculum	1.35
PrDL	Pre-dorsal length	Distance from tip of snout to anterior margin of the dorsal fin base	9.01
DFL	Dorsal fin length	Longest distance from tip to tip of dorsal fin	2.03
DFBL	Dorsal fin base length	The longest horizontal distance of the dorsal fin base	1.26
PrAL	Pre-anal length	Distance from tip of snout to anterior margin of the anal fin base	9.09
AFL	Anal fin length	Longest distance from tip to tip of anal fin	2.02
AFBL	Anal fin base length	The longest horizontal distance of the anal fin base	1.36
PFH	Pectoral fin height	Distance from the lower end to the upper end of the pectoral fin	2.99
HBD	Highest body depth	The deepest distance from the lower end to the upper end of the body	3.20
CpD	Caudal peduncle depth	The minimum depth of the caudal peduncle	0.69

L. spadiceus is characterized by an aggressive invasion capacity, which has facilitated its spread beyond its native range. As one of the earliest lessepsian fish to enter the Mediterranean through the Suez Canal (Kosswig, 1950), this species has shown a remarkable ability to adapt to diverse environmental conditions, making it a highly resilient invader (Vidthayanon & Premcharoen, 2002). This adaptability allows *L. spadiceus* to thrive in various habitats, from sandy substrates in shallow waters to deeper, enhancing its potential to establish in new ecosystems.

The warming of the Black Sea, often described as the "Mediterraneanization" of the region (Baltaci & Turk, 2024; Oğuz & Öztürk, 2011), has significant implications for the distribution and habitat suitability for non-native species like *L. spadiceus*. This process reflects an ongoing transformation where the Black Sea ecosystem increasingly resembles the warmer, saltier, and more biodiverse Mediterranean environment. Over the last decade, the Black Sea's surface temperatures have risen by an anomalous 1.8°C, with projections suggesting a continued increase of 13.3% in sea temperatures over the next 25 years (Baltaci & Turk, 2024). The ongoing Mediterraneanization thus allows *L. spadiceus* and other non-native species to form established populations in the Black Sea (Aydın, Karadurmuş, Verep, & Gözler, 2024; Bilecenoğlu, Yokeş, & Aydın, 2023; Öztürk, Karadurmuş, & Aydın, 2022; Öztürk, 2021; Uzer, Karakulak, & Kabasakal, 2024), contributing to shifts in the region's biodiversity and ecosystem dynamics. This warming trend, attributed to climate change, not only enables *L. spadiceus* to tolerate northern waters but also promotes its active dispersal through natural migration routes, such as the Istanbul Strait from the Sea of Marmara. The Sea of Marmara functions as a crucial biological corridor and climatic transition zone, enabling the spread of Mediterranean-origin invasive species, including *L. spadiceus*, into the Black Sea (Öztürk, 2021). This transitional zone enables species to gradually adjust to changing salinity, temperature, and oxygen levels as they spread northward, facilitating their geographical range expansion.

The presence of *L. spadiceus* in the Black Sea, though currently undocumented as a fully established population, poses a significant risk of colonization due to the species' remarkable resilience. *L. spadiceus* has shown the ability to traverse high-salinity barriers, such as the Bitter Lakes, and adapt to the lower salinity of the Nile estuary (Bianchi & Morri, 2003). Its tolerance to the cooler winter temperatures of the Mediterranean Sea further suggests that it may acclimate well to the Black Sea's unique conditions, highlighting its potential for rapid colonization and expansion in this new environment. The specimen's size in the Black Sea also indicates maturity, as *L. spadiceus* typically reaches reproductive maturity at around 9 cm and is capable of breeding twice annually (Naik & Jalihal, 1988). This suggests that, if additional individuals of the opposite sex are present, reproduction could begin soon, initiating a population that may be self-sustaining. If *L. spadiceus* becomes established, its generalist feeding strategy which includes a diverse diet of crustaceans, fish, molluscs, algae, and ophiuroids (Xu et al., 2024) could significantly impact the Black Sea's food webs. As an aggressive predator, *L. spadiceus* may disrupt local ecosystems by competing with native species for prey and altering the abundance and distribution of key species within the benthic and pelagic zones. This competition could potentially harm local fisheries, affecting commercially important species and altering ecosystem balance. These concerns underscore the importance of monitoring *L. spadiceus* spread and considering management strategies to mitigate its potential impact on the Black Sea ecosystem.

CONCLUSION

The first confirmed record of *L. spadiceus* in the Black Sea highlights an expanding distribution facilitated by warming seas and the Mediterraneanization of Black Sea waters. As a resilient and highly adaptable lessepsian migrant, *L. spadiceus* has leveraged the Sea of Marmara as a biological corridor, enabling its spread into northern waters via the Istanbul Strait. The species' presence poses potential ecological and economic challenges, including disruption to native food webs and competition with local species. Continued monitoring and targeted management interventions are recommended to mitigate its impact and protect the Black Sea's ecological balance.

Conflict of Interest: The authors declare that they have no actual, potential, or perceived conflict of interests for this article.

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Short Communication

Efficacy of Hydroxychloroquine Sulphate for Treating Disease Caused by *Cryptocaryon irritans* Brown, 1951 in Marine Ornamental Fish

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ABSTRACT

Cryptocaryon irritans is a ciliate protozoan parasite of wild and cultivated marine fish and causes the clinical signs of white spot disease. It results in significant losses for aquarists and commercial fishermen worldwide and infects various marine teleosts. This study reports on the efficacy of the administration of hydroxychloroquine sulphate as a treatment for white spot disease caused by *C. irritans*. Nine marine ornamental fish of different species showed behavioural changes such as flashing and had white spots on their skin and fins. Four fish with higher numbers of white spots were subjected to skin scraping to confirm the diagnosis under a microscope, through which the presence of *C. irritans* was confirmed. The aquarium containing these fish species was treated with 20 mg L^{-1} of hydroxychloroquine sulphate for fifteen days. One day after the addition of hydroxychloroquine sulphate to the tank, positive behavioural changes were observed among the fish. On the third day, none of the nine fish had signs of white spots visible, and no parasites were found on the skin scraping sample. In the study, it was concluded that hydroxychloroquine improved the clinical signs and prognosis of the disease caused by *C. irritans* in the fish species examined.

Keywords: Marine ornamental fish, Aquatic health, Parasite, Ciliated protozoan, Hydroxychloroquine

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INTRODUCTION

The protozoan parasite *Cryptocaryon irritans* (Brown, 1951) belongs to the class Prostomatea. This ciliated organism infects marine fish, both in wild populations and aquaculture settings, and is responsible for a disease commonly referred to as white spot disease or cryptocaryoniasis. This disease leads to significant economic losses for aquarists and commercial fisheries worldwide and infects various marine teleost species (Chi et al., 2017). Through the infestation of epithelial tissues, it produces numerous tiny white spots (Li et al., 2022). The invasion of the epithelial tissues in the skin, gills, and fins can interfere with the host's osmotic balance and respiratory functions. Additionally, damage to these tissues may pave the way for secondary infections by bacteria, viruses, or fungi, which can cause significant mortality in various species of farmed marine fish (Gao et al., 2022; Colorni & Burgess, 1997).

White spots or nodules can be challenging to detect on fish with light-coloured scales, making it difficult to confirm the absence of *Cryptocaryon* spp. infection through visual inspection alone. This highlights the necessity of diagnostic tests for accurate identification. When fish are infected with *Cryptocaryon* spp., mortality rates can escalate quickly within a few days. The intensity of the outbreak is determined by several factors, including the salt water temperature, the strain's virulence, and the host previously exposed to the ciliated parasite (Yanong, 2009).

The parasite *C. irritans* completes its life cycle within 8 to 10 days at temperatures ranging from 24 to 25 °C. However, has also been observed to complete its cycle in as little as 7 days. The duration of the life cycle can significantly fluctuate, from a few days to several months, influenced by factors such as specific host species and water temperature (Yanong, 2009).

The *C. irritans* life cycle has four morphologically and physiologically different stages: parasitic trophont, protomont, reproductive tomont and infective theront (in the environment) (**Figure 1**). Trophonts are commonly found in marine fish. They appear as white spots present in the host's gills, fins, skin, and eyes. In this stage, the parasite penetrates the epithelial layer, forming a cavity that shields it from the host's immune defences while granting it access to body fluids, tissue particles, and whole cells, which serve as its food source. Microscopic can range in size from about 27 μm to 452 μm and have spherical to pear-shaped, ciliated and rolling in the slide. Mature trophonts leave the fish, becoming protomonts. After a few hours, protomonts reduce their movements, slowing down and encysting in the tissue, and then transform into tomonts, which are the reproductive stage. Tomonts undergo successive asymmetric binary fissions, dividing into numerous tomites that develop into pear-shaped theronts. The exact mechanism triggering the release of trophonts from the host remains unclear, but evidence suggests that the photoperiod plays a significant role, as trophonts are typically released into the water just before dawn. After fully developing, theronts are released from the tomonts, leaving the cyst through a small opening, thus beginning the infective free-swimming stage. Theronts actively search for a new fish host, swimming quickly until reaching their destination. They have a lifespan of approximately 24 h or more, but their infectivity quickly decreases after they leave their original host (Li et al., 2022; Yanong, 2009).

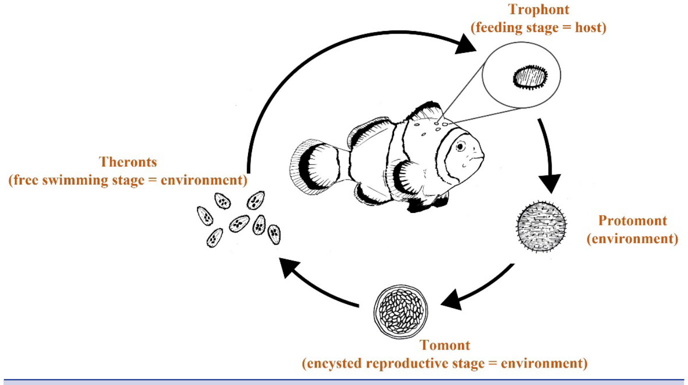

Figure 1. Life cycle of *Cryptocaryon irritans* (Yanong, 2009).

If not treated early, white spot disease can yield a mortality rate of 100% in a few days. Currently, the treatments available within veterinary practice are chloroquine and cooper prolonged immersion in the theronts phase. The use of copper sulphate ($CuSO_4$) at a therapeutic concentration of 0.2 mg L^{-1} for two to three weeks was found to help in removing *Cryptocaryon sp.* infections in marine hosts but presented high toxicity to fish (Noga, 2010). Hydroxychloroquine sulphate is another possible treatment, but its use is not very common in Brazil. This compound disrupts the processes of endocytosis and haemoglobin breakdown in vulnerable parasites; it also increases the pH of acidic cytoplasmic vesicles and, therefore, inhibits the activity of lysosomal enzymes and other organelles (Schlesinger & Krogstad, 1987; Al-Bari, 2017).

This study reports the results and efficacy of the administration of hydroxychloroquine sulphate as a treatment for the disease characterised by white spots and caused by *C. irritans* in nine marine ornamental fish that belonged to an aquarist in São Paulo, Brazil.

MATERIALS AND METHODS

Case history

In this case, nine marine ornamental fish of different species [*Acanthurus achilles* Shaw, 1803 (n=1), *Acanthurus nigricans* Linnaeus, 1758 (n=1), *Amphiprion ocellaris* Cuvier, 1830 (n=2), *Chelmon rostratus* Linnaeus, 1758 (n=1), *Pygoplites diacanthus* Boddaert, 1772 (n=1), *Siganus vulpinus* Schlegel & Müller, 1845 (n=1), *Zanclus cornutus* Linnaeus, 1758 (n=1), *Zebrasoma desjardinii* Bennett, 1836 (n=1)] showed behavioural changes such as flashing and had white spots on their skin and fins.

At the beginning of the present case, none of these ornamental fish had shown any clinical signs of disease. The translocation of *C. rostratus* and its introduction into the new habitat probably generated stress and changed the dynamics in the already established environment, causing imbalance and favouring the emergence of diseases. Thus, three days after the introduction of an apparently healthy specimen of *C. rostratus* to the existing population of the aquarium, all the fish began showing behavioural changes. In addition to behavioural changes, the species *A. achilles, C. rostratus*, *P. diacanthus*, *Z. desjardinii* (**Figure 2**) presented white spots on their bodies, and these fish were chosen to verify the diagnosis by microscopically identifying the presence of the parasite. The other fish *Acanthurus nigricans*, *Amphiprion ocellaris*, *Siganus vulpinus*, *Zanclus cornutus*, *and Zebrasoma desjardinii* did not show visible signs of white spot disease, like the previous ones, but because they were in the same environment they were considered to have the same disease.

Treatment practice

The fish were housed in a 0.3 m³ reef aquarium with a recirculation system and were fed three times daily with a commercial feed (Aloe large granules, Dr. Bassler Biofish-food®, Belgium) provided *ad libitum*. The water condition metrics of the aquarium were monitored weekly and maintained as follows: salinity at 33 g/L, temperature at 26 °C, alkalinity at 9 dKH, dissolved oxygen at 5 mg/L , pH at 8.3, total ammonia levels below 0.25 mg/L, nitrite at 0.25 mg/L, and nitrate at 10 mg/L. The aquarium was treated once with 20 mg L^{-1} of hydroxychloroquine sulphate (Reuquinol® Aspen) for 15 days (Dhayanithi et al., 2022). No water changes were made during the treatment: only the substrate was cleaned using a gravel vacuum twice a day, and an external filter was used. The temperature was controlled through a thermostat. Right at the end of the treatment, 100 g of Hw® activated charcoal was placed in the filter to remove residual hydroxychloroquine sulphate from the water.

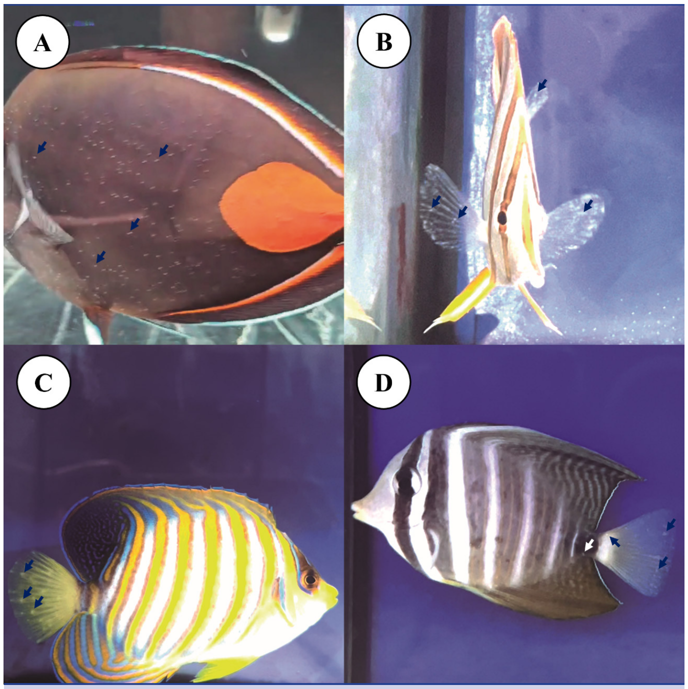

Figure 2. Clinical signs of white spots in fish: A. achilles (A), *C. rostratus* (B), *P. diacanthus* (C), and *Z. desjardinii* (D).

RESULTS AND DISCUSSION

After skin scraping, trophonts were observed in the microscopic had range in size from about 150 to 250 μm and have spherical to pear-shaped, ciliated and rolling in the slide which confirmed the presence of *C. irritans* (**Figure 3**) according to Yanong, (2009). One day (24 hours) after the addition of hydroxychloroquine sulphate to the tank, positive behavioural changes could be seen among the fish; also, another skin scraping was done, and the trophonts present in the sample showed less and slower movement than before. On the second day (48 hours) the trophonts did not move and had small vacuoles in the cell. On the third day (72 hours), no one of the nine fish expressed any manifestation of white spot visible in clinical inspection, in addition no parasites were found on a skin scraping sample. Nonetheless, the administration of the medication was continued until the end of the 15-day treatment cycle, without water change. Thus, the application of hydroxychloroquine for the treatment of *C. irritans* resulted in a significant reduction in the clinical signs of infection within 72 h, with improved fish behaviour and complete elimination of parasites observed under microscopic analysis. No apparent adverse effects were detected in the treated fish during the observation period, and mortality was reduced to zero. The 20 mg L^{-1} concentration demonstrated superior efficacy compared with alternative treatments reported in the literature, highlighting its potential as a safe and effective antiparasitic for ornamental marine fish.

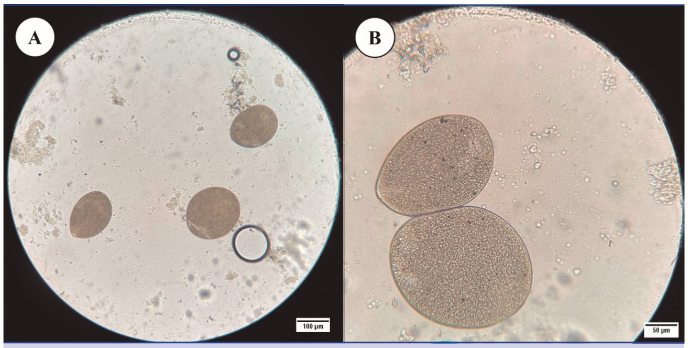

Figure 3. Cryptocaryon irritans on wet-mount preparations under a light microscope at magnifications of 10 x (A) and 20 x (B).

The research conducted by Van and Nhinh (2018) demonstrated a significant difference in the frequency of *C. irritans* in distinct fish species. Certain species, such as *Chaetodon adiergastos* Seale, 1910; *Paracanthurus hepatus* Linnaeus, 1766; *Amphiprion frenatus* Brevoort, 1856; *Diodon holocanthus* Linnaeus, 1758; *Platax teira* Forsskål, 1775; *Pterois volitans* Linnaeus, 1758; *Plectorhinchus vittatus* Linnaeus, 1758; *Siganus guttatus* Bloch, 1787; and *Pygoplites diacanthus* Boddaert, 1772, show high susceptibility to the parasite, with infection rates reaching up to 70%. In contrast, other species like *Rhinecanthus aculeatus* Linnaeus, 1758, *Zanclus cornutus* Linnaeus, 1758, *and Zebrasoma veliferum* Bloch, 1795 exhibited much lower susceptibility, with infection rates under 50%. These insights are valuable for aquarists managing ornamental fish in controlled environments and addressing outbreaks of this parasite. Although the previous study evaluated the susceptibility of several species, we cannot state in this study that *A. achilles, C. rostratus, P. diacanthus, and Z. desjardinii*, which had white spots early, are less or more susceptible than the other inhabitants of the aquarium (*A. nigricans, A. ocellaris, S. vulpinus, Z. cornutus, Z. desjardinii*). We chose to perform skin scrapings only on the animals with the most evident clinical signs so as not to stress the other fish and to begin treatment as soon as possible to ensure that all of them survived this lethal parasite.

Prompt diagnosis of *C. irritans* infection in aquarium fish is crucial, as the disease can lead to significant mortality in a brief timeframe (Colorni & Burgess, 1997). Light-coloured fish are particularly vulnerable and may not survive without early detection. The challenge in identifying this infection often arises from the difficulty of observing white spots, which can go unnoticed even by experienced professionals who lack specific training in recognising these signs (Cardoso et al., 2019).

Hydroxychloroquine acts on *Plasmodium* spp (the protozoan that causes malaria) by accumulating in the acidic food vacuole, where it raises the pH and interferes with the digestion of haemoglobin, which is essential for the parasite. In addition, it inhibits the polymerisation of toxic haem into hemozoin, leading to the intracellular toxicity of the protozoan. This mechanism compromises the survival of the protozoan and the continuity of the infection. In addition, there are numerous other immunomodulatory effects of hydroxychloroquine that are still unclear (Ben-Zvi et al., 2012). There are no studies of this information in relation to the protozoan *C. irritans*, but taking into account the various positive effects against other comorbidities already described (Abdel-Aziz et al., 2022; Ben-Zvi et al., 2012) and the observation of the evolution of the disease in the present case study, it is suggested that hydroxychloroquine sulphate is a possible drug for the healing of this disease in ornamental marine fish. However, more advanced and robust studies should be conducted in the laboratory to confirm the clinical findings.

Picón-Camacho et al., (2011) conducted in vitro tests for the control of *C. irritans* theronts and found that hydroxychloroquine at 20 mg L^{-1} eliminated over 90% of theronts within 1.5 h, while doses of 50 mg L^{-1} and 80 mg L^{-1} eliminated more than 93% and 96%, respectively. Leethochavalit (2011) found that at a dose of 20 mg L^{-1} of hydroxychloroquine, 100% of the theronts were not moving after 30 min of treatment. For chloroquine at a dose of 10 mg L^{-1} , after 60 min, more than 96% of the theronts were not moving.

The use of a drug based on chloroquine to treat parasites of the phylum Ciliophora was found to be effective at doses of 10 and 20 mg kg^{-1} orally and presented low toxicity for the freshwater fish *Cyprinus carpio* Linnaeus, 1758 , *Ictalurus punctatus* Rafinesque, 1818 and *Ctenopharyngodon idella* Valenciennes, 1844. Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) was treated with a prolonged immersion bath at 10 mg L^{-1} for 24 h and the results indicated moderate efficacy, suggesting that longer exposure time or dose adjustments may be necessary for optimal results (Yevtushenko, 2019). The therapeutic concentration of chloroquine was used in the form of prolonged immersion for treating protozoan parasites in aquarium fish at doses of 10 to 20 mg L^{-1} for up to 21 days and is nontoxic to fish but highly toxic to micro-and macroalgae and to various invertebrates (Noga, 2010). A study using hydroxychloroquine phosphate at a dose of 10 mg L^{-1} demonstrated that this was effective for controlling the parasite *Amyloodinium ocellatum* Brown, 1931 in orange clownfish (*Amphiprion percula* Lacepède, 1802) and was also effective for increasing the survival of the affected fish when compared to traditional treatments, such as malachite green and formalin, which can be toxic at therapeutic doses. A single dose of 10 mg L^{-1} of hydroxychloroquine was applied for a period of 15 days, and no significant side effects or evident toxicity were observed in the treated fish during the study. The authors assert that the relative safety of hydroxychloroquine in this dose may be a viable therapeutic option for the management of parasites in ornamental fish (Dhayanithi et al., 2022).

The present study corroborated previous investigations, and the use of hydroxychloroquine sulphate at a dose of 20 mg L^{-1} for the treatment of *C. irritans* in nine species of ornamental fish demonstrated highly positive results. No deaths were recorded during the 15-day treatment period, and the parasites were completely eliminated within 72 h after the start of therapy. In addition, no clinical signs or side effects were observed in the treated fish throughout the study. These findings suggest that hydroxychloroquine, at the dose used, is a safe and effective option for the management of *C. irritans* in multispecies ornamental systems, providing improvement in fish behaviour and the absence of relapses until the end of the observation period.

Hydroxychloroquine degradation in aquariums occurs mainly due to microbial action in the biofilms in the pipes, where microorganisms use the drug as a source of carbon and nitrogen. This process can reduce the therapeutic concentration of the drug, compromising antiparasitic treatment and favouring the selection of resistant parasites. In addition, degradation increases operational costs due to the need for redosing and can impact water quality and animal health. Strategies such as biofilm management, water flow adjustments, and the development of more stable drugs are essential to mitigate these problems and ensure effective treatments (Hu et al., 2022). The researchers' previous findings led us to believe that a dose of 20 mg L^{-1} lasting more than 15 days is a good alternative to prevent the complete degradation of hydroxychloroquine.

Unfortunately, there is no test on the aquarium market that measures the concentration of hydroxychloroquine sulphate in aquarium water, as we have for copper sulphate, for example. However, considering that hydroxychloroquine sulphate is a promising drug for treating *C. irritans* and other possible parasitic protozoa, a test that measures the concentration gradient in the water to check whether the drug remains stable over the days of treatment would be very useful for professionals who treat these animals. Although chloroquine and hydroxychloroquine are widely used worldwide by aquarists, scientific publications are scarce; therefore, our findings are extremely valuable and contribute to increasing scientific and technical dissemination. Larcombe et al., (2024) highlighted that the ornamental fish trade represents a multi-billion dollar global industry, carrying significant responsibility for ensuring the welfare of countless fish species regularly traded across international borders. Disease-related mortality and morbidity pose serious threats to fish welfare and result in considerable economic losses for the industry, highlighting critical gaps in scientific knowledge that demand immediate attention.

As soon as there is any suspicion of a *C. irritans* infection, it is essential for aquarists to consult a qualified professional. This ensures accurate identification of the disease and timely implementation of the appropriate treatment. *C. irritans* is known to cause mortality rates of up to 100% in infected fish populations if left untreated, posing significant economic and ecological challenges to aquarists and the ornamental fish industry (Colorni, 1987; Yanong, 2009; Dickerson, 2006; Fridman, 2022).

Therefore, the prompt diagnosis and timely application of therapeutic strategies involving hydroxychloroquine-based treatments can enhance both the clinical outcomes and the overall prognosis of infections caused by *C. irritans*. The application of this therapeutic approach should be carefully tailored and monitored according to the specific fish species being treated.

CONCLUSION

In this context, it was concluded that the administration of hydroxychloroquine sulphate contributed to an improvement in clinical signs and the overall prognosis of the disease caused by *C. irritans* in the species studied *A. achilles*, *A. nigricans*, *A. ocellaris*, *C. rostratus*, *P. diacanthus* , *S. vulpinus*, *Z. cornutus* and *Z. desjardinii* in a home aquarium. Further studies are necessary to evaluate whether other species of marine ornamental fish might exhibit sensitivity to hydroxychloroquine sulphate at the dose used in this study. Additionally, future research should explore the broader application of this therapeutic approach on a larger scale while assessing its potential ecotoxicological impacts across diverse environmental contexts. Such investigations will provide critical insights into the safety and efficacy of hydroxychloroquine in managing parasitic infections in ornamental aquaculture.

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Conflict of Interest: The author states that there are no conflicts of interest to disclose.

Ethics Committee Approval: As no experimental animals were involved in this study, approval from an ethics committee was not necessary.

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Review

Building a Collaborative Aquaculture Research Ecosystem with APIs and AI

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ABSTRACT

Recently, the mission of the aquaculture production sector in achieving sustainable development goals has become increasingly critical. Synthesizing large data sets with advanced technological tools in aquaculture is no longer a luxury but a necessity for significant progress. This article examines the pivotal role of Application Programming Interface (API) integration in advancing open science and collaborative research in aquaculture. It also explores the use of Artificial Intelligence (AI) to facilitate data analysis across disparate databases and proposes the establishment of a ChatGPT-like virtual environment to catalyze seamless global collaboration among researchers. A comprehensive overview is presented on the feasibility of a unified AI-driven database that collects, analyzes, and shares data, overcomes geographical constraints, and supports a shared information ecosystem. The article scrutinizes current implementations, identifies gaps in existing infrastructures, and outlines a robust framework for API integration that could significantly enhance innovation and operational efficiency in aquaculture research.

Keywords: Artificial intelligence, Aquaculture, Application Programing Interface, Internet of things

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INTRODUCTION

Every year, population growth, rising income levels, and increasing awareness of quality nutrition contribute to a significant rise in the demand for global food production. Aquaculture plays a critical role in meeting this demand. Consequently, the aquaculture industry has experienced remarkable growth in recent years. This growth is evident in the rapid increase in production, making it the fastest-growing sector in the global food industry, with an average annual growth rate of 6.9 percent over the past three decades (Ottinger et al., 2021). Furthermore, the industry's average annual growth rate from 1970 to 2005 was around 8.9%, significantly outpacing the growth rate of capture fisheries, which stood at only 1.2% (Abdul Kari et al., 2020). This growth has positioned aquaculture as the fastest-growing food-producing industry globally, driven by the increasing demand for food fish consumption (Pridgeon & Klesius, 2012). The expansion of the industry is also reflected in the development of aquaculture areas, with significant increases observed in regions such as China and Türkiye (Zhou et al., 2022; Erol, 2022; Wang et al., 2022). Additionally, the growth of the aquaculture industry has led to new trends, such as the increased production of hybrids in the aquaculture sector of some species, like grouper, due to improved growth and disease resistance compared to parent species (Nankervis et al., 2021).

However, this rapid development has not been without challenges. The accelerated growth of finfish aquaculture has resulted in developments detrimental to the environment and human health (Cabello, 2006). The industry's expansion has led to an increase in waste generation, raising concerns about environmental sustainability (Zhou et al., 2021).

Challenges such as preventing an increase in aquaculture production costs, the obligation to use resources rationally, waste management, and minimizing losses (even production with zero discharge) still maintain their significance. These challenges highlight the need for the industry to innovate toward low environmental impact systems to ensure sustainable growth (Gephart et al., 2020).

In the burgeoning field of aquaculture, technological innovation is not just a tool but a necessity to address the challenges of sustainability and efficiency. Aquaculture, a cornerstone of global food security, faces the dual challenge of meeting rising demand while maintaining ecological and economic sustainability. The industry's growth is intrinsically linked to its ability to innovate and adapt. In this pursuit, the seamless integration of Application Programming Interfaces (APIs) and Artificial Intelligence (AI) stands as a transformative force, offering new avenues for research and operational efficiency. The current landscape of aquaculture is characterized by fragmented data and knowledge systems. This fragmentation hinders the synthesis of multifaceted data ranging from environmental impacts to supply chain logistics, essential for informed decision-making and sustainable practices. There is a critical need for a paradigm shift towards open science—an ethos that promotes the accessibility, sharing, and collaborative use of research data and methodologies, fostering a culture of transparency and cooperative progress.

Artificial Intelligence in Aquaculture; Artificial Intelligence (AI) is transforming aquaculture by optimizing breeding programs, feed formulation, and disease treatment protocols. AI's ability to analyze large datasets allows for predictive analytics, which can improve operational efficiency and sustainability.

Internet of Things (IoT) in Aquaculture; The Internet of Things (IoT) connects various devices and sensors in aquaculture systems, enabling real-time monitoring of water quality, temperature, and other critical parameters. This connectivity enhances data collection and management, leading to better decision-making.

API Integration in Aquaculture; Application Programming Interfaces (APIs) facilitate seamless data exchange between different systems in aquaculture. By integrating APIs, researchers and practitioners can access and share data more efficiently, breaking down data silos and promoting collaborative research.

The integration of APIs and AI in this context is more than a mere technological enhancement; it is a strategic imperative. APIs facilitate the flow of information, breaking down the barriers of data silos, while AI offers advanced tools for interpreting complex datasets, driving insights that could revolutionize fish farming practices from breeding to health management and waste reduction. The integration of Application Programing Interfaces (APIs) and Artificial Intelligence (AI) offers a transformative approach to aquaculture research, fostering enhanced data sharing and collaborative efforts across global scientific communities.

The integration of artificial intelligence (AI) into the aquaculture sector has significant promise for revolutionizing efficiency, sustainability, and global food security. Leveraging AI technologies, such as machine learning and deep learning, in conjunction with modern information technologies, including the Internet of Things (IoT), big data, and cloud computing, has enabled aquaculture to optimize resource utilization and enhance long-term sustainability (Kaur et al., 2023). Furthermore, the application of AI in aquaculture systems has the potential to address critical water quality control challenges, thereby improving the overall efficiency of prawn harvesting from freshwater ponds (Kaur et al., 2023). Additionally, the utilization of AI-based systems for regulating key water quality factors, such as salinity, dissolved oxygen, pH, and temperature, demonstrates the transformative potential of AI in ensuring optimal aquaculture conditions (Kaur et al., 2023). Moreover, the incorporation of AI services using computer vision and deep learning recognition models in cloud-based autonomous drones has facilitated scalable and advanced aquaculture surveillance, further enhancing operational efficiency and sustainability (Ubina et al., 2021). These advancements underscore the pivotal role of AI in driving innovation and progress within the aquaculture sector, offering solutions to challenges and paving the way for sustainable and secure global food production.

Integrating AI technology can revolutionize aquaculture research by automating data collection and analysis processes. For example, AI systems can regulate key water quality factors such as salinity, dissolved oxygen, pH, and temperature, ensuring optimal conditions. This automation translates to significant time and cost savings, as it enables continuous and real-time monitoring, reducing the need for manual intervention. Furthermore, AI is transforming the development of intelligent fish farming systems by integrating with the Internet of Things (IoT) and big data technology. This integration facilitates the monitoring of aquaculture environments, leading to improved sustainability and productivity. Additionally, AI-driven advancements in aquaculture include the use of computer vision and deep learning models in cloud-based autonomous drones for scalable and advanced aquaculture surveillance, enhancing operational efficiency and sustainability. These examples illustrate how AI technology is reshaping aquaculture, offering efficient and cost-effective solutions that contribute to the industry's sustainable development.

Aquaculture's current state is marked by data silos and segmented research efforts, impeding the flow of information and hindering innovation. Collaborative efforts are often hampered by incompatible data formats and the lack of a centralized system for data management. This scenario underscores the need for a paradigm shift toward open science—an approach that advocates for the accessibility and sharing of research data and methodologies to foster collaboration and transparency.

Interoperability, that is, the ability of different information systems, devices, and applications to access, exchange, integrate, and cooperatively use data in a coordinated manner within and across organizational boundaries, is fundamental to actualizing open science in aquaculture. It promises to streamline research processes, reduce redundancies, and lead to more robust and rapid scientific discoveries. Aquaculture, with its diverse data sources ranging from satellite imagery to genomics, stands to gain enormous value from a framework that facilitates open science and interoperability. This section sets the stage for a discussion of how API and AI integration can catalyze this transformation.

This chapter lays the foundation for a discussion on how the fusion of API and AI technologies can catalyze a significant transformation within aquaculture, shifting the industry toward data-driven sustainable practices that can reshape not only the sector but also its relationship with the global ecosystem.

AI as a Data Analysis Catalyst

Artificial Intelligence (AI) in aquaculture is a game-changer for managing and interpreting the vast amounts of data generated by modern scientific methods. AI, through machine learning algorithms and data analytics, can identify patterns and insights that are beyond the scope of traditional data analysis methods.

AI methodologies suitable for aquaculture research range from neural networks for image recognition—useful for monitoring fish health and biomass—to machine learning models that can predict optimal feeding schedules. These AI-driven tools enhance the accuracy and efficiency of data analysis, leading to better informed aquaculture management decisions.

It also provides an overview of smart aquaculture systems, with a focus on machine learning and computer vision applications, demonstrating the potential of AI in interpreting complex aquaculture data and, in addition, AI-based data to minimize production costs and maximize fish production. The role of artificial intelligence innovation technology is very important in creating an intelligent cage culture management system containing modules (Chang,et al.,2021; Kaur,et al., 2023). Collectively, these references support the view that artificial intelligence, through machine learning algorithms and data analytics, will indeed be a game changer in managing and interpreting the large amounts of data generated by modern scientific methods in aquaculture research. The evidence presented in these studies underscores the transformative potential of AI in revolutionizing data analysis and decision-making in the aquaculture industry. The use of AI for predictive analytics allows real-time monitoring and forecasting of environmental conditions, disease outbreaks, and production outcomes. This foresight enables aquaculture practitioners to proactively manage risks and improve operational efficiency, ultimately leading to more sustainable practices.

ChatGPT-like Virtual Environment for Global Cooperation

To prepare a ChatGPT-like virtual environment for global collaboration in aquaculture, several important steps and considerations must be considered. Understanding the global potential of seafood farming is important for identifying regions with high potential for cooperation and collaboration. Gentry et al. (2017) highlighted the unknown global capacity for increased aquaculture production in the ocean and the relative productivity potential between countries. This study is important for mapping the global potential of marine aquaculture. Understanding the role of aquaculture in improving food security on a global scale is crucial for shaping collaborative efforts in aquaculture. Troell et al. (2014) highlighted the impact of aquaculture on global food security and resilience. Understanding the global potential of seafood farming is essential for identifying regions with high potential for cooperation and collaboration. Discussing the role of aquaculture in sustainable development and its potential to reduce poverty and improve food security on a global scale, Subasinghe et al. (2009) provided valuable insights into the broader implications of aquaculture for global collaborative efforts. Ottinger et al. (2018) stated that based on the significant contribution of aquaculture to global food security and protein intake, understanding the global importance of aquaculture is important for developing international cooperation in this field. Providing information on the Dual-Mode Underwater Smart Sensor Object for Precision Aquaculture Based on AIoT Technology, Chang et al. (2022) discussed the use of AIoT technology for precision aquaculture, highlighting the potential for integrating advanced technologies into aquaculture practices. Leveraging artificial intelligence and Internet of Things (IoT) technologies can improve global collaboration by facilitating data-driven decision making and information sharing. Aquaculture in China Presenting the current status, challenges, and outlook of industrial aquaculture, Li et al. (2011) provide insights into the current status of the aquaculture industry in China and its global implications. Understanding aquaculture experiences and challenges in different regions, such as China, can shed light on best practices and collaboration strategies on a global scale.

Referring to the Visual Aquaculture System Using Cloud-Based Autonomous Drones, Ubina et al. (2021) stated that combining computer vision and artificial intelligence services using cloud-based autonomous drones can improve aquaculture surveillance and monitoring. Applying advanced technologies to data collection and analysis can support global collaboration by providing real-time insights and information sharing.

Envisioning a ChatGPT-like virtual environment for the aquaculture research community requires a platform that uses natural language processing to understand and respond to researcher questions. This AI-driven environment will facilitate the exchange of ideas, data, and methodologies and collaborative efforts worldwide. The key features of such a platform include intuitive user interfaces, scalable infrastructure for processing large datasets, and customizable tools to suit various research needs. Real-time translation capabilities will further enhance the collaboration of researchers from different linguistic backgrounds. Implementing a virtual environment brings challenges such as ensuring data security, ensuring fair access, and preserving system integrity. Overcoming these obstacles requires careful planning, sound technical design, and ongoing community engagement to ensure the system meets the diverse needs of the aquaculture research community.

Building a Unified AI-Driven Database

Creating a unified AI-driven database for aquaculture research is an ambitious yet feasible goal. Such a database should integrate various forms of data, from genomic sequences to satellite imagery. The framework for this integration should be modular and flexible, allowing for the incorporation of new data types and sources as they become available.

Ensuring rigorous data entry and validation standards is essential for maintaining data quality. AI can play a significant role in this by using machine learning algorithms to detect and correct anomalies. Security and privacy are critical concerns, especially when handling proprietary or sensitive information. Therefore, encryption, access controls, and regular security audits should be integral components of the framework.

The potential of such a database is vast. By providing researchers with access to a rich, well-curated repository of data, it could accelerate discovery and foster collaboration on a scale previously unseen in aquaculture research.

In creating a unified AI-driven database for global collaboration in the field of aquaculture production, it is essential to consider the following steps and insights gained from the information provided. To enable autonomous data generation, a unified data representation and transformation method based on Adversarial generative network (GAN) models was designed (Zheng et al. 2023). In addition, a data integration system was utilized to provide users with access to distributed heterogeneous databases through a unified interface for information sharing (Lu et al., 2015).

To achieve explainable, robust, and general AI, human knowledge is integrated with data-driven machine learning (Zhuang et al., 2017), which involves combining data-driven models with structured models of logic rules and moving from task-oriented intelligence to artificial general intelligence in the general context.

Semantic Data Modelling follows the principles of Linked Data and uses the Resource Description Framework (RDF) data model to create a unified data model for description labels and visual features (Tran et al., 2022). Although the Resource Description Framework is a World Wide Web Consortium (W3C) specification designed as a metadata model, it is a general method used to model information in various syntax styles. This approach provides a standardized and interoperable representation of data for global collaboration.

A bidirectional architecture that integrates knowledge and data to create a framework for AI can be developed to promote independent learning and update knowledge and AI models (Deng et al., 2022)

By emphasizing the prevalence of data-driven artificial intelligence in autonomous and hybrid systems, such AI can be used for clinical decision support (Montani and Striani, 2019). This approach can be adopted to support decision-making in aquaculture production.

The Berlin Indexing and Harvesting Toolkit (B-HIT) can be used to collect web services and create a unified index database (Holetschek et al., 2019). This tool can facilitate the collection and organization of various data sources for collaboration with aquaculture applications.

Large amounts of data must be collected to create big data databases and use data mining to generate information (Li et al., 2023). This process is critical for extracting valuable insights and patterns from the combined database. By combining these steps and insights, a unified AI-driven database for aquaculture can be created to support global collaboration, knowledge sharing, and data-driven decision-making in aquaculture.

A unified database focused on artificial intelligence for global cooperation in the field of aquaculture can lead to several significant benefits. By integrating various technologies such as the Internet of Things (IoT), big data, cloud computing, and artificial intelligence, the database can facilitate sustainable aquaculture development (Ubina & Cheng, 2022). This integration can enable the real-time monitoring of aquaculture sites through computer vision, thus enhancing production and reducing labor while being environmentally friendly (Ubina et al., 2021; Vo et al., 2021). Furthermore, the use of artificial intelligence technologies, such as intelligent feeding techniques based on predicting shrimp growth, can improve water quality prediction and early warning systems, making aquaculture practices more accurate and efficient (Chen et al., 2022). Additionally, the application of modern technologies, including sensors, robots, drones, and artificial intelligence, can contribute to the development of aquaculture (Mustafa et al., 2021).

Global cooperation in the field of aquaculture, facilitated by a unified database, can address challenges such as the need for large amounts of labelled data to train artificial intelligence systems, which has been a bottleneck in further aquaculture applications (Yang et al., 2020). Moreover, the database can support the establishment of an aquaculture industry with world-class competitiveness, thereby contributing to sustainable development and seafood security (Chang et al., 2021). It can also help reduce the negative effects of aquaculture wastewater on global sustainability by identifying ways to apply modern technologies to existing aquaculture production methods (Han et al., 2019; Mustafa et al., 2021).

The benefits of a unified database focused on AI for global aquaculture cooperation align with the broader context of the application of AI in various fields. The framework for global cooperation on artificial intelligence and its governance aims to ensure that humanity can enjoy the benefits of artificial intelligence while minimizing its risks (Ala-Pietilä & Smuha, 2021). Furthermore, the use of AI technologies in aquaculture is part of the broader trend of applying modern technologies to improve production methods and sustainability in various sectors, including agriculture and fishery (Song, 2020; Subasinghe et al., 2009).

Concrete examples of how artificial intelligence-supported analysis can be used in aquaculture research are provided. "By deeply analyzing information from large data sets, AI can provide valuable insights into topics such as water quality, fish health, and feeding strategies. Feed efficiency plays a crucial role in aquaculture practices, particularly in industrial and offshore applications. Concepts such as the quantity, timing, and frequency of feed given to fish, as well as the decision to feed based on changing environmental conditions, are of vital importance in farm management. Achieving this management with minimal errors or, ideally, without any errors relies heavily on comprehensive observations and measurements. Instantaneous feeding of fish and subsequent adjustment of feed based on live weight gain are also essential. Typically, these values need to be continuously updated by engineers based on specific formulas and assumptions. Successful aquaculture processes depend on ensuring optimal fish feeding. Otherwise, fish may experience inadequate growth or overfeeding. One of the most effective ways to optimize this process is to manage operations by considering numerous assumptions with the assistance of artificial intelligence.

The integration of AI into the aquaculture sector represents a transformative leap that will propel scientific research and applications in this field to unprecedented levels. By harnessing AI, aquaculture can benefit from enhanced precision, efficiency, and sustainability. AI technologies enable the automation of critical processes such as data collection, analysis, and decision-making, leading to faster and more accurate results. This integration has the potential to revolutionize various aspects of aquaculture, including feed management, water quality control, disease detection, and growth monitoring. Moreover, the utilization of AI in aquaculture research extends to the development of intelligent fish farming systems by integrating IoT and big data technology to monitor aquaculture environments, leading to improved sustainability and productivity. The seamless integration of AI into aquaculture operations can significantly contribute to global food security by optimizing resource utilization, minimizing environmental impact, and enhancing the overall efficiency of food production. As the aquaculture industry continues to embrace AI-driven innovations, the future holds promise for sustainable and secure global food production, ensuring a steady supply of high-quality seafood to meet the demands of a growing population.

In conclusion, the creation of a unified database focused on artificial intelligence for global cooperation in the field of aquaculture can lead to numerous benefits, including enhanced monitoring, improved production efficiency, and sustainable development. This aligns with the broader trend of applying modern technologies, including artificial intelligence, to address challenges and improve practices in various industries.

Current Implementations and Gaps

An analysis of existing platforms reveals a landscape in which several initiatives have made strides toward unified data management; however, significant gaps remain. Current systems often suffer from issues like limited interoperability, inconsistent data standards, and inadequate user interfaces. These challenges lead to inefficiencies and barriers to entry that can discourage collaboration and slow innovation.

To bridge these gaps, it is crucial to identify and adopt best practices from successful implementations. This involves not only technological aspects such as the use of cloud-based storage and sophisticated data analytics tools but also the fostering of a culture that values data sharing and open science. Future developments must focus on creating more user-centric platforms that address the needs of researchers while maintaining high standards of data integrity and security.

To comprehend the current implementations and gaps in ChatGPT-like virtual environments for global collaboration, it is essential to consider the existing literature on virtual environments, global cooperation, and the use of virtual teams for collaboration. Virtual environments have been increasingly utilized for global cooperation, with studies emphasizing the potential to create global networks of employees and facilitate communication across international boundaries (Mueller et al., 2010). Additionally, the use of virtual teams has become prevalent due to the development of communication tools and the need to address the effectiveness of virtual teams (Shwartz-Asher & Ahituv, 2019). Furthermore, the potential for virtual team communication and cooperation in a virtual environment of computer technology has been highlighted (Kukytė, 2021).

In the context of global cooperation, it is crucial to consider the implications of virtual interactions and the potential for stimulating discussions that foster greater understanding and effective interaction in real-world collaborations (Lewis et al., 2010). Moreover, the promotion of cooperation among countries on global issues, such as climate change mitigation, has been observed in the context of initiatives like the Belt and Road Initiative (BRI) (Sultan et al., 2022). These references collectively underscore the growing significance of virtual environments and virtual teams in facilitating global cooperation and addressing transboundary concerns.

To understand the current practices and gaps in Collaborative Virtual Environments (CVEs) for global collaboration, it is crucial to consider the existing literature on virtual environments, global collaboration, and the use of virtual teams for collaboration. CVEs play a significant role in supporting collaborative work across distributed teams by providing a common virtual space for real-time interaction and manipulation of virtual artifacts (Cunha et al., 2008). These environments are particularly relevant for global virtual teams working across geographical and cultural boundaries (Larsson, 2003). In the context of open science data platforms in different countries, the success and areas that require improvement can be analyzed by looking at platforms like the Global Nutrition and Health Atlas (GNHA) and the European Open Science Cloud (EOSC). The GNHA serves as an interactive tool for sharing information, fostering collaborations, and driving innovation (Zhou et al., 2022). On the other hand, data platform providers often struggle to aggregate data to meet user needs and establish high-intensity data exchange in collaborative environments (Laufs et al., 2022). This highlights the need for improved data aggregation and exchange mechanisms in open science data platforms to enhance collaboration and knowledge sharing. Moreover, the emergence of platforms like the EOSC, provides researchers and professionals with a virtual environment in which to store, share, and reuse large volumes of information generated by the big data revolution (Kaivo-oja & Stenvall, 2022). Such platforms offer new interaction styles and sharing approaches that can lead to novel results and regulations, including data and analysis platforms, scientific social networks, and new forms of collaboration (Kondoro et al., 2017). In conclusion, leveraging Collaborative Virtual Environments and open science data platforms can significantly enhance global collaboration by providing shared virtual spaces for real-time interaction and facilitating data sharing and knowledge exchange. However, there is a need to address challenges related to data aggregation, user needs alignment, and efficient data exchange mechanisms to further improve collaboration among global teams in virtual environments.

However, as virtual environments continue to evolve, emerging challenges and gaps must be addressed. For instance, the shift toward real-time voice and photorealistic digital personas in virtual systems like ChatGPT raises concerns about the potential threats posed by conversational AI as a vector for targeted influence (Rosenberg, 2023). Additionally, the effectiveness of virtual teams and the antecedents of virtual team effectiveness remain areas that require further empirical research to bridge existing gaps (Shwartz-Asher & Ahituv, 2019). Furthermore, the need to disclose and address issues such as the formation of clusters in virtual reality, the development of network relationships, and cooperation in the global digital space has been highlighted as areas requiring further exploration (Kraus et al., 2021).

The current literature reflects the increasing utilization of virtual environments and virtual teams for global cooperation while also highlighting emerging challenges and gaps that must be addressed. As technologies like ChatGPT continue to advance, it is essential to consider the potential implications for global cooperation and address the evolving needs and challenges of virtual collaboration.

Framework for Robust API Integrations

To galvanize the aquaculture research community toward unprecedented levels of collaboration, a robust framework for API integration is indispensable. Such a framework must prioritize scalability to ensure that APIs can handle increasing loads and complexity as the community grows. Efficiency must be engineered into the system to minimize latency and maximize response times, while user-friendliness is paramount to encourage widespread adoption by researchers with varying levels of technical expertise.

From a technical perspective, the proposed framework should adopt a microservices architecture that allows independent scaling and iterative development. Containerization technologies like Docker and Kubernetes are pivotal for deployment and management. Organizational considerations include the establishment of cross-functional teams comprising developers and end users to ensure that the APIs are aligned with researcher needs.

Policy implications are vast and critical. There must be a concerted effort toward standardization to ensure that APIs can communicate effectively across diverse systems. Initiatives such as the OpenAPI Specification can provide a foundation to create consistent and standard API descriptions, which are vital for interoperability.

In addition, security protocols must be embedded into the API design to safeguard sensitive data. OAuth (Open Authorization) and other authorization frameworks can ensure that data access is controlled and that user permissions are managed appropriately. It is also essential to consider the implications of data sovereignty and the regulations that govern data transfer across borders, which may impact the architecture of a global research platform.

To promote widespread adoption, policies should encourage open standards and provide clear guidelines for API documentation and versioning. This ensures that as APIs evolve, researchers can adapt without losing access to vital data and functionality. The integration framework should also foster an environment that supports the development and sharing of API-related tools and best practices.

Implementing such a framework requires a balance between innovation and regulation, necessitating collaboration between technologists, researchers, and policymakers. By building consensus on standards, the aquaculture research community can create a resilient API ecosystem that promotes the science and practice of aquaculture.

To develop a solid framework for API integrations in the aquaculture industry, it is important to consider the following aspects and insights: First; It is necessary to ensure compliance with state-of-the-art API policies. Best practices that facilitate scalability and robustness in this regard can be found in Rodríguez et al. (2016). Subsequently, the aquaculture industry can achieve increased environmental, economic, and social acceptability and contribute to sustainability by adopting integrated polytrophic practices (Chopin et al., 2001) . In support of this issue, the integration of monitoring methods combined with the Internet of Things (IoT) and Internet+ frameworks has been proposed as robust tools for tactical decision-based management in marine farming and aquaculture (Zhou et al. al., 2019) . The framework must include tools and practices for responsible AI engineering to ensure robustness, scalability, and ethical considerations in API development (Soklaski et al., 2022). Extending the framework to integrate nature-based solutions and marine spatial planning could identify opportunities to increase food production and reduce environmental damage in the aquaculture sector (Hughes, 2021). The framework should emphasize interoperability and the use of standards-based smart city data platforms to ensure seamless integration of various aquaculture data sources (Jeong et al., 2020). The framework should address occupational safety and health standards, including best practices and capabilities to ensure worker safety in the aquaculture industry (Fry et al., 2019). An ecosystem-based framework can provide a structured methodology for assessing the impacts of aquaculture and integrating best management practices (Cranford et al., 2012). The framework should include APIs to process environmental images and integrate time series data with modeling systems to realize comprehensive data analysis and decision making (Mac Coombea et al.,2017). A multi-layered system robustness testing strategy based on abnormal parameters and fault injection can ensure the robustness of the API framework (Xiang et al., 2013). By integrating these considerations into a comprehensive framework, a robust and scalable API integration system can be established for the aquaculture industry to support sustainability, environmental responsibility, and operational efficiency.

To optimize an API gateway using Docker and Kubernetes, organizations can leverage microservices and container technologies to enhance data flow in the API architecture. By breaking down the monolithic structure into smaller, independent services, greater flexibility, scalability, and efficiency in managing API requests and responses can be achieved. The following studies are presented as examples of work done on this topic.

a. Containerization with Docker: Docker can be used to containerize microservices within the API gateway. Docker containers encapsulate each service and its dependencies, ensuring consistency across different environments (Li et al, 2021). - Docker containers allow developers to package applications and their dependencies into standardized units, providing a lightweight and portable solution for deploying microservices (Malić et al., 2019).

b. Microservice architecture: Implementing a microservice architecture involves designing each service as an independent, self-contained unit responsible for a specific business capability (Li et al., 2021). - Microservices promote loose coupling, allowing for independent development, deployment, and scaling of services, which aligns well with the principles of containerization (Dragoni et al., 2017).

c. Orchestration with Kubernetes: Deploying Docker containers containing microservices on a Kubernetes cluster allows for efficient orchestration and management of services (Saboor et al., 2022). Kubernetes automates the deployment, scaling, and management of containerized applications, ensuring high availability and optimal resource utilization (Chandrasekaran et al., 2022).

d. API gateway optimization: Kubernetes can be used to scale the API gateway horizontally by adding more instances of the gateway to handle increased traffic and improve performance (Muzumdar, 2024). - Kubernetes provides features like service discovery and load balancing, which are essential for optimizing the API gateway and ensuring seamless communication between microservices (Muzumdar, 2024).

e. (CI/CD): Implementing CI/CD pipelines automates the testing, building, and deployment of microservices within the API gateway (Donca et al., 2022). - CI/CD practices help maintain the quality of services, accelerate the release cycle, and ensure smooth integration of new features into the API architecture (Donca et al., 2022). By combining Docker for containerization, Kubernetes for orchestration, and a microservice architecture, organizations can streamline data flow in the API architecture. These technologies enable efficient service management, enhanced scalability, and improved overall performance of the API gateway, leading to a more robust and agile system for handling API requests and responses.

Future of Aquaculture Research with API and AI Integration

Long-term Impacts of Technological Integration

The integration of APIs and AI into aquaculture research is expected to have profound and far-reaching consequences. Enabling seamless data sharing and sophisticated analysis will accelerate the pace of discovery, leading to more rapid advancements in sustainable aquaculture practices. In the long term, AI can automate and optimize breeding programs, feed formulation, and disease treatment protocols, enhancing yields and improving the overall health of aquaculture stocks (Arcelay et al.,2021; Jogdand, 2024). Moreover, the predictive power of AI could lead to better anticipation of environmental impacts and market demands, ensuring the resilience of aquaculture systems against the uncertainties of climate change and economic fluctuations.

Furthermore, the long-term effects of technological integration in aquaculture research are expected to extend to workforce development and skill requirements. AI and API technologies will become integral to aquaculture operations, and the demand for a competent workforce with expertise in AI-driven aquaculture management is likely to increase. This necessitates a strategic approach to identify and address future skills needs in the aquaculture sector, aligning with the evolving technological landscape (Arcelay et al., 2021). The ethical implications of AI and API integration in aquaculture research are also paramount. The responsible and ethical use of AI technologies, coupled with robust API integrations, will be critical in ensuring patient-centered care, environmental sustainability, and worker safety within the aquaculture industry (Ayling & Chapman, 2021; Watterson et al., 2020). Additionally, the future of aquaculture research with AI and API integration is expected to benefit from academia-industry collaboration, as highlighted in the context of software engineering education. The instrumental role of AI in shaping the trajectory of aquaculture research and industry practices underscores the potential for synergistic partnerships between academia and industry to drive innovation and knowledge creation (Wang, 2023).

To provide an example to better understand the Future of Aquaculture Research with API and Artificial Intelligence Integration, the use of real-time monitoring and diagnosis systems using computer vision on artificial intelligence-supported disease detection systems in aquaculture can be examined. Malik et al. (2017) proposed an automated fish identification model based on image processing techniques. Their study specifically addressed Epizootic Ulcerative Syndrome (EUS), a disease caused by a fungal pathogen that is often mistaken for ulcers by observers. The researchers employed a series of image processing methods, starting with histogram equalization for image segmentation and then Canny edge detection. They utilized two feature descriptor techniques, namely Histogram of Oriented Gradients (HOG) and features from an acceleration segment test (FAST), to extract pertinent image features. Classification tasks were then conducted using two types of neural networks alongside the K-Nearest Neighbors (K-NN) algorithm (Mia et al.2022). The steps taken to automatically recognize fish disease are shown in Figure 1.

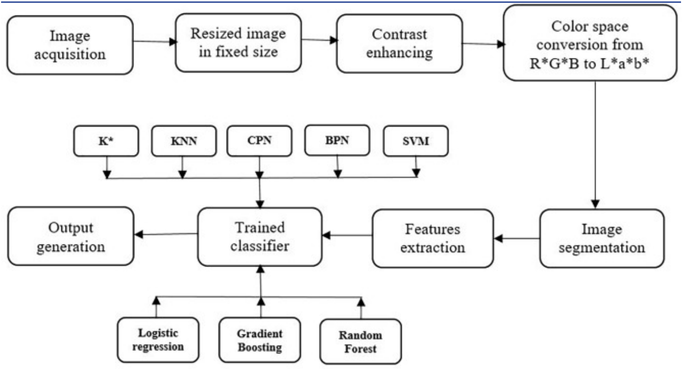

Figure 1. The working process of an automatic fish disease recognition system (Mia et al.2022).

Challenges and Solutions

The widespread adoption of APIs and AI is not without challenges. A major concern is data privacy and security. As aquaculture databases become increasingly interconnected, sensitive information could be at risk of breach. Ensuring robust cybersecurity measures and establishing clear data governance policies are essential. In addition, there may be resistance to adopting new technologies due to the cost of implementation and the need for technical expertise. To address these challenges, aquaculture communities should focus on developing user-friendly platforms and providing comprehensive training programs. It is also important to advocate for scalable solutions that can be adapted to various operational scales, from small-scale farms to large commercial enterprises.

Another challenge is the potential for data homogenization, which might overlook localized or context-specific knowledge that is crucial for certain aquaculture systems. To address this need, AI systems should be designed to be adaptable and learn from diverse data sources, including indigenous and local knowledge systems, to ensure that the insights generated are nuanced and applicable across different contexts.

To address the challenges and solutions related to the integration of APIs and AI into aquaculture, it is essential to consider the multifaceted nature of aquaculture and the potential benefits of integrating AI technologies. The challenges of AI integration with big data have been discussed in various studies (Dwivedi et al., 2021). Among Aquaculture 4.0 technologies, AI is receiving increasing attention, indicating its potential relevance in addressing aquaculture challenges (Mustafa et al., 2021).

In the aquaculture context, the challenges and solutions related to AI integration can be further understood by considering the broader applications of AI in various domains. For instance, in radiology, aspects such as system integration, vendor compatibility, and the availability of vendor-neutral solutions are crucial considerations when implementing AI (Adams et al., 2020). In addition, data quality is a critical factor that influences the implementation of AI applications (Lu et al., 2022). The potential benefits of integrating AI into existing workflows, such as enhanced automation and the utilization of feedback for further improvement, have been highlighted in the context of radiology (Dikici et al., 2020). Moreover, the challenges faced by developers when recommending APIs include mashup-oriented APIs, time-consuming processes, and limited usage of code (Nawaz et al., 2022).

In summary, the integration of APIs and AIs into aquaculture presents both challenges and opportunities. Leveraging AI technologies, such as IMTA and Aquaculture 4.0, can contribute to more sustainable and efficient aquaculture practices. However, challenges related to data quality, system integration, and developer considerations must be addressed to realize the full potential of AI integration in aquaculture.

Cultivating an International Collaborative Community

Fostering a global community centered on open science and collaboration requires concerted efforts from multiple stakeholders. International consortia could be established to set common goals, share best practices, and coordinate research efforts. Such consortia would also play a key role in standardizing APIs and AI applications in aquaculture to ensure compatibility and interoperability across different systems.

Encouraging open access to research findings and datasets is another strategic way to enhance collaboration. This could be facilitated through incentives for researchers who publish in open-access journals or contribute to shared databases. Additionally, global conferences and symposia dedicated to API and AI in aquaculture provide platforms for knowledge exchange and networking.

The integration of artificial intelligence (AI) and application programing interfaces (APIs) in aquaculture has the potential to foster the development of an internationally cooperative community. Technological advancements in AI and API integration offer opportunities for collaboration, knowledge sharing, and sustainable development within the aquaculture industry. As the aquaculture sector continues to embrace AI and API integration, considering the multifaceted implications and potential long-term effects of this technological convergence is essential.

The challenges and solutions in aquaculture research with AI and API integration are multifaceted and encompass various aspects, including data management, ethical considerations, security, workforce development, environmental sustainability, and technological limitations. Addressing these challenges requires advanced data management tools, ethical frameworks, cybersecurity measures, training initiatives, sustainable practices, and technological advancements.

The potential for international cooperation in aquaculture research through AI and API integration is evident in the growing body of literature that emphasizes the global significance of aquaculture production and the need for sustainable development. The rapid growth in aquaculture production, the expansion of the aquaculture industry worldwide, and the increasing demand for aquatic products underscore the importance of international cooperation and collaboration to address the challenges and leverage the opportunities presented by AI and API integration in aquaculture.

The references provided offer valuable insights into the global status and trends of aquaculture, the impact of aquaculture on fish supplies, the role of beneficial bacteria in aquaculture, and the potential for sustainable development in marine finfish aquaculture. These references contribute to our understanding of the challenges and opportunities associated with AI and API integration in aquaculture and provide a foundation for fostering international cooperation in the industry.

Thus, the integration of AI and APIs into aquaculture research presents an opportunity to develop an internationally cooperative community that promotes sustainable development, knowledge exchange, and collaborative efforts to address challenges and opportunities in the aquaculture industry.

CONCLUSION

As we navigate the currents of innovation, the integration of Application Programming Interfaces (APIs) and Artificial Intelligence (AI) stands as a beacon for the future of aquaculture research. The explorations within the preceding chapters have charted a course towards a more interconnected, efficient, and collaborative research environment, underpinned by these technologies.

The transformative potential of API and AI integration in aquaculture research cannot be overstated. APIs promise a new era of collaboration, where data flows freely across institutional and geographic boundaries, empowering researchers to build upon each other's work. AI offers the tools to make sense of the deluge of data, providing insights that drive sustainability and productivity in aquaculture practices.

This paper serves as a clarion call to action for the aquaculture research community. Researchers are urged to champion the adoption of open science principles, technologists to develop more advanced and accessible platforms, and policymakers to create frameworks that nurture this technological growth while safeguarding data privacy and integrity. The collective efforts of these stakeholders are critical in realizing the vision of a technologically empowered aquaculture research community.

Looking ahead, the landscape of aquaculture research is set to be reshaped by the adoption of the proposed technological solutions. The integration of APIs and AI represents more than just an enhancement of research capabilities; it signifies a transformative step towards a future where the aquaculture sector thrives on innovation, data-driven decisions, and a global network of shared knowledge. Future research should focus on developing scalable AI models, improving data interoperability, and fostering international collaboration through open science initiatives. These efforts will ensure sustainable practices, enhanced fish health and yields, and better environmental management. The potential benefits are immense, including more sustainable practices, enhanced fish health and yields, and better environmental management. Yet, the true success of this integration will be measured not only in scientific advances but also in how it fosters a collaborative culture that embraces transparency and inclusivity. As we cast our nets wider to the possibilities of tomorrow, let us also anchor our endeavours in the collaborative spirit that API and AI integration inherently promotes. It is through the shared pursuit of knowledge and the collective wisdom of the aquaculture community that we can ensure a bountiful and sustainable future for both the industry and the ecosystems it depends upon.

In conclusion, we advocate the integration of Kubernetes and AI technologies to enhance the scalability and efficiency of aquaculture research. Citing successful implementations like those at the Broad Institute and NOAA, we underscore the potential for these technologies to transform aquaculture research practices to become more sustainable and productive.

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