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Cyber Security of the Maritime ICTs, Threat Vectors and Implications on Global Sea Lanes of Commerce (SLOC)

By Md Ziaul Haque

Synopsis- This paper examines the cyber security aspect of the Maritime Transportation System (MTS) to understand the scope of the MTS, the different ways in which a hacker can infiltrate the computer systems of maritime, logistics, and port infrastructures, and the potential consequences and financial impact of a marine cyber disaster on businesses, states, and individuals.

Glossaries: CISA (cybersecurity and infrastructure security agency), AI (artificial intelligence).

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Cyber Security of the Maritime ICTs, Threat Vectors and Implications on Global Sea Lanes of Commerce (SLOC)

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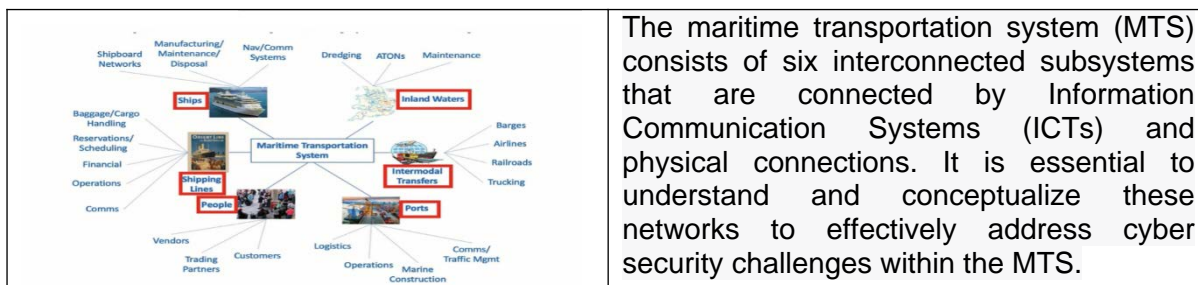
I. INTRODUCTION

Cybersecurity refers to the protection of information, computer systems, and networks from unauthorized access or attacks. The threat level of cyber-attacks on the Maritime Transportation System (MTS) has increased by 400% in recent months. The CISA identifies 16 essential infrastructures critical to national economic security which can be physical or virtual assets, systems, networks, or assets. Maintaining cyber security in the MTS is crucial for the functioning of the Sea Lanes of Commerce (SLOC) and supply chain resilience. Cybersecurity is the responsibility of regulatory authorities and all other stakeholders, as the

MTS is dependent on every supply chain. This study aims to inform the maritime audience about the threat vectors in the upcoming Artificial Intelligence (AI) era, highlighting the various components of cyber security. This article uses experimental methodology on how data/instruction is passed from LBCC LAN to SBCC LAN to conceptualize the cyber essentials using conventional equipment, protocols, and configurations. The ship's LAN is created.

II. MARITIME TRANSPORTATION SYSTEM (MTS) MODEL

The US maritime transportation system (MTS) encompasses approximately 95,000 miles of coastline, 25,000 kilometres of waterways and 361 ports. It is a complex system of interconnected physical and modern ICT networks that must be considered to address cyber security challenges. Governments, regulators, maritime stakeholders, and commercial organizations must work together to understand and address the underlying networks within the MTS. (CISA, 2020).



(CISA, 2020), (Atlantic Council,2023)

Figure 1: Maritime Transportation System (MTS)

III. COMPARATIVE CONTEMPLATION OF BRIDGE MODELS

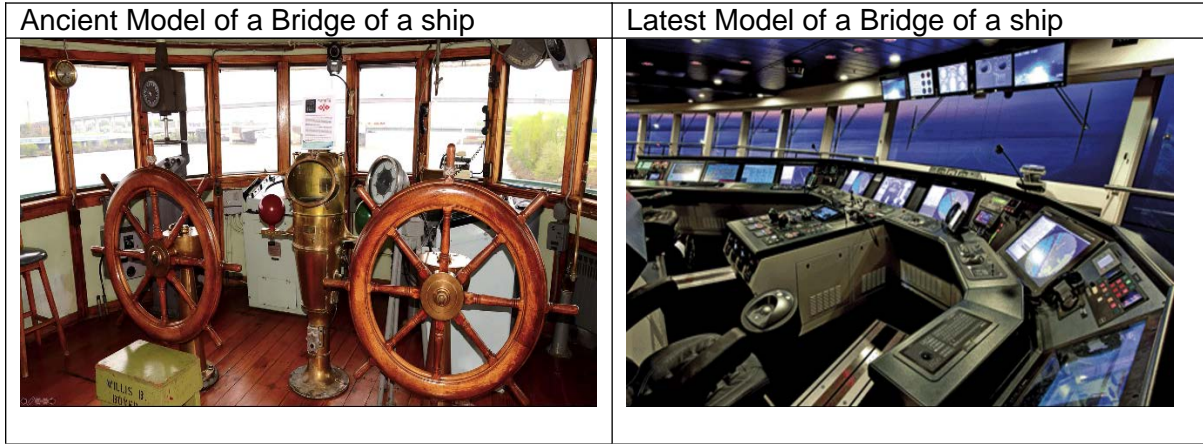


Figure 2: Ancient Vs Latest Bridge Model (Loomis et al., 2021, pp.1–50)

The key difference between ancient and the latest models is the presence of ICTs. The latter consists of networks of satellites, computers, routers, and servers in the latter. The figure illustrates the underlying network, with LBCC connected to SBCC in the latest Model.

cyber security literature. If supply chain disruptions were to occur, the monetary value could reach trillions of dollars within a matter of hours.

IV. SHIP'S INFORMATION & COMMUNICATION TECHNOLOGY (ICT)

a) Systems

These systems are central in the Worldwide Area Network (WWAN) and play a significant role in

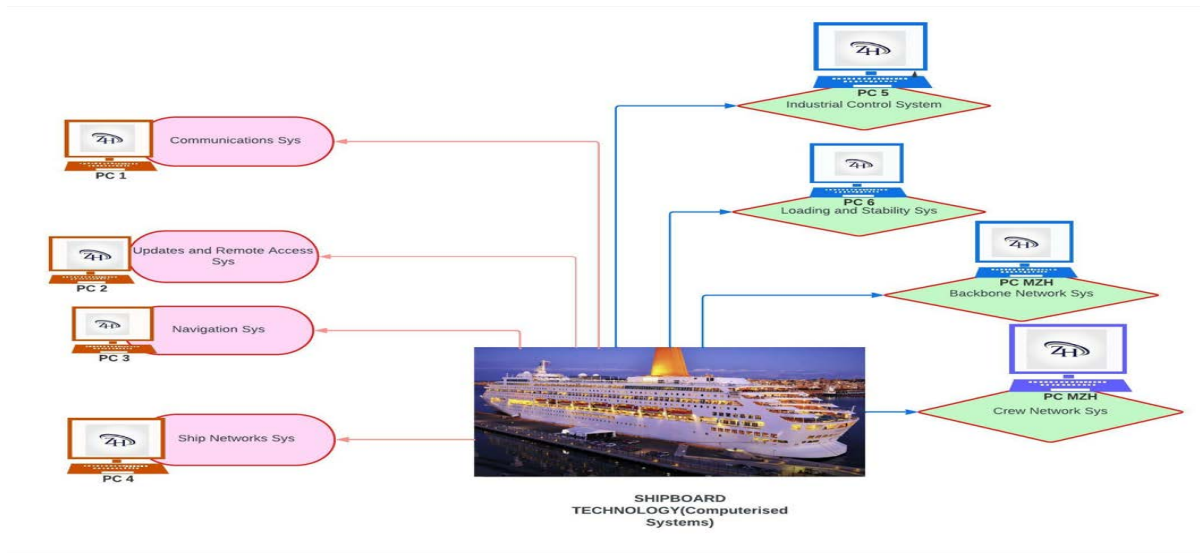


Figure 3: Ships' Core ICT Systems (Loomis et al., 2021, pp.1–50)

Therefore, ensuring the security and reliability of these ICT systems in the maritime transportation system is essential.

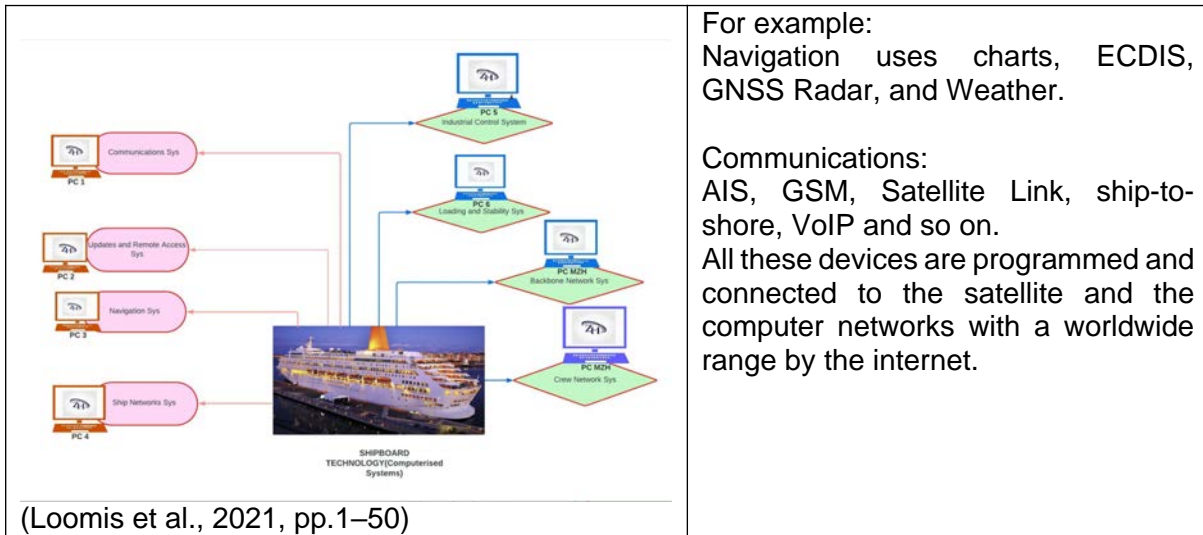


Figure 4: ICT Equipment

V. SHIP'S NETWORK LAN (LOCAL AREA NETWORK)

The diagram illustrates a typical ship network connected to a shore satellite that is invisible to human eyesight. The network has some components

connected in series and others in parallel and is interdependent and interconnected. The network topology is a star topology, which is scalable and can easily be extended, or new ICT systems can be included.

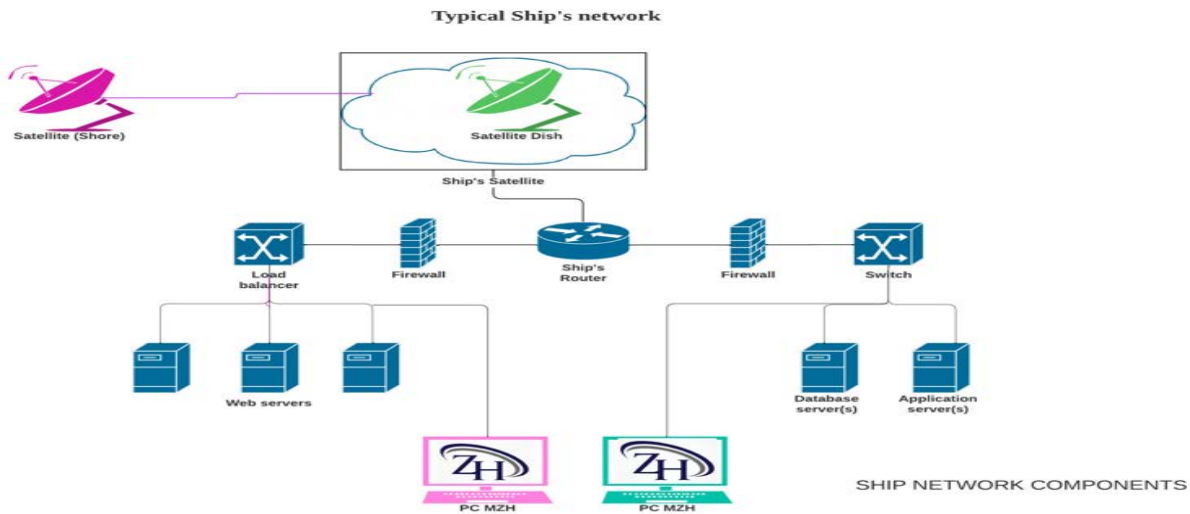


Figure 5: Ship Network Topology

However, this topology also has a drawback, where if a computer clicks a malicious link, it can install malware despite firewall security arrangements, which can have cascading impacts on servers' computers and IoT devices, leading to data being stolen, malware replication, and connecting to botnets with malicious intentions. All devices in this network require regular updates and security patches to keep the Local Area Network (LAN) safe and secure.

VI. SHIP NETWORK TOPOLOGY (LBCC LAN TO SBCC LAN)

The simulation illustrates an autonomous model where a central PC sends instructions to the SBCC's central PC and the LBCC, using IPSec VPN tunnelling to secure data from malicious actors. However, there is a risk that some data may not be able to be encrypted due to commercial pressures, increasing the risk of data breaches. Connecting ship systems to port and logistics systems increases the risk vectors and matrix as cross-industrial network volume increases.

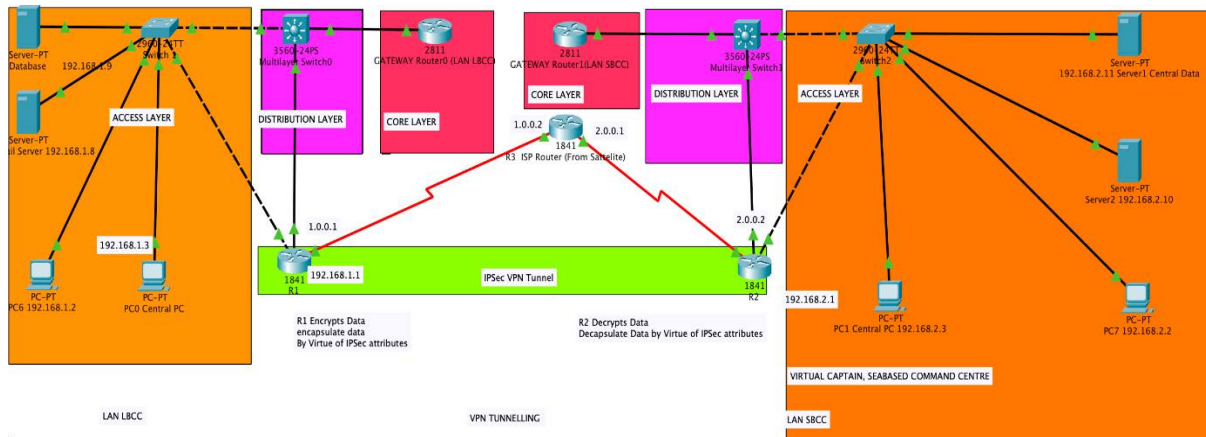


Figure 6: LAN to LAN Network Simulation

In the cyber world, all parties compete to control assets, infrastructures, and services in the supply chain (ship, port, and logistics), leading to a tug of cyber war.

chain disruptions and thwarting terrorist attacks or criminal and malicious intent on critical sea lanes of commerce (SLOC).

VII. COMPUTER RISK MANAGEMENT: (UOB, 2021)

The subsequent paragraphs focus on every network administrator's critical cyber security challenges. These challenges include threat vectors such as hacking, malware, and phishing, among others. Suppose shipboard management can effectively deal with these challenges. In that case, they will successfully handle maritime cyber challenges, avoiding supply

VIII. KEY MARITIME CYBER CHALLENGES

The MTS computer network is a combination of LAN, WAN, and WWAN. Its main cyber security challenges include ensuring the security of networks and hardware, implementing threat monitoring, developing alternative networks, raising user awareness, and managing and configuring interfaces between networks to secure the whole network.

IX. WHAT DOES IT MEAN CYBER SECURITY

<p>Funda of Computer Security=Cyber Security</p> <ul style="list-style-type: none"> • Identify the common sources of malwares • Identify common password threats • Understanding the function of Malware • Four Common Wireless Security Threats • Skillful Network Administration • Understand the Domain name system. • Eavesdropping = Looking from your back • Piggbacking= Neighbour hijack your router connection. • Snipping= Interception inbetween router and your computer). • Phishing=by unsolicited email, data stealing • Spam=Nuisance • Updating the softwares(system, applications), software intalling patches, 	<p>The left pane lists some points to keep in mind to ensure day-to-day cyber threats</p>
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Figure 7: Fundamentals of Computer Security

a) *Overlapping*

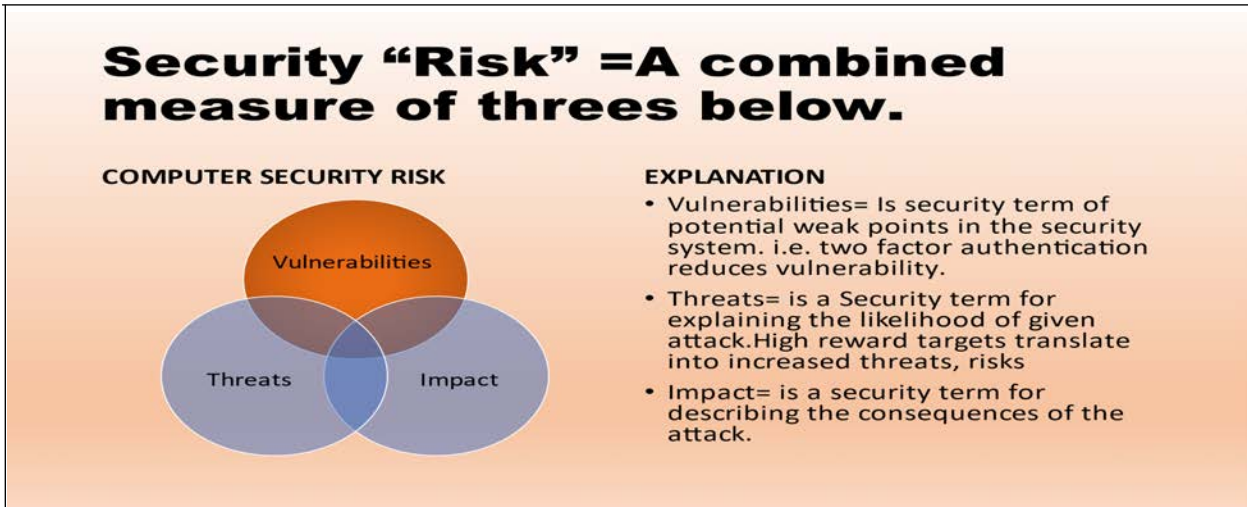


Figure 8: PC RISK MODEL IN Venn Diagram (UoB,2021)

b) *Viruses*

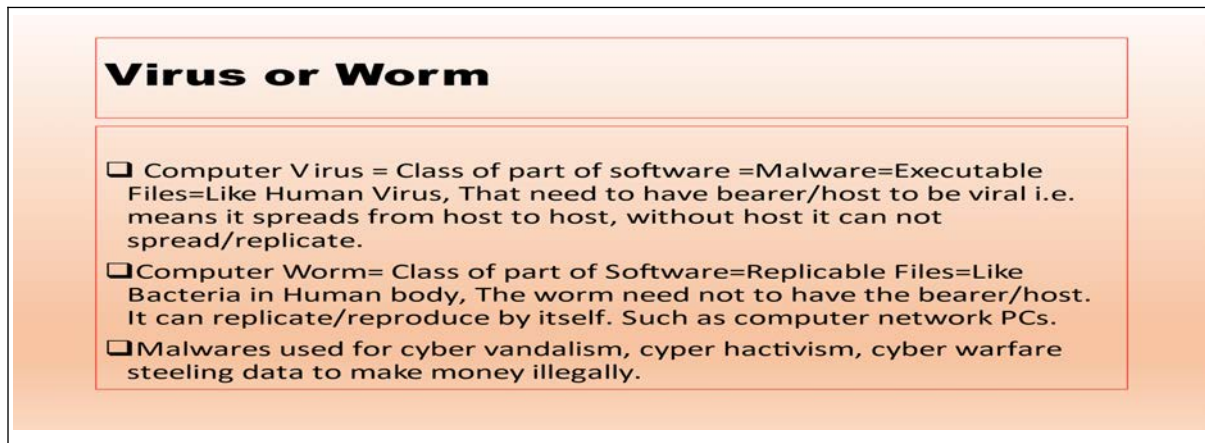


Figure 9: Virus or Worm Explanation

c) *Attack Definition*

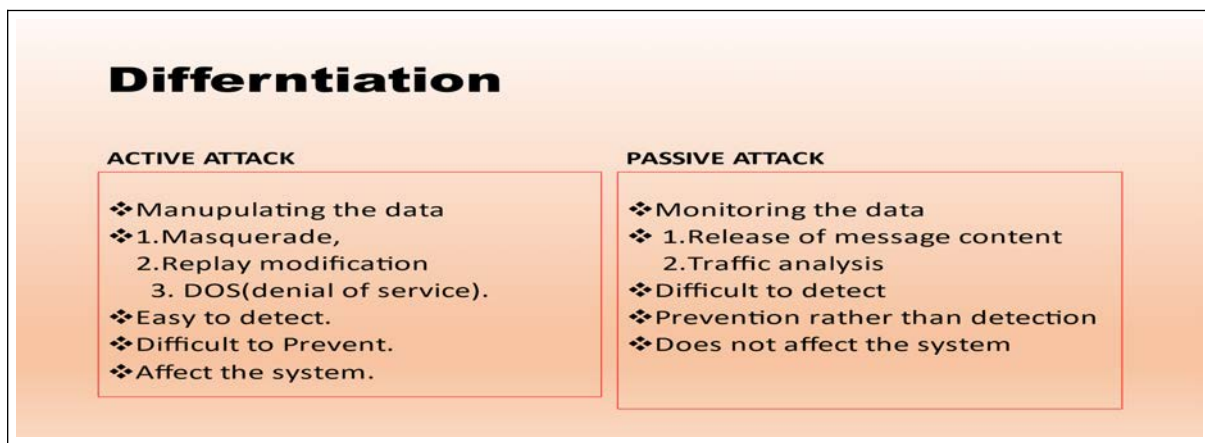


Figure 10: Attack Definition

d) *Intrusion*

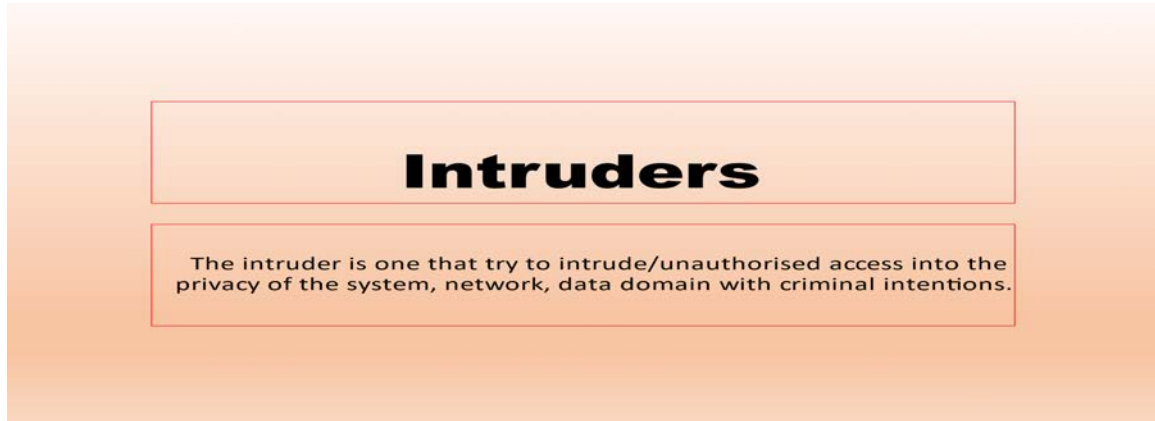


Figure 11: Intruder Definition

i. *Types of Intruders*

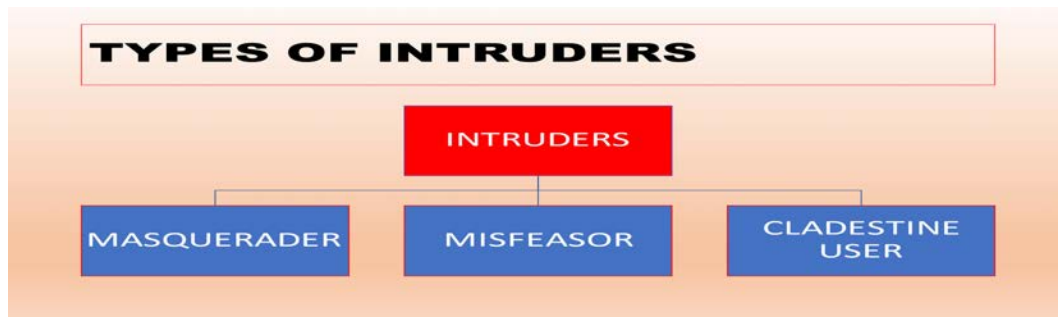


Figure 12: Types of Intruders

X. *ATTACK MODEL: (IAPH, 2020)*

Types of Techniques	How Delivered and Deployed
Social Engineering	Phycological Manipulation to click, tempting to click on the social media posts.
Ransomware	Email phishing, Remote Desktop Protocol (RDP), Downloads, Pirated Software, Removable Media. (Cawthra et al., 2020)
Spoofing	Domain Spoofing, Email Spoofing, geolocation GPS spoofing, TCP/IP Spoofing
Unauthorized Access	Gaining access to a company's network, endpoint, application, or device without permission is often due to flawed or misconfigured authentication measures.

XI. ATTACK ON MODEL

Attack on IT System

Information Technology (IT) is used to create, process, store, securely transmit, and electronically exchange data, computers, networking, storage, and other devices.

Attack on OT System

Operational technology primarily engages with the physical world by controlling industrial equipment via hardware and software.

Attack on PNT Systems

Positioning, Navigation and Timing (PNT) is a system that includes three core capabilities: positioning, which is the ability to determine the ship's location precisely and reliably, and orientation in two dimensions (or three-dimensionally when necessary).

(Loomis et al., 2021, pp.1–50)

a) Navigation System

Modern ships use three types of navigation systems and 30 different navigational tools and resources, and communication and vessel status equipment. The ship has evolved into a floating computer network, but advanced IT interactions also increase vulnerability to specific threats as no computer equipment is 100% safe by default.

b) ICT (Information Communication Technologies)

The Internet is a complex network made up of millions of other networks. The Maritime Transportation

System (MTS) is similar in that it is a system of systems. Information and Communication Technologies (ICT) play a vital role in these systems as they allow for data storage and communication between different parts of the system. However, vulnerabilities may surface when various components interact, such as from a ship to a satellite to a base station to a command and control and tracking center. The figure illustrates real-time global ship traffic, which is too vast to account for without using ICT and its applications, with red representing cargo ships.



Figure 16: Satellite Overview of Global Fleets (Marine Traffic, 2023)

Clicking on a vessel allows easy access to shipping details via satellite. Still, malign actors/hackers can alter the data and send false information to the satellite, base station, and vessel tracking centre, leading to inaccurate vessel location data.

c) Spoofing Attack

The spoofing attack on the global navigation satellite system (GNSS) aims to trick a GNSS receiver by transmitting fake signals that mimic real GNSS signals or by rebroadcasting real signals at a different location

or time. The spoofing can cause the receiver to estimate its position incorrectly or at a different time, as decided by the attacker. One common type of GNSS spoofing is a carry-off attack, which starts by broadcasting signals in sync with the real signals that the receiver sees and then gradually increasing the strength of the fake signals. (Ball, 2020)

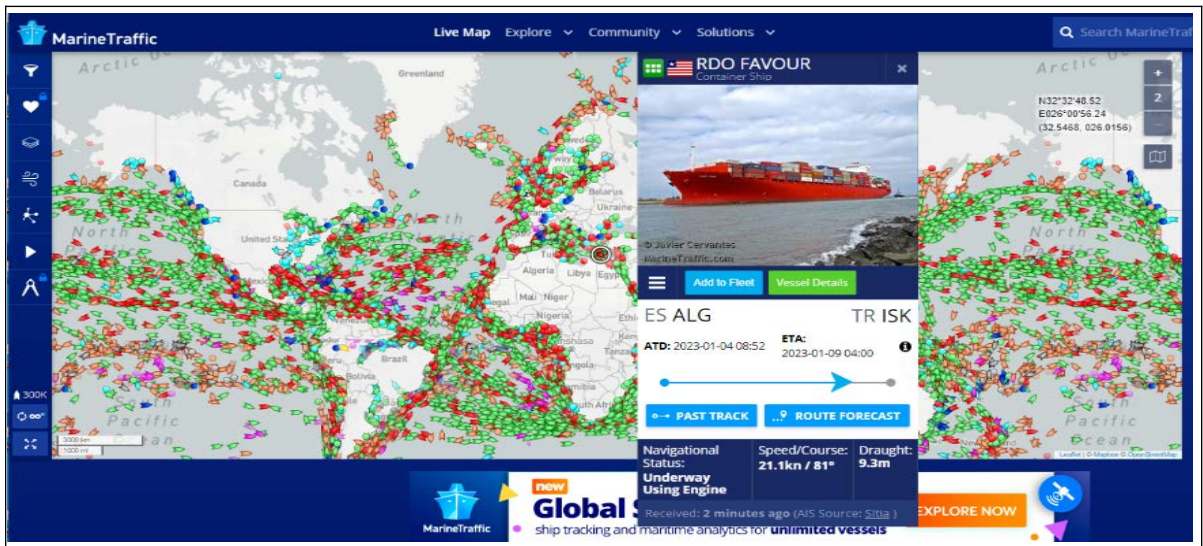


Figure 17: Ships' Data from Satellite Captures (Marine Traffic, 2023)

Ships have computerized devices connected to satellites and networks that allow for the exchange of information. However, the interconnected nature of the maritime transportation system means that when one component breaks down, it can significantly impact the entire system. (Brewin, 2013)

how breakdowns can affect the entire system. The Defra Impact Calculator and methodology can be used to predict and calculate the monetary costs and impact of such breakdowns and help identify critical areas and interdependencies to mitigate risks.

XII. IMPLICATIONS OF THE CYBER DISASTER

The implication model illustrates the relationship between computer systems and the supply chain and

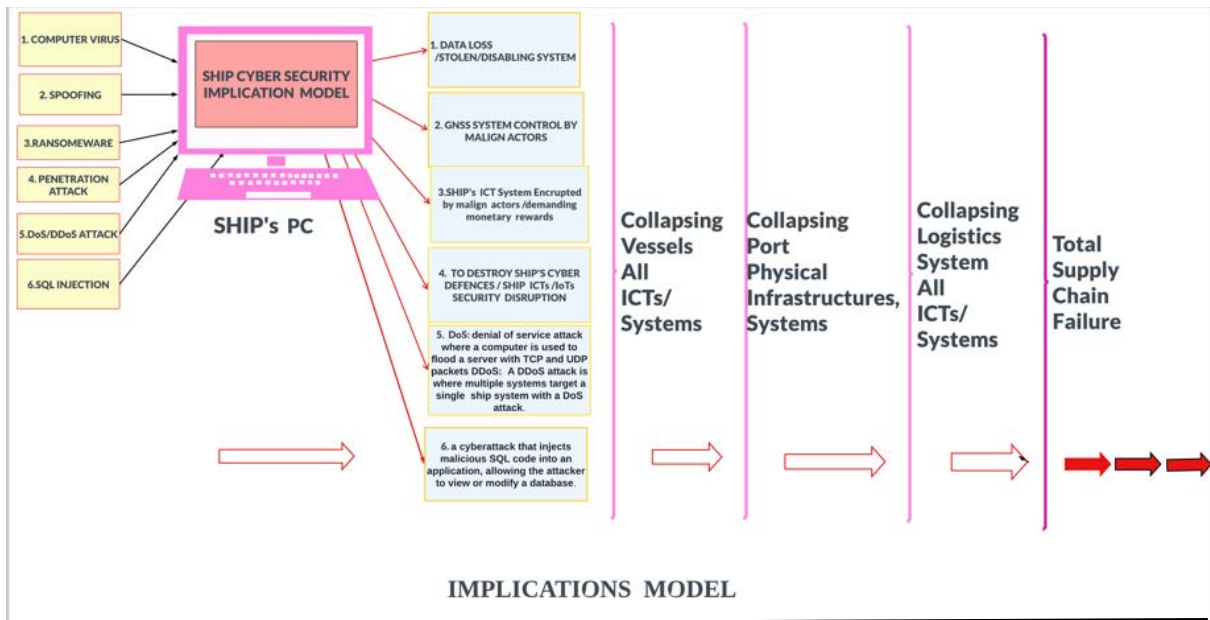


Figure 18: Cyber Disaster Implication Model

Shortages of essential items, price increases, industrial closures, unloaded shipping containers, and other factors can negatively impact a country's economic health.

XIII. CONCLUSIONS

It is important to remember that computer viruses need hosts to harm a system, service, or infrastructure. Cyber security can be effectively managed by understanding how malicious actors can take control of an organization's assets and services. The objectives of this article are looked at /conceptualized from various angles. They will help maritime professionals promote their awareness of cyber responsibilities in their workplace to uphold supply chain resilience and the resilience of its stakeholders.

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Status of River Cauvery – Water Quality and Riverine Environment

By A. K. Das, D. N. Singh, D. S. Krishnarao, S. Manoharan, M. Naskar & J. Canciyal

Abstract- The river Cauvery was explored from origin to confluence at BoB (into two stretches) to unravel its eco-physiography, limno-chemical profile, biotic components, pollution scenario, fish species composition, abundance and fish bio-diversity. Data thus collected was processed through different statistical tools to have conspicuous relationship with the abiotic and biotic components, productivity and the fish species. Water and sediment quality parameters revealed that the river is low productive in Karnataka stretch with increment in productivity in downstream in Tamil Nadu because of innate characters having moderate acidic basin in the upper stretch as validated by its alkalinity, hardness, water and sediment reaction (pH) getting reflected in primary productions. Most of the tributaries showed productive criteria contributing moderately to limno-chemical profiles of main Cauvery. Local pollution was not phenomenal as revealed by chloride content in some of the tributaries especially encountered with the first monsoon flood. The fish catch in the entire Cauvery system has been dwindled drastically with a great reduction in catch structure as well as faunistic diversity. The study is a modest attempt, will be of immense help in unravelling the various facets of riverine ecology and their bearing on productivity, and overall riverine eco-health.

Keywords: cauvery river, ecology, limno-chemistry, productivity, riverine eco-health.

GJSFR-E Classification: DDC Code: 628.4 LCC Code: GE300



Strictly as per the compliance and regulations of:



Status of River Cauvery – Water Quality and Riverine Environment

A. K. Das ^α, D. N. Singh ^σ, D. S. Krishnarao ^ρ, S. Manoharan ^ω, M. Naskar [¥] & J. Canciyal [§]

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I. INTRODUCTION

The most vibrant sector under the umbrella of 'Agriculture' is the aquaculture and fisheries sector in India contributing 6.11% to Agriculture GDP and 1.12% of National GDP with annual increment at 7.11% growth achieved in 2018-19 having all time high production of 13.646 million tonne (mt) of Fish, with major share from inland resources (68%) (GOI, 2020). Imbibing with the spectacular rise in this sector that are providing nutritional security (9.5 kg fish consumption per capita per year) to around 60% of 137.44 crores Indian populace with direct involvement of 15 million fishers and fish farming communities, Govt of India has opened up a new Directorate of Fisheries in February, 2019 under Ministry of Agriculture & Farmers' Welfare with an immediate budget layout of more than Rs. 10,000 crores – Rs. 3,000 crores for 'Blue Revolution Schemes' and Rs. 7,322 crores as Fisheries Infrastructure Development Fund (IFDF) and subsequently a separate ministry 'Ministry of Fisheries,

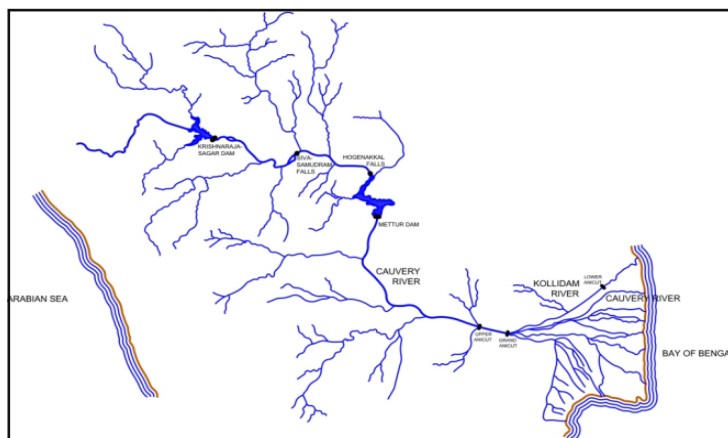
Animal Husbandry and Dairying' as to strengthening the sector in a more pragmatic way.

Though, India is blessed with unique inland open water resources under diversified geographical locations, is in declining trend in capture fisheries from our rivers is obvious with intense anthropogenic interventions resulting in habitat loss, thereby many fish species have become highly endangered associated with target species for capture. Habitat destruction (Cuizhang *et al.*, 2003), heavy siltation, water demand abstraction for industries, irrigation (Szollosi-Nagy, 2004), faulty river embankment strategies for flood control, rapid urbanization, pollution (Lima-Junior *et al.*, 2006), construction of dams, barrages, anicuts, weirs obstructing migration of fishes coupled with over exploitation and unscrupulous fishing practices, invasion of exotics (Copp *et al.*, 2005) gets further aggravated with global climate change (Leveque *et al.*, 2005; Mas-Marti *et al.*, 2010). The present communication, first of its kind has been explored from origin of R. Cauvery to confluence at BoB (into two stretches) to unravel its eco-physiography, limno-chemical profile, biotic components, pollution scenario. Data thus collected was processed through different statistical tools to have conspicuous relationship with the abiotic and biotic components, productivity, a modest attempt to unravel the present ecology of this important peninsular river having a peep through in assessing the environmental flow to sustain riverine eco-health and fishery.

R. cauvery is fed with more than 15 tributaries, the important ones in Karnataka stretch, joining on the left are Harangi, Hemavathi, Shimsha and Arkavathi while on the right are Lakshmanatirtha, Lokapavani, Kapila, Honnuhole, Suvarnavathi and Kabini; barring Shimsha and Arkavathi, all the tributaries rise in Western Ghats characterized by dense forest and high rainfall and all the tributaries (except Lakshmanatirtha) have impoundment constructed on them diverting less water to the main R. Cauvery other than monsoon; while Bhavani, Amaravathi, Noyyal etc. are in Tamil Nadu joining from the left, diverting substantial volume of water into the main river specially in monsoon as almost all the tributaries do have dams and in Cauvery basin there are 96 dams and 11 weirs (CWC, 2020) abstracting huge volume water. Cauvery receives run-off water from its total catchment of 89,600 km² and a mean rainfall of 1560 mm, the run-off contribution per unit of catchment is about 0.140 MCM/Km².

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II. CAUVERY RIVER BASIN



(Source: URL. <https://sandrp.in/tag/grand-anicut/>)

Fig. 1: River Cauvery

a) Sampling programme and methodology

River course was divided into two stretches – upper stretch (Karnataka) and lower stretch (Tamil Nadu). In the first stretch R. Cauvery from its origin point at Talacauvery to Arkavathi Sangama near Kanakpura in Karnataka spreading over 300 km in length was chosen with 10 sampling stations viz., Bhagamandala, Kannege, Koodige, Ramnathapuram, K.R.Nagar, Srirangapatnam, T. Narasipur, Muduthere, Talakadu and Mekedatu while in the second stretch the sampling stations (14 nos) in Tamil Nadu were – Hogenakkal, Mettur, Bhavani, Thirumukkudal (Karur), Upper Anicut, Grand Anicut (Thiruvayur), Kumbakonam-Cauvery, Kumbakonam-Coleroon, Kollidam, Grand Anicut Canal, Vettaru, Vennaru, Palaiyar and Poompuhar, selected basically based on domestic, agricultural and industrial activities in the vicinity of river basin, recreation and ritual practices with the river body and also river - tributaries confluence points (Fig 1). Geographical details of the sampling locations and possible sources of contamination are given in Table 1. Samplings were also done in tributaries at the site in the upstream of confluence point. At each centre, sampling was done for various limnological parameters and assessment of phytoplankton primary production.

Samplings were done during 2001-02 covering post-monsoon (POM, Oct-Nov), pre-monsoon (PRM, May-June) and monsoon (MON, September). All samples were collected at 9.00 am. Sediment samples were collected from five to six locations across the river from each sub sector randomly. Barring nutrients, other physico-chemical features of water were analysed *in situ* subsequently after collections. Water samples were preserved with respective preservatives and analysed in the laboratory without much time lag. Chemical analyses of water were performed following 'Standard Methods' (APHA, 2002) and soil analysis by Standard Methods

(Tandon, 1993). The primary production was estimated up to one meter depth using dark and light technique (Vollenweider, 1969) keeping incubation period of 4 hrs (10 to 14 hrs). Plankton samples were collected both from sub-surface and preserved with Lugol's iodine solution. The drop-count method was used (APHA, 2002) for planktonic enumeration. Benthic and periphytic samples were collected following APHA (2002).

III. STATISTICAL ANALYSIS

For statistical analysis, several multivariate analyses (Buyan, 2005) were carried out to characterize the environment, species community structure and their plausible interaction to be reflected in management aspects (Lee et al., 2001; Regunath et al., 2002; Singh et al., 2006; Hayal and Hiilya, 2009; Pejman et al., 2009). The multivariate canonical correlation analysis was carried out to investigate the relationship between water quality and soil quality parameters. The hierarchical cluster analysis based on euclidean distance measure was applied to classify sampling sites according to environmental distance. One of the aims in the present study was to evaluate the influence of soil-water parameters on the productivity. To accomplish this, the immediate choice was to carry out the classical multiple regression. To circumvent this problem, Partial Least Square Regression (PLS) method (Marten, 2001) has been applied. Essentially, all the soil-water quality parameters were designated as predictor variables, and GPP was designated as response variable, to carry out PLS regression. The method quantifies the contribution of soil-water quality on GPP as well as derives latent variables (components) that explains reasonable amount of variability of the soil-water parameters. Selection of optimum number of components is the key to effective implementation of PLS method, and it has been selected by using the Root Mean Square Error of

Prediction (RMSEP) criterion with Leave-One-Out (LOO) cross validation. Thereafter, the PLS was fitted with the selected number of components. Finally, relative contribution of each variable is computed by using following formulae:

$$RC_j = \frac{b_j^2}{\sum_{j=1}^p b_j^2};$$

Where, b_j is the standardized estimate of the j -th regression coefficients. The 'PLS' package (Mevik et al., 2019) under R software (R Core Team, 2019) environment was used to implement the PLS method.

IV. PHYSICAL FEATURES

Depth and width: The catchment of R. Cauvery at Brhamagiri hills (1355m asl) is under Western Ghats (12°25'N) which is covered with evergreen deep forest with forest origin red soil. At Bhagamandala, 19 km down to Talakaveray, the river is 4-6 m width with 2-3 m depth in monsoon with steep gushing of water but in post-monsoon it is narrowed down to 2-3 m width having sheet flow. The river width is maximum in the downstream after Shivasamudram onwards up to Karur. Kannige, the first tributary, however, even with low volume of water being diverted into main river, the tributaries of R. cauvery are exerting tremendous impact on the sediment and water chemistry of the main river which are more vivid during monsoons (Table 1).

Temperature: Ambient water temperature (WT, °C) fluctuated sinusoidally keeping parity with air temperature, varied from a low of 20 & 26°C to a high 30° & 30.5°C in the first and second stretches respectively. However, the mean annual temperature fluctuated over a wide range of 22-29.5°C in the entire river system of Cauvery. Accordingly, higher WT was noticed in PRM (25.0-31.0) followed by MON (21.0-30.5) and POM (20.0-29.4) in the entire river course. More temperature was encountered in the riverine sheet flow compared to deeper waters (lotic of downstream reservoirs, anicuts, barrages etc.) even in the same season. As R. Cauvery basin especially in Karnataka is predominantly covered with forest origin red soil having low heat retentive capacity getting reflected in the ambient water rendering low water temperature unlike other Indian rivers.

Transparency: Maximum water transparency (Secchi-depth, cm) was noticed in PRM with bottom exposed in many occasions followed by POM (22.0-140.0) and lowest in MON (20.5-135.0) in the entire river course. Due to sheet flow of water during summer in some sampling sites both in the up- and down-streams, bottom was viewed (exposed). However, transparency was very high in pre-monsoon months in most of the sampling sites in the entire river course due to quicker sedimentation of red soil (colloid micelles) especially in

the up-stream with reduced flow and stable condition. Statistical analysis by the boxplot reflects, temperature and transparency significantly differs from summer than monsoon and post monsoon season.

Sediment characteristics: Soil in R. Cauvery basin is red in Karnataka stretch while it is alluvium and sandy in Tamil Nadu stretch. River bed is rocky at some parts of Kudige, Ramnathpuram, Srirangapatnam, Sivasamudram, Mekedatu and Hogenakkal in the upper stretch. Soil texture is generally sandy to sandy-loam in the entire river stretch with predominance of sand in the down stretch. Both in stretch I and II, structure of the river bed has been modified due to reservoirs, barrages and weirs. Intense agricultural activities around the riverine catchment have their impact to some extent in modifying the texture of basin soil in both the stretches. Soil reaction was low in the up-stream 5.74-7.8, 5.95-6.95 and 4.08-6.81 while these values were increased gradually towards downstream 7.66-8.32, 7.13-8.28 and 6.25-7.50 in PRM, MON and PRM respectively. Moderate values of specific conductance (mScm^{-1}) were also noticed in the entire river course (0.062-1.26), (0.11-1.94) and (0.09-1.51) in PRM, MON and MON respectively. Organic carbon content (%) was fairly rich in MON (0.20-2.26) and POM (0.20-2.05) than PRM (0.02-1.75). More C/N ratio was encountered in MON (3-48) followed by POM (1-40) and PRM (4-36) in the entire stretch with low values in estuarine parts. Available nitrogen was in moderate range ($\text{mg}/100 \text{ g}$) 3.40-37.50, 2.80-44.80 and 2.50-82.50 in PRM, MON and POM respectively. Available-P ($\text{mg}/100 \text{ g}$) was fairly moderate in R. cauvery like other Indian rivers and ranged from 0.47-16.97, 0.32-14.75 and 0.38-8.50 in MON, POM and PRM respectively.

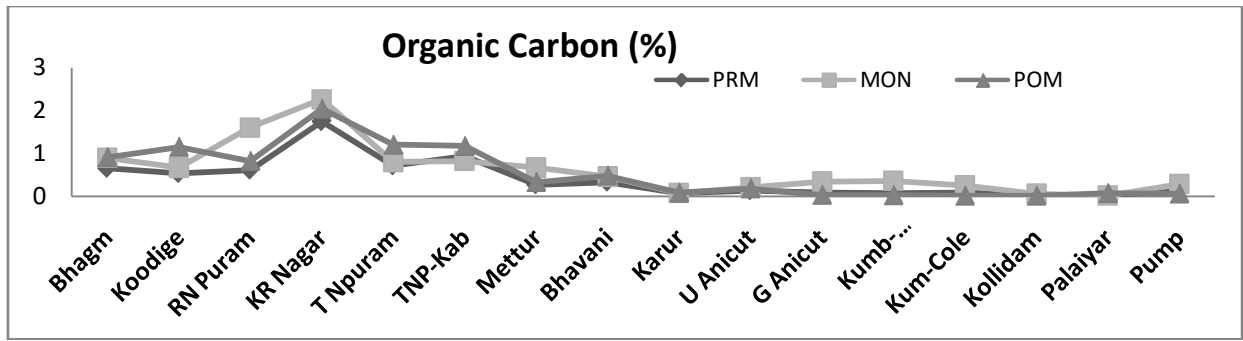


Fig. 2: Organic matter in sediment of R. Cauvery

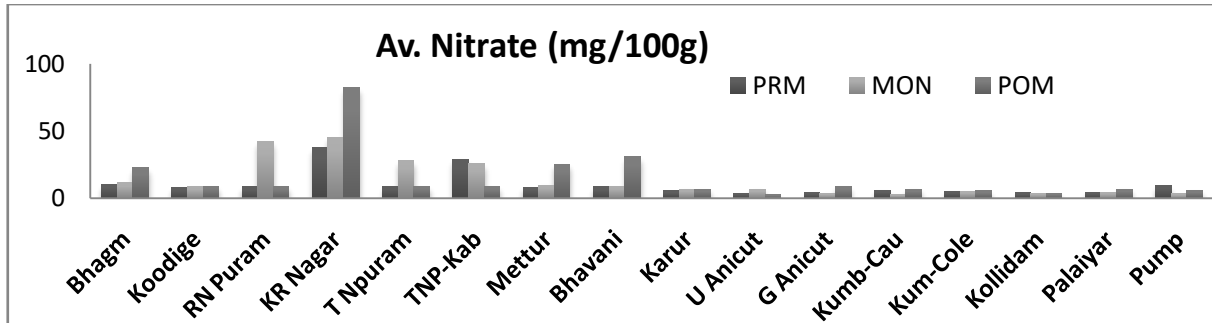


Fig. 3: Available nitrogen in sediment of R. Cauvery

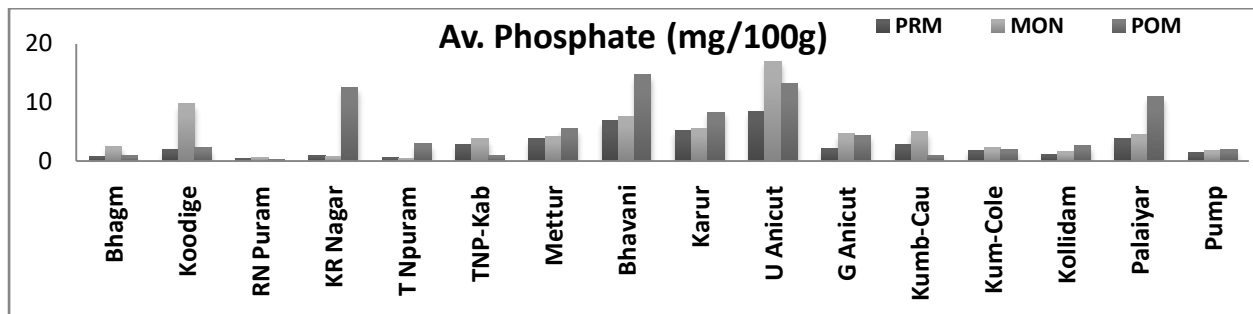


Fig. 4: Available phosphate in sediment of R. Cauvery

V. LIMNO-CHEMICAL FEATURES OF WATER

Water reaction was moderately acidic to near neutral in the upper four sampling sites (pH 6.07-7.17) at Coorg district, Karnataka due to innate character of catchment predominately covered with forest origin red soil. Neutral (pH 6.79) to moderately alkaline (pH 8.6) is followed after that in the entire river stretch. In general, pH was low in MON (6.00-8.50) followed by POM (6.18-8.60) and in slightly higher order during PRM (6.07-8.50). Sp. conductance (μScm^{-1}) was on increasing trend from the origin with higher values noticed in the down-stream and fluctuated widely in tributaries; more in PRM (90-2870) followed by POM (60-2210) and lowest in MON (28-2130). Pumpuhar, the extreme lowest estuarine centre in the south showed highest values of sp. conductance (2870, 2210 & 2130 μScm^{-1} in PRM, POM and MON respectively). Total dissolved salts followed the same trend as observed for specific conductance.

Amongst dissolved gases, DO was moderately rich in R. Cauvery water in many sampling sites. The lowest DO (mg l^{-1}) recorded at Mettur outfall (4.0) in PRM with highest at Hogenakkal (8.7) in POM in this study. Free CO_2 (mg l^{-1}) remained absent in the downstream from Sivasamudram onwards barring Hogenakkal (3.0) in monsoon, in all the seasons; first four sampling sites in Coorg, Karnataka, it was found to the tune of 2.0-8.0 year round due to forest cover in the catchment. Like river Krishna (Das et al, 2017) high total alkalinity with predominant presence of carbonates was encountered in the entire Tamil ndu strch of R. Cauvery irrespectiv of seasons to the tune of 166-320 mg l^{-1} barring few sampling sites. Very low alkalinity was noticed in the extreme two stations Bhagamandala and Kannege (6-34 mg l^{-1}) after which it is in increasing order. Total hardness (TH, mg l^{-1}) also follos the same pattern as of total alkalinity, more in the Tamil Nadu stretch (104-237) barring Hogenakkal (60) and Mettur lotic (84) in monsoon. The estuarine sites showed higher values of

hardness (680-3670) due to saline water ingress. Calcium content (mg l^{-1}) was moderately rich in Cauvery, like Krishna (Das et al, 2017) registered low values in MON (1.25-25.65) and PRM (4.00-33.14) as compared to PRM (3.38-44.89). Magnesium content (mg l^{-1}) also was moderately rich and ranged from 1.94-48.78 in the entire river stretch during the study period barring Uppar Anicut (PRM 112.22); the estuarine parts recorded highest Ca and Mg content (66.18-917.83 & 74.52-700.00 mg l^{-1}) irrespective of seasons. In many occasions, the results were in agreement with the findings of Jayaram 2000, Begum 2008, Venkatesharaju et al. 2010, Shivakumar et al. 2014, and Sivakumar et al. 2017.

Local pollution was not well pronounced in R. Cauvery other than Mekedatu, the last sampling station in Karnataka where tributary Arkavathy confluences with R. Cauvery diverting local sewage in the river as getting reflected in chloride content (mg l^{-1}) to the tune of 127.80, 38.89 & 138.00 in PRM, MON & POM seasons. In general moderate values of chloride (mg l^{-1}) were encountered in R. Cauvery more pronounced in POM (14.20-49.40) followed by PRM (10.94-41.40) and MON (12.70-54.80). Palaiyar (120-178) and Pumpuhar (138-166) registered the highest values of chloride) in all the above three seasons respectively due to estuarine parts.

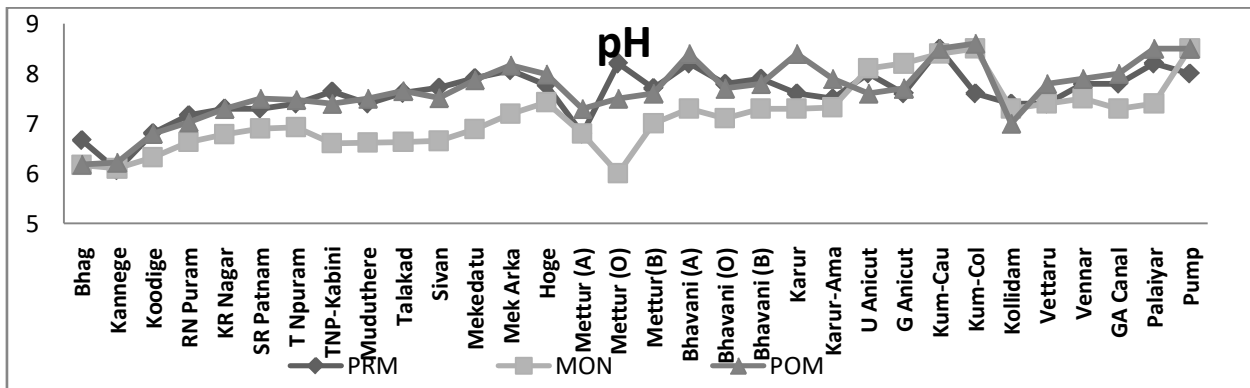


Fig. 5: Water reaction (pH) of R. Cauvery

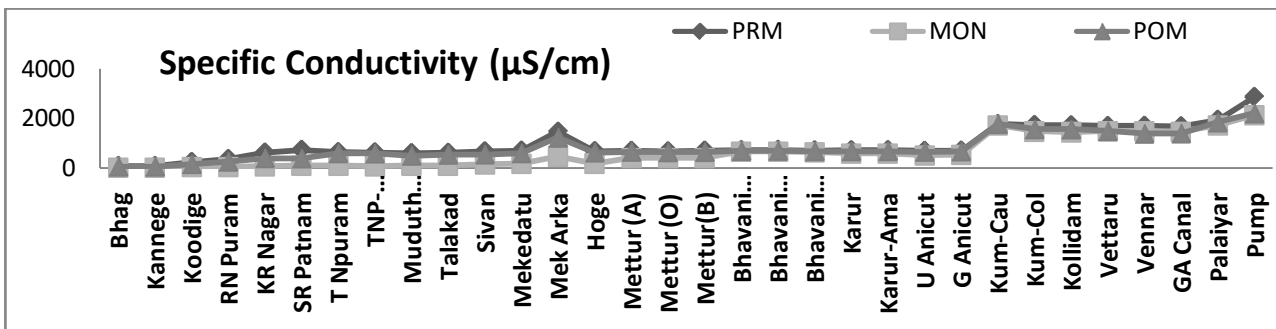


Fig. 6: Specific conductivity of water in R. Cauvery

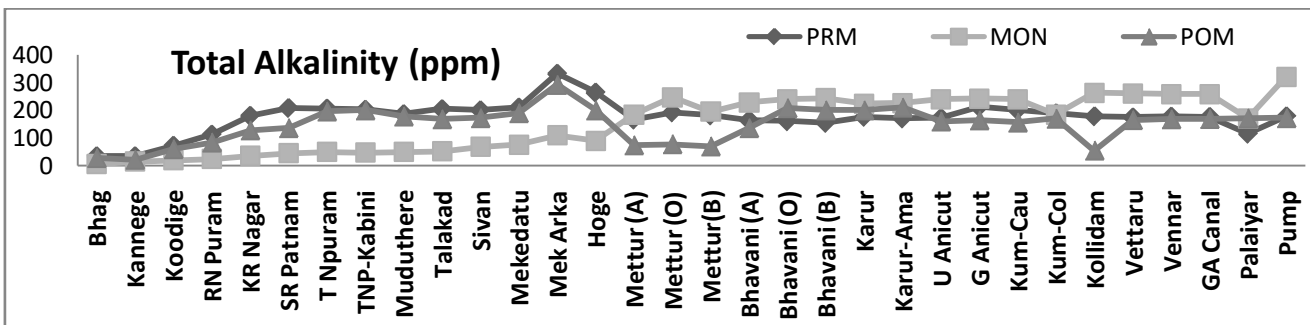


Fig. 7: Total alkalinity of water in R. Cauvery

VI. NUTRIENT STATUS OF WATER

Amongst dissolved nutrients, fluctuation of nitrate – N ($\mu\text{g l}^{-1}$) was to the tune of 20 -345, 20-312

during MON & POM respectively in the stretch between Mekedatu in upper stretch up to estuarine part in Tamil Nadu with lowest presence or in traces in the entire river system in PRM. The upper stretch beyond Mekedatu in

Karnata represented very low dissolved nitrate in water irrespective of seasons. Soluble reactive phosphorus was evenly distributed in the entire river course with some significant presence in most of the sampling sites in the downstream, more in MON and POM than PRM. It ($\mu\text{g/l}^{-1}$) was in the range 1-390, 10-350 & 1-230 in MON, POM, PRM seasons respectively in the entire river course. Forest origin red soil in the catchment of up-stream prevents its availability in this stretch. Moderate presence of total – P was observed in the entire river

system. Silicate – Si maintained a moderate productive range (mg/l^{-1}) in the downstream (4.35-9.92, 3.90-10.20 & 3.62-7.51) as compared to up-stream (0.30-5.25, 2.00-5.75 & 0.36-9.99) in MON, POM & PRM seasons respectively; the estuarine parts represented low silicate-silicon content. Overall, the R. Cauvery showed moderate values of dissolved nutrients in water unlike R. Krishna having more dissolved nutrients in its water (Das et al, 2017).

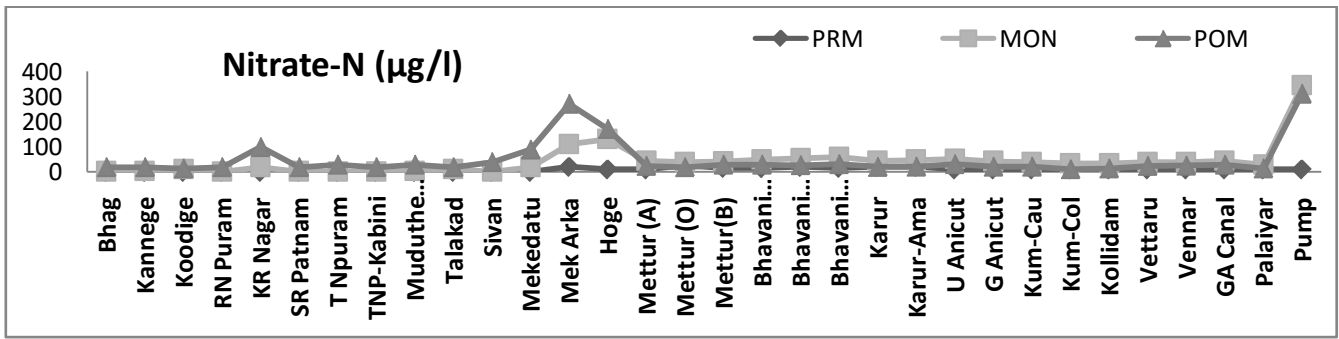


Fig. 8: Nitrate nitrogen in water of R. Cauvery

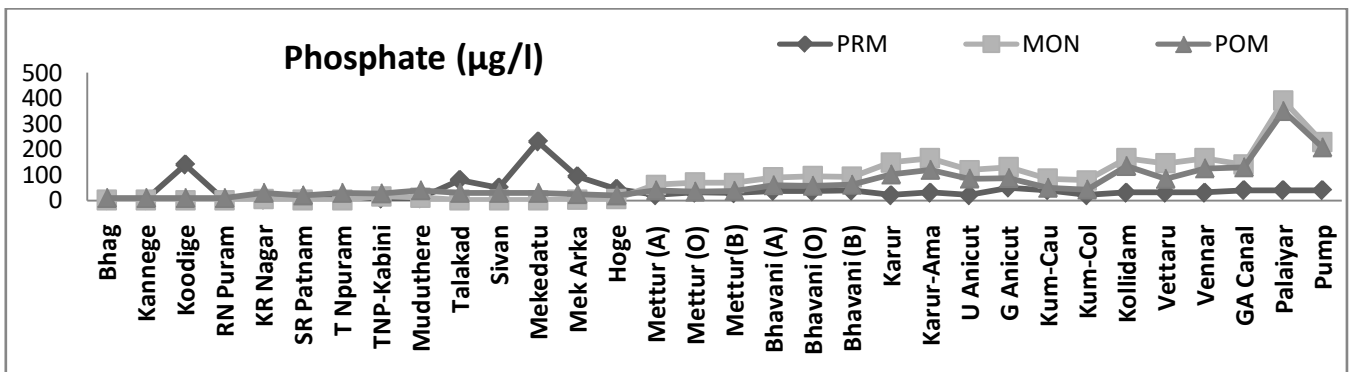


Fig. 9: Phosphate-P in water of R. Cauvery

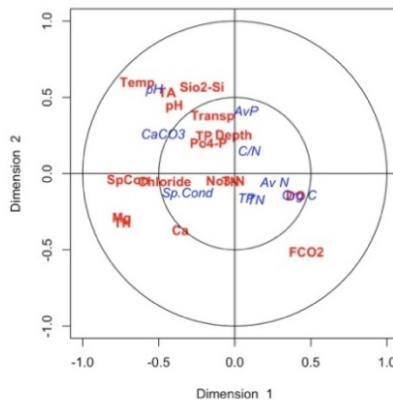


Fig. 11: Relationship between water and sediment qualities. Blue colour denoted sediment qualities

VII. ASSOCIATION BETWEEN WATER AND SEDIMENT QUALITIES

The dimension 1 separates most of the water quality parameters. Considering 0.5 as the threshold contribution, sediment pH and CaCO₃ of sediment were positively associated with temperature TA, SiO₂-Si and pH of water. Though weakly related dissolved nutrients, especially SiO₂-Si was negatively related to the sediment nutrients.

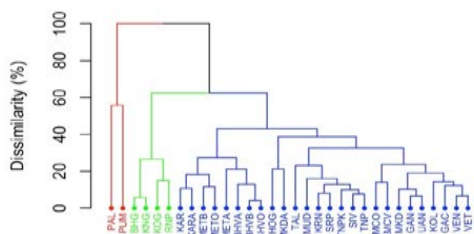


Fig. 12: Dendrogram tree of sites according to environmental dissimilarity. Three different colours denoted the three clusters at 60% dissimilarity

VIII. ENVIRONMENTAL CLASSIFICATION OF SITES

The results of hierarchical cluster analysis have been depicted in Fig.12. It indicates that environment comprising physical, limno-chemical features of water distinguishingly divides the river into three stretches with 60% dissimilarity in the environment. The three distinct environmental site-groups were as follows: Group1 of two sites (Palaiyar and Pumpuhar); Group 2 of four sites (Bhagamandala, Kannege, Koodige and Ramnathapuram) and Group 3 of 26 sampling sites (KRNagar, Srirangapatnam, TNarasipur, TNarasipur-Kabini, Muduthere, Talakadu, Mekedatu, Mekedatu-Arkavathy, Shivasamudram, Hogenakkal, Mettur-Above, Mettute-Outfall, Mettur-Below outfall, Bhavani-Above, Bhavani -Outfall, Bhavani-Below out fall, Karur, Karur-

Amaravathy, Upper Anicut, Grand Anicut Kumbakonam-Cauvery, Kumbakonam-Coleroon, Kollidam, Grand Anicut Canal, Vettaru, Vennaru), which is obvious from the fact that the Gr 1 is comprising the extreme estuarine parts unlike any other sampling stations. Group 2 covers the upstream four sampling sites in the serene hilly terrains with red cover forest soils. Rest of the 26 samplings sites under Group 3 are more or less homogeneous in respect of limnological parameters existing between upstream hilly terrain and extreme estuarine sectors with deep pools, flattened river basins with influence of reservoirs and weirs/anicut.

IX. PRIMARY PRODUCTIVITY

The gross primary production (GPP, mgCm⁻³h⁻¹) increased progressively with the river flowing downwards. Significantly, higher production was registered in the downstream of the river in PRM months followed by MON and POM. The overall range of GPP in PRM, MON and POM seasons were 20-306, 28-296 and 27-250 respectively in the entire river stretch. Down-stretch of river zone is always more productive due to accumulation of more nutrients and ions as reflected this study also.

Net production (NPP, mgCm⁻³h⁻¹) also, in most centres, followed the same trend as observed for GPP and attained the value of around 60-65% of GPP in most occasions irrespective of seasons. Community respiration (CR, mgCm⁻³h⁻¹) varied widely amongst the sampling stations and ranged from 16-85, 17-125 & 9-75 mg in PRM, MON and POM seasons respectively. P:R ratio (GPP:CR), an indicator of organic pollution, registered moderate amplitude of variation (1.30-3.66, 1.27-1.51 & 1.47-5.20) in up-stream and higher values (2.25-6.65, 1.50-7.00 & 1.39-5.30) in downstream during PRM, MON & POM periods respectively, reflecting that contribution to respiration component was predominantly by phytoplankton biomass.

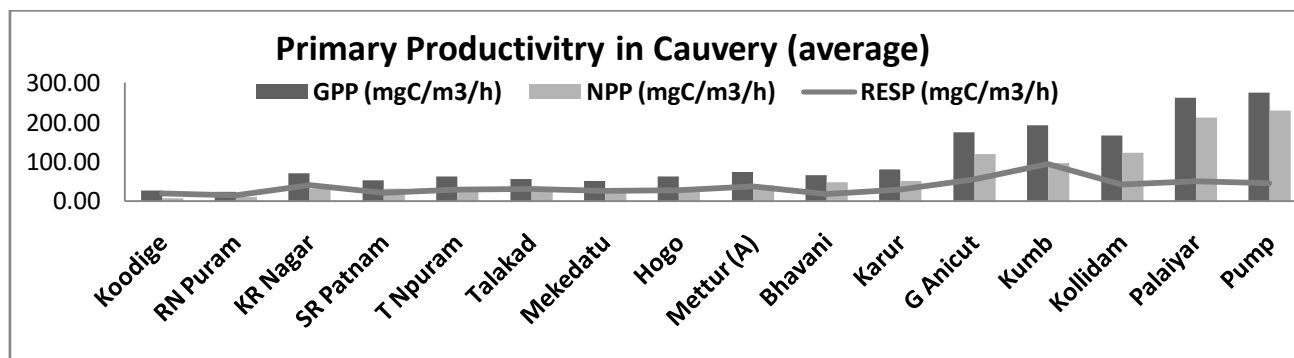


Fig. 10: Primary productivity of R. Cauvery

X. INFLUENCE OF SOIL-WATER QUALITIES ON GPP

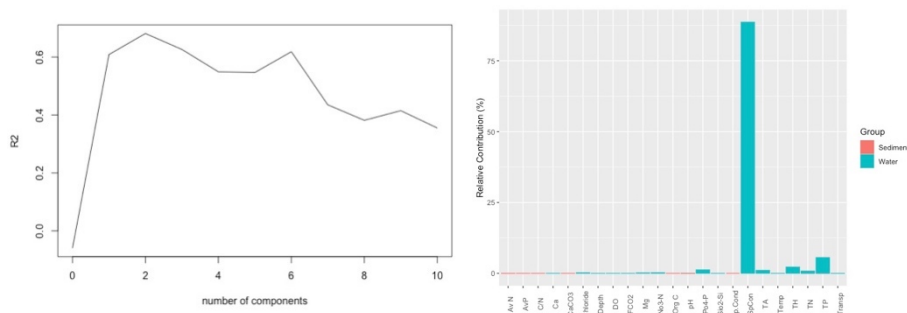


Fig. 13: R² against the number of components for latent variable selections (Left panel). Relative contribution of soil-water quality on GPP in R. Cauvery

The PLS with two components resulted in maximum R² (Fig. 13), suggesting two components suffice to explain GPP. These two components explain 95.8% variability in the soil-water qualities and 76.6% variability in GPP, which are quite good for data interpretation. The relative contribution of soil-water quality variables to the GPP revealed that specific conductivity of water has the highest influence as compared to other parameters (Fig13; Right panel). In order of relative magnitude, the top five influential variables on GPP are as follows: specific conductivity (88.7%) > TP (5.5%) > TH (2.2%) > PO₄-P(1.2%) > TA (1%), all of which represent the water qualities. This implies that water quality parameters precisely specific conductivity is more influential to GPP than the soil quality parameters.

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Table 1: Sampling Stations in R. Cauvery with GIS locations and their habitats

No.	Stations	Lat (N)	Long (E)	State	Habitat
1	BHG	12°23'4.7"	75°32'4.4"	Karnataka	Very narrow, sheet flow in summer
2	KNG	12°17'50.6"	75°47'54.4"	Karnataka	Kannige confluence, narrow sheet flow
3	KOG	12°27'28.7"	75°57'34.41"	Karnataka	Flowing through gorgy site, sheet flow
4	RNP	12°36'23.07"	76°5'21.18"	Karnataka	A sanctuary, deep pool
5	KRN	12°28'7.62"	76°23'54.15"	Karnataka	Backwaters of KRS reservoir
6	SRP	12°24'12.26"	76°43'26.26"	Karnataka	Deep pool, gorgy sporadic pool
7	TNP	12°12'5.19"	76°54'53.06"	Karnataka	Deep pool, river flattened
8	TNPK	12°6'32"	76°20'17"	Karnataka	Kabini confluence point with R. cauvery
9	MUD	12°13'32.07"	77°02'10.11"	Karnataka	A deep pool, year round water, fish hub
10	TAL	12°11'27.08"	77°1'42.88"	Karnataka	Deep pool type with low depth
11	SIV	12°17'38.40"	77°10'4.80"	Karnataka	Rocky widened gorgy falls
12	MKD	12°16'20"	77°26'25"	Karnataka	Gorgy river basin, fish sanctuary
13	MKDA	12°17'19.48"	77°25'54.67"	Karnataka	Arkavathy confluences diverting sewage
14	HOG	12°06' 54.0"	77°46'42.5"	T Nadu	Flattened river, rocky bad, falls, fish hub
15	META	11°48'58"	77°48'38"	T Nadu	Lotic of Mettur reservoir, flattened, rocky
16	METO	11°47'10.51"	77°48'2.81"	T Nadu	Meettur reservoir outfall point
17	METB	11°47'59.99"	77°47'59.99"	T Nadu	One km down to Mettur outfall, flattened
18	BHVA	11°28'15"	77°6'50"	T Nadu	Bhavani reservoir lotic, flattened
19	BHVO	11°28'44.95"	77°8'2.89"	T Nadu	Bhavani outfall point in R. cauvery
20	BHVB	11°30'39"	77°12'57"	T Nadu	One Km down to out fall point of Bhavani
21	KAR	10°57'27.76"	78°4'51.42"	T Nadu	Out fall point of R. Noyyal at Cauvery
22	KARA	10°24'38.39"	77°15'36"	T Nadu	Amaravathy confluence point, flattened
23	UAN	10°52'58"	78°34'58"	T Nadu	Upperanicut, flattened, mixed terrain
24	GAN	10°49'54.5"	78°49'22.2"	T Nadu	Grandanicut, flattened, mixed terrain
25	KMCV	10°57'35.33"	79°22'51.23"	T Nadu	Kumbakonam Cauvery alluvial basin
26	KMCO	10°57'43.52"	79°23'28.46"	T Nadu	Kumbakonam Coleroon alluvial basin
27	KOL	10°58'5.7"	79°22'41.5"	T Nadu	Kollidam fertile basin flattened

28	VET	10°48'41.41"	79°48'35.39"	T Nadu	Vettaru-narrow flattened fertile basin
29	VEN	11°48'58"	77°48'38"	T Nadu	Vennaru-narrow flattened fertile basin
30	GAC	10°49'43.3"	78°49' 2.6"	T Nadu	Grandanicut canal-narrow, fertile basin
31	PAL	11°2'12.41"	79°32'49.75"	T Nadu	Eastuarine, sandy basin Palaiyar
32	PUM	11°8'36.60"	79°51'25.29"	T Nadu	Eastuarine, sandy basin Pumpuhar

Table 2: Pre-monsoon physical characteristics of R. Cauvery

Sampling stations	Width (m)	Depth (m)	Flow (cm/sec.)	Temp. (AW) (°C)	Transp. (cm)
Bhagamandala	3.0-3.5	0.25-0.50	15	30/25	Bottom
Kannige T	2.0-2.5	0.25-0.50	10	30/25.5	Bottom
Koodige	30-40	0.8-1.0	35		Bottom
R.N. Puram	100-150	0.5-0.7	30	32/28.5	Bottom
K.R. Nagar	80-100	0.25-3.0	25	33/30	100
S.R. Patnam	70-90	0.5-2.0	35	32/29	85
T.Narasipura	80-100	0.5-1.5	20	32/29	100
TNP Kabini T	50-70	0.5-2.0	25	32/28	80
Muduthere	60-80	3-5	20	32/28	130
Talakadu	70-90	0.5-2.5	25	31/28.5	200
Mekedatu	15-20	0.5-0.8	40	31/29	bottom
Arkavathy T	15-18	0.3-0.4	30	31/29.5	bottom
Hogennakal	100-150	1-1.5	40	32/30	90
Mettur (A)	500-550	2.0	50	29.5/26.2	200
Mettur (O).	500-560	0.5-0.8	60	29.5/26.8	25
Mettur (B)	520-570	2.0	50	29.5/26.4	140
Bhavani (A)	350-400	1.0	50	31.5/29	100
Bhavani (O)	400-450	1.3	60	31.5/29.1	95
Bhavani (B)	400-480	1.5	45	31.5/29.0	97
Karur	500	0.7-0.8	70	30.4/29.1	70
Upper Anicut	20-25	0.5	nil	30.2/27	50
Grand Anicut	75	0.5	60	30.5/26.2	30
Kumbakonam-Cauvery	Dry				
Kumbakonam-Coleroon	100	1.0	35	30.7/26.2	30
Kollidam	200	1.0-1.5	35	30.5/27	40
Palaiyar	650	7-7.5	Sheet flow	30.8/30	500

A=Air, W=Water



Relationship between Fish Length and Otolith Shape of *Sargocentron Spiniferum* (Forsskål, 1775 from Shalatin, Red Sea, Egypt

By Yassein A.A. Osman, Samia M. El-Mahdy, Ashraf S. Mohammad & Kélig Mahé

Abstract- Otolith morphology analysis is one of the main tools used for fish or stock identification. Moreover, otolith shape can also be used in the fish feeding studies (stomach content) for the identification of prey fish and their size according to the relationship between fish and otolith sizes. In the present study, the relationship between fish length and otolith morphological dimensions was investigated for *Sargocentron spiniferum* (Forsskål, 1775) (family: Holocentridae). The samples (185 fish and 370 sagittal otoliths) were collected from the coast of the Red Sea, Egypt. The statistical analysis was undertaken in two steps using generalized linear models for the relationship between body length and weight and otolith morphology descriptors (length, width, area, and perimeter) and shape indices (aspect ratio, compactness, form factor, rectangularity, roundness, ellipticity, squareness, sulcus, and ostium). From the relationships between total length (TL) of fish and fourteen morphology descriptors and shape indices, three are significantly correlated with TL (otolith length, cauda, and squareness) where the side effect were $p < 0.05$. The our results provide more information for the relationship between otolith morphometric and fish length.

Keywords: fish length, otolith shape, *S. spiniferum*.

GJSFR-E Classification: LCC Code: QL638.H64



RELATIONSHIPBETWEENFISHLNGTHANDOTLITHSHAPEOFSARGOCENTRONSPINIFERUMFORSSKALL1775FROMSHALATINREDESEAEGYPT

Strictly as per the compliance and regulations of:



RESEARCH | DIVERSITY | ETHICS

Relationship between Fish Length and Otolith Shape of *Sargocentron Spiniferum* (Forsskål, 1775 from Shalatin, Red Sea, Egypt

Yassein A.A. Osman ^α, Samia M. El-Mahdy ^ο, Ashraf S. Mohammad ^ρ & Kélig Mahé ^ω

Abstract- Otolith morphology analysis is one of the main tools used for fish or stock identification. Moreover, otolith shape can also be used in the fish feeding studies (stomach content) for the identification of prey fish and their size according to the relationship between fish and otolith sizes. In the present study, the relationship between fish length and otolith morphological dimensions was investigated for *Sargocentron spiniferum* (Forsskål, 1775) (family: Holocentridae). The samples (185 fish and 370 sagittal otoliths) were collected from the coast of the Red Sea, Egypt. The statistical analysis was undertaken in two steps using generalized linear models for the relationship between body length and weight and otolith morphology descriptors (length, width, area, and perimeter) and shape indices (aspect ratio, compactness, form factor, rectangularity, roundness, ellipticity, squareness, sulcus, and ostium). From the relationships between total length (TL) of fish and fourteen morphology descriptors and shape indices, three are significantly correlated with TL (otolith length, cauda, and squareness) where the side effect were $p < 0.05$. The our results provide more information for the relationship between otolith morphometric and fish length.

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I. INTRODUCTION

The sabre squirrelfish *Sargocentron spiniferum* (Forsskål, 1775) is a member of the family Holocentridae, which is mainly distributed in the Indo-Pacific from the Red Sea and East Africa to the Hawaiian Islands and Ducie Islands extending south to Australia. It is also distributed in New Caledonia, north to southern Japan and the Ogasawara Islands (Randall, 1998; Randall and Greenfield, 1999). This species inhabits different reef areas between reef flats in lagoons and seaward reefs at a depth of 122 m. This fish occurs under ledges during the day (Lieske and Myers, 1994), and when smaller in size, this fish inhabits shallow protected areas. It is a nocturnal fish that feeds on crabs, shrimp, and small fishes (Kuitert and Tonzuka, 2001).

Many researchers have been focusing on the study age and growth of fishes due to the clear and distinct growth rings of sagittal otoliths. Otolith has calcified structures and located at the right and left inner

ears of fishes and it is useful in the study of fish biology, ecology, and fisheries science (Lecomte-Finiger, 1999; Tuset *et al.*, 2003; Jawad *et al.*, 2017; Mehanna *et al.*, 2019; Serdar and Derya, 2020). Also, the otoliths are used to estimate movement, varied habitat, population dynamics, and trophic ecological level of ecosystem for fish species (Campana and Casselman, 1993; Tuset *et al.*, 2003a; Cardinale *et al.*, 2004; Rooker *et al.*, 2008; Zorica *et al.*, 2010; Morat *et al.*, 2012; Yilmaz *et al.*, 2014; Osman *et al.*, 2020).

Otolith shape and dimension are used to identify fish species or stocks. However, a recent study showed that the directional asymmetry between right and left otoliths within individuals could affected the results from the otolith shape analysis as tool to identify the stocks (Mahé *et al.*, 2018b). So, the aim of the present work was to estimate the relationship between fish size and otolith size (length, width, weight, form factor, and aspect ratio) to identify this species from the Egyptian water of the Red Sea.

II. MATERIALS AND METHODS

Fish species were randomly collected from the southern Red Sea at the Shalateen fishing port, which is located 520 km south of Hurgada (Fig. 1), Egypt, during March 2018 to February 2019. The fish were obtained from the commercial catch of the hook and line fishery at Shalateen fishing ground. In the laboratory, total fish length (TL) was measured to the nearest 0.1 mm, and fish weight (W) was recorded to the nearest 0.01 g. Then, the sex was determined. The total length and body weight of the species ranged between 17.7 and 45.8 cm and 101.5 and 1632 g respectively. Sagittal otoliths (370 left and right otoliths) were extracted from the inner ear of 185 *S. spiniferum*, cleaned and dried. Otolith weight (OW) for each head side was measured using a digital balance AS220 k/1 to the nearest 0.0001 g. Otolith outlines were realised using a Euromex-CMEX- 10 PRO camera with a stereomicroscopic. Otolith length (OL, mm), otolith area (OA, mm²), otolith perimeter (OP, mm), sulcus (SU), ostium (OS), Form factor (FF), Aspect ratio (AR), circularity (CI) rectangularity (RE), Round (RO), Ellipticity (EL), Compactness (C), and Squareness (SQ) were extracted using Image J analysis software (Rohlf, 2006) (detailed

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descriptions is in table 1 and Figure 2). The shape index factors (FF, C, AR, CI, RO, RE, EL, and SQ) were calculated according to different formulae (Table 1) (Russ, 1990; Tuset et al., 2003b; Pavlov et al., 2015; Mahe et al., 2016; Zischke et al., 2016; Jawad et al.,

2017; Kabakli and Ergüden, 2018; Mahé et al., 2018a; Osman et al., 2020). The main step was modelled the relationship of body length with the otolith outline indices according to the side (S):

$$\log LT \sim OL + OW + OH + OA + OP + SU + OS + FF + AR + CI + RE + RO + EL + C + SQ + OL:S + OH:S + OW:S + OA:S + OP:S + SU:S + OS:S + FF:S + AR:S + C:S + SQ$$

Statistical analyses were performed in the statistical environment R (Fox and Weisberg, 2011) stats package (R Development Core Team 2016).

The otoliths of *S. spiniferum*, shape of the sulcus acusticus, sulcus type, ostium, the caudal, dorsal and ventral marginal have similar descriptions, and the characteristics of an otolith of *S. spiniferum* were detailed descriptions are in Table 2). A general pattern of *S. spiniferum* sagittae can be recognized in adult individuals.

Otolith shape of *S. spiniferum* is ovate with sinuate margins and the otolith is very thick. The sulcus acusticus is ostial with the Heterosulcus and ostium formed by a short funnel-like ostium that opens to the anterior margin and closed tubular cauda at least two times larger than the ostium (Figure 2 and Table 2). The statistical description and paired t-test results for left and right otoliths of *S. spiniferum* are given in Table 3.

The analysis of the relationship between fish length and fourteen otolith shape descriptors using a generalized linear model showed that there is a significant relationship between eight otolith parameters (ostium, cauda, otolith area, otolith perimeter, compactness, form factor, circularity, and squareness), with the total length of fish. Only the relationship of body length with the otolith length, cauda and squareness ($P < 0.05$) was significant for right and left otolith (Table 4).

The correlation between fish total length and otolith morphology showed that with the increase of the total length, the otolith morphology such as otolith length, width, sulcus, ostium cauda, area, and perimeter also increase (Figure 3). These relationships between body length and otolith measurements (left and right) were best fitted as linear regression. On the other hand, the relationship between body length and the aspect ratio was close to 1; confirming that the otolith of *S. spiniferum* was oval. There is a positive relationship between total length and the aspect ratio. The same was noticed for the compactness and circularity values where they increased as the TL increased. The form factor, rectangularity, roundness, ellipticity, and squatness values decreased as the TL decreased.

III. DISCUSSION

The sabre squirrelfish *Sargocentron spiniferum* (Forsskål, 1775) contribute to important fisheries, specially the small artisanal fisheries at Shalatin fishing

ground, Red Sea, Egypt. From this point, basic data on the biology and dynamics of the species are essential for successful stock assessment and consequently in fisheries management.

The observed fish length and shape of the otolith in this study should encourage more research to verify the essential role of otolith morphometric measurements in fish stock identification. The strong correlation between the somatic length and otolith size suggests that somatic growth has a significant influence on the otolith growth (Jockusch, 1997; Cardinale et al., 2004). The results of this study were not similar to those previously obtained for the redcoat *S. rubrum* (Kabakli and Ergüden, 2018), where the present study have max otolith length, weight and height than *S. rubrum*. Previous studies have focused mainly on the relationship between otolith measurements and fish length (Harvey et al., 2000; Fossen et al., 2003; Lychakov et al., 2006; Morat et al., 2008; Pavlov, 2016; Mehanna et al., 2016; Osman et al., 2020). In our study, the results of generalized linear model showed the relationship among these parameters as the otolith length could be affected by the choice of the otolith (significant asymmetry between right and left otoliths). Based on the present data, the relationship between TL and AR, CO, and CI was determined as a linear relationship, despite the relationship among TL and FF, RE, RO, EL, and SQ being determined as a nonlinear relationship.

On the other hand, the shape of otolith from different geographical areas is influenced by abiotic ecological parameters (e.g salinity, temperature) and biotic parameters for examples prey availability, and depends on individual genotype (Cardinale et al. 2004; Gagliano and McCormick, 2004; Swan and Palmer, 2006; Vignon and Morat, 2010). So, an interaction of environmental and genetic fluctuation generates the morphological variance in shape of otolith that may allow the differentiation of stock units. However, the factors that affects the shapes are not fully understood and have not been investigated deeply yet (Burke et al., 2008). An on-going recent work displayed that the ontogenetic trajectory of otolith shape could be impacted by the environmental disturbance during early life stage (Vignon, 2018).

The relationships between fish size and otolith shape indices demonstrate the high variability in fish length and morphometric parameters, indicating that the otolith of *S. spiniferum* is rectangular to oval. As results

in the current study, the fish size and otolith morphometric parameters are useful for further research on verifying the role of otoliths identification, discrimination and taxonomic classification of fish. Also, the results showed that the otolith shape indices significantly differed from species to species, although the indices indicate a similar pattern for otoliths. The results are reliable with that illustrated that otoliths are widely used for the discrimination and variation of fish species because of their form, diet, weight, and growth (Tuset *et al.*, 2008; Bacha *et al.*, 2010).

Finally, the estimation of the generalized linear model supposed in the present work may be good tool to study the relationship between fish and otolith morphometric features used to fish population dynamics, stomach contents of piscivorous predators, paleontological composition, and yield estimates.

IV. CONCLUSION

Finally, the estimation of the generalized linear model supposed in the present work may be good tool to study the relationship between fish and otolith morphometric features used to fish population dynamics, stomach contents of piscivorous predators, paleontological composition, and yield estimates.

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Table 1: Otolith shape indices (OL: length; OH: width; OA: area; OP: perimeter)

Otolith shape indices	Formula
Aspect ratio, AR	(OH / OL)
Compactness, CO	(OP ² / OA)
Form factor, FF	$4 \pi \text{ OA} / \text{OP}^2$
Rectangularity, RE	OA / (OL * OH)
Roundness, RO	$4 \text{ OA} / \pi \text{ OL}^2$
Ellipticity, EL	(OL - OH) / (OL + OH)
Circularity, CI	OP ² / OA
Squareness, SQ	OA / (OL*OW)

Table 2: Summary of descriptive statistics and paired t-test results for left and right otoliths of *S. spiniferum*

		N	Minimum	Maximum	Mean	Std. Deviation	Paired T Test
OL	Left	185	9.54	14.11	11.91	0.91	0.0880
	Right		9.56	14.11	11.88	0.93	
OH	Left		5.75	9.97	7.55	0.86	0.3790
	Right		6.04	9.97	7.57	0.83	
OW	Left		0.0365	0.1574	0.0648	0.0017	0.1931
	Right		0.0255	0.1572	0.0643	0.0017	
OSL	Left		8.18	13.75	10.47	1.11	0.0000
	Right		8.15	13.73	10.31	1.13	
OOs	Left		2.56	5.86	4.21	0.60	0.1120
	Right		2.58	5.98	4.26	0.64	
OCu	Left		4.84	8.04	6.27	0.72	0.0000
	Right		4.87	8.03	6.05	0.72	
OA	Left		43.44	91.29	66.89	0.729	0.18
	Right		44.44	91.33	66.90	0.727	
OP	Left		25.01	40.01	32.278	0.194	0.17
	Right		25.01	40.00	32.276	0.194	

Otolith parameters are following OL, length- mm; OH- width, OW- otolith weight; mm; OSL- otolith salcus length, mm; OOs- otolith ostium, mm, OCu- otolith cauda; OA- area; OP- perimeter.

Table 3: Description of the otolith morphological structure of *S. spiniferum*

Characters	<i>S. spiniferum</i>
Otoliths shape	Ovate
Otolith width	Thick
Posterior	Round
Anterior	Lobed
Sulcus acusticus	Ostial
Sulcus types	Heterosulcioid
Ostium	Flared, shallow, floor
Cauda	Flared, deep, floor
Dorsal margin	Sinuate
Ventral margin	Crenate



Figure 1: Egyptian Red Sea map showing the studied Hurghada area

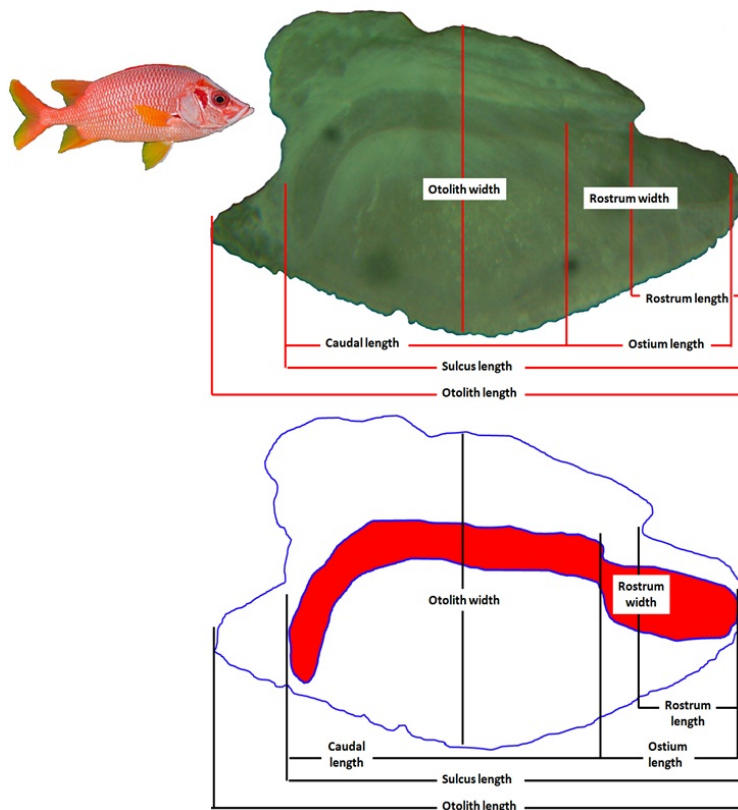


Figure 2: Diagram and scheme of the otolith of *Sargocentron spiniferum* illustrating various features of the otolith measurements

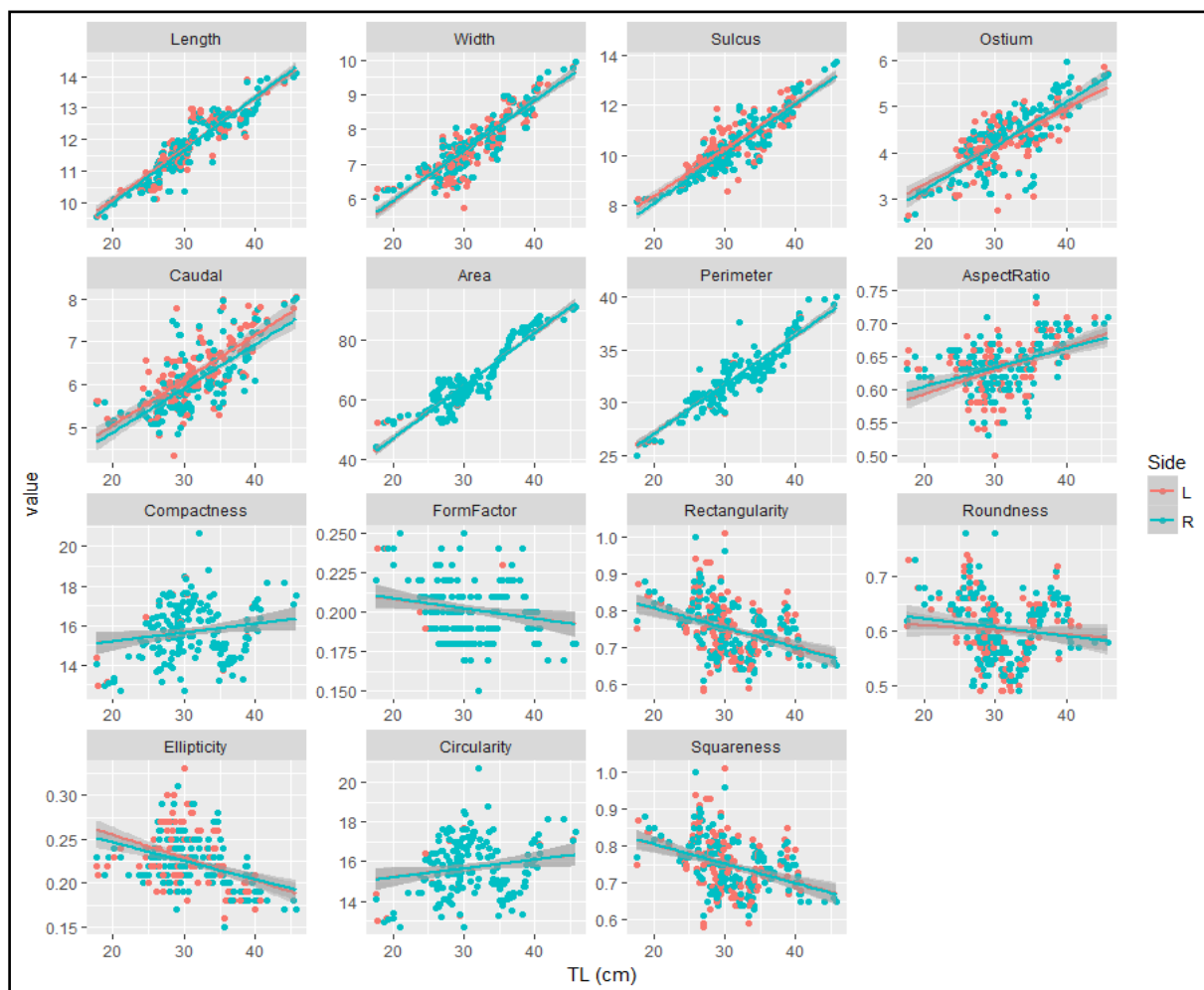


Fig. 3: Relationships of body length with the otolith morphological structures (points red, left otolith and points green= right otolith) according to the side of *S. spiniferum* captured from Hurghada, Red Sea, Egypt

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Effects on DNA and Antioxidant System of *Anabas Testudineus* on Experimental Exposure to a Sublethal Concentration of Methylmercury

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Abstract- Mercury is the most toxic in the organic form like methylmercury (MeHg), with an ability to accumulate in the cells of aquatic organisms like fish. This has been known to adversely affect the functions of almost every aspect of life. This study examined the effects of MeHg exposure on certain antioxidant defences in the liver and muscle of climbing perch (*Anabas testudineus*) adults and juveniles; the effect on DNA of erythrocytes were also studied. After 15 d of MeHg exposure ($87.6 \mu\text{g L}^{-1}$), the effect of MeHg on DNA of the fish were analysed using comet assay and the activities of SOD (superoxide dismutase), catalase (CAT) and glutathione (GSH) alongwith lipid peroxidation levels (LPO) in the form of TBARS were used as antioxidant markers. The juvenile erythrocytes showed more DNA damage in the form of comets than adults. The activities of SOD significantly increased in all the experimental groups and tissues whereas catalase (CAT) activity increased in adult liver and muscles but juvenile liver reported a decrease. Glutathione (GSH) levels showed difference in induction depending on the age of the fish with adult liver and muscle showing decrease and juveniles showing significant increase at 15 days of exposure.

Keywords: mehg, SCGE, antioxidants, comets, anabas testudineus.

GJSFR-E Classification: DDC Code: 572.86 LCC Code: QH450.2



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Swetha, S. ^α & Pramod Kiran, R. B. ^σ

Abstract- Mercury is the most toxic in the organic form like methylmercury (MeHg), with an ability to accumulate in the cells of aquatic organisms like fish. This has been known to adversely affect the functions of almost every aspect of life. This study examined the effects of MeHg exposure on certain antioxidant defences in the liver and muscle of climbing perch (*Anabas testudineus*) adults and juveniles; the effect on DNA of erythrocytes were also studied. After 15 d of MeHg exposure (87.6 $\mu\text{g L}^{-1}$), the effect of MeHg on DNA of the fish were analysed using comet assay and the activities of SOD (superoxide dismutase), catalase (CAT) and glutathione (GSH) alongwith lipid peroxidation levels (LPO) in the form of TBARS were used as antioxidant markers. The juvenile erythrocytes showed more DNA damage in the form of comets than adults. The activities of SOD significantly increased in all the experimental groups and tissues whereas catalase (CAT) activity increased in adult liver and muscles but juvenile liver reported a decrease. Glutathione (GSH) levels showed difference in induction depending on the age of the fish with adult liver and muscle showing decrease and juveniles showing significant increase at 15 days of exposure. whereas exposure to MeHg did not remarkably affect CAT activity. The levels of lipid peroxidation (LPO) increased in a time dependent manner showing tissue specificity with the higher levels in the liver than muscles. Overall, higher sensitivity to oxidative stress induced by MeHg was detected in the liver than the muscle. These findings improve our understanding of the tissue-specific accumulation of heavy metals and their roles in antioxidant responses in marine fish subjected to MeHg exposure.

Keywords: mehg, SCGE, antioxidants, comets, anabas testudineus.

I. INTRODUCTION

Aquatic ecosystems act as a sink for many contaminants that could cause various physiological damage which is not restricted to molecular, biochemical, cellular and physiological damage[1]. According to International Agency for Research on Cancer (IARC) methylmercury (MeHg) is classified as an element belonging to the group 2B, making it a possible human carcinogen[2]. Many studies have been conducted on the effect of such toxic chemicals upon fish genome and antioxidant system[3]. The high toxicity of heavy metals along with their

bioaccumulative property make them very important when it comes to environmental contamination as they are able to induce damage in the genes[4]. Genotoxicity studies are extremely important in aquatic ecosystems, as the toxic substances can accumulate in water [5] and fish respond in manner similar to higher vertebrates, and may be used to estimate genotoxic effects that such compounds may cause in animals especially the humans [6]. However, research into clastogenic or mutagenic effects in tropical air-breathing fishes exposed to contaminants is scarce [7].

Mercury and some organomercurial compounds cause genomic damage due to their effect on tubulin, the component of spindle fibers and important for cytoplasmic organization. The impairment of polymerization causes metaphasic chromosome contraction, delay of centromeric division with slow anaphasic movement[8]. MeHg also increases the reactive oxygen species (ROS) or free radicals which accelerates reactions that induce genotoxicity.

The main line of defence against pollutants and xenobiotics is the antioxidant system, with components that prevent ROS from being formed or removing it when formed, preventing damage to the vital components of the cell [9]. ROS are also involved in cell functions like signalling; hence, antioxidant system does not entirely remove oxidants, but instead, keeps the quantity at the optimum level [10]. MeHg is known to produce ROS and generate oxidative stress leading to stress damage [11]. Inorganic mercury showed high oxidative-stress-inducing potential in *B. amazonicus*[12]. Such changes in the enzyme activity have been used as an early warning system to detect the potential to cause adverse effects well before the onset of serious pathological damage in the animals exposed to toxicants [13]. The cell defences against ROS include scavenger compounds such as glutathione and enzymes with antioxidant activities such as SOD, CAT and GPx[14]. The ROS have the capacity to react with lipids, proteins and nucleic acids, which leads to several biochemical injuries. The changes in the antioxidant activities show a shift in the equilibrium in the organism [15] and the induction or decrease in the enzyme activities in relation to oxidative stress is used as the marker for the presence of hazardous substances [16]. These changes are more pronounced in aquatic

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organisms as they are more sensitive compared to those on land [15].

This study aimed to evaluate genotoxicity by using comet assay on the erythrocytes and the impact on a few components of the antioxidant system in muscles and liver of *Anabas testudineus* (Bloch, 1840) exposed to a sublethal concentration of MeHg.

II. MATERIALS AND METHODS

a) Test Animal

The test organism *A. testudineus* (Fig. 1.) was selected as per the criteria put forth by Rand and Petrocelli [17].

b) Test Chemical (Toxicant)

In the aquatic environment, methylmercury (MeHg) is produced by the action of bacteria on elemental and inorganic mercury. MeHg is a very potent neurotoxin with potential to accumulate in the body of the organism. An organic compound of mercury is used in the experiment. The chemical in the form of the iodide salt (MeHgI) of 98% purity was acquired from Alfa Aesar, England. The stock solution of 100 ppm was prepared from the salt which was diluted as needed for the experiment.

c) Median lethal concentration

The median lethal concentration (96h LC₅₀) for the animal was observed to be 438.21 µg L⁻¹ (95% fiducial CI 314.65 – 610.32 µg L⁻¹) or 0.438 mg L⁻¹ by probit analysis [18].

For the experimental exposure, 25 fish each of juvenile (average length 9.27 ± 0.62 cm and weight 11.96 ± 2.36 g) and mature (average length 13.96 ± 0.93 cm and weight 36.86 ± 10.10 g) life stages were utilised as experimental and control groups. The sublethal concentration was (LC₅₀/5); 87.6 µg L⁻¹ of MeHg. The fish were exposed as per the semi-static renewal test with the toxicant water renewed every four days and with intermittent feeding.

d) Comet Assay

At the end of the 15-day exposure period, the exposed fish were sampled along with the control fish. Five fish each were used for the analysis. The blood from the fish was obtained by cardiac puncture and transferred into heparinized tubes. For the comet assay, the method of Singh *et al.* [19] was used. The slides were photographed using inverted epifluorescent microscope (Olympus CKX41) attached with camera (Opitka Pro5 CCD). Comets were scored using Tritex comet scoring software and correlated statistically.

e) Antioxidant assay

After exposing to MeHg, five fish from exposure groups along with those from the control experiment were sacrificed by spinal dislocation. The fish were weighed, and the liver and muscle were removed and

weighed. The tissues for the biochemical analyses were stored at –80°C until use. The dissected tissues were homogenized in a 1:15 ratio (1 g tissue:15 mL buffer), using a chilled glass homogenizer with chilled phosphate buffer (0.1 M, pH 7.4). The homogenate was used for LPO and total GSH quantification, and to prepare the post-mitochondrial supernatant (PMS). The homogenate was centrifuged at 13,400 g for 20 minutes in a refrigerated centrifuge (Eppendorf 5415R) and the supernatant is the PMS for antioxidant enzyme analyses. Aliquots of PMS were stored at –80°C until analysis. The antioxidant analyses were conducted as detailed below at the Vizhinjam Centre of Central Marine Fisheries Research Institute. The assays were performed by standard methods as follows. SOD was estimated [20]. The CAT assay was conducted by standard protocol [21]. The activity of CAT is expressed as picomoles of hydrogen peroxide decomposed per minute per milligramme protein. The standard method [22] was used to estimate the MDA contents formed due to lipid peroxidation. The results were calculated using the extinction coefficient of the pink chromophore (1.56 × 10⁵ M⁻¹ cm⁻¹) and expressed as nanomoles of MDA formed per milligramme protein. Total GSH was also determined [23]. The GSH content was calculated with the help of the standard graph and expressed as nanomoles per milligramme protein. The values (mean ± SD) are graphically represented.

III. RESULT

a) Comet Assay

The results showed an increase in the number of comets in both the juveniles and the adults when compared with those of the control. The DNA damage could be visualised as comets with a tail and a head, and the juveniles had more pronounced comets, whereas the control showed minimal comets but pronounced heads. In the case of the length of comets (Fig. 2) the juveniles showed more induction when compared to the adults. The lengths of the comets of the juveniles are significantly longer when compared to the controls and so are those of the adults. Both results showed high significance at $p < 0.01$ revealing that the MeHg exposure for 15 days induces significantly higher DNA damage in the erythrocytes of both juveniles and adults. Even though there are more comets formed in the juveniles than in adults, the results do not show significant difference when compared to each other.

The results do not show significant difference except in the case of juveniles compared to controls. The tail lengths are not significantly different in this case. The erythrocytes of control juvenile fish showed few comets and more of the pronounced (Fig. 3). The comets in the juvenile fish were pronounced (Fig. 4), which are caused by the single strand and double strand damage causing the smaller DNA fragments to

move out into the matrix creating the illusion of tails when stained. After 15 days of exposure to sublethal concentration of MeHg, comets were formed in most of the cells in the adults and the DNA damage was pronounced in most cases with the formation of "hedgehogs" (Fig. 6) that are considered to indicate necrotic and apoptotic cells and may not be analysed by the software used for the analysis hence, the insignificant values. Such damage was not observed in the control fish (Fig. 5).

b) *Antioxidant Enzyme Levels*

i. *Superoxide dismutase activity*

The results of the SOD activity (mean \pm SD) are presented in Fig. 7. All the values increased and are highly significant when compared with the control data. The liver and muscle of the juvenile fish were analysed and found to exhibit increase at 15th day of exposure. In the case of the adults, the liver and muscle showed trend in the SOD levels similar to that in the juveniles.

ii. *Catalase activity*

The juvenile liver showed a decrease in activity after 15 days of exposure in comparison with the control values. In the adult liver, activity is significantly increased when compared to the control. The adult muscle showed an increase over the entire time of exposure similar to that seen in the adult liver. After 15 days of exposure, the values kept increasing in both juvenile and adult muscle (Fig. 8). All the values are significant ($p < 0.01$) with all tissues other than juvenile liver showing increase in the values at 15 days of exposure.

c) *Non-enzymatic Antioxidant Levels*

i. *Lipid peroxidation activity via TBARS levels*

The quantity of MDA (measured as TBARS) formed in the control liver of the juvenile fish was

analysed to be at 276.97 $\mu\text{mol mg}^{-1}$ protein and in the mature liver, it was 541.33 $\mu\text{mol mg}^{-1}$ protein. The formation of TBARS increased in both the juvenile and adult fish liver. The juvenile liver had the value at 7706.7 $\mu\text{mol mg}^{-1}$ on day 15, whereas in adult liver it increased to 7719.9 $\mu\text{mol mg}^{-1}$ protein on day 15. The same was the case with the muscles from both the groups showing increase in the levels of TBARS formed; from values of 66.73 $\mu\text{mol mg}^{-1}$ protein to 990 $\mu\text{mol mg}^{-1}$ protein in juvenile muscle and 33.28 $\mu\text{mol mg}^{-1}$ protein to 729.30 $\mu\text{mol mg}^{-1}$ protein in adult muscle (Fig. 9). All the values obtained for TBARS are highly significant ($p < 0.01$) when compared to the control values and showed an increase which means the rate of lipid peroxidation also increased during exposure.

ii. *GSH activity*

GSH was observed to be 0.02 mmol mg^{-1} tissue in the liver of juveniles and 0.185 mmol mg^{-1} in the adult liver at the start of the experiment. With the increase in the duration of exposure to 15 days, GSH levels in the juvenile liver increased, whereas in the adult liver, it decreased. The change in the values is significant ($p < 0.01$) when compared to the control. The GSH content in the muscles of the juvenile fish in the control group was found to be 0.00974 mmol mg^{-1} tissue weight and in the mature fish, the value was 0.0178 mmol mg^{-1} (Fig. 10). The values decreased in the case of both mature and juvenile fish with significant values ($p < 0.01$) and ($p < 0.001$), respectively.

Figures



Fig. 1: Test animal - *Anabas testudineus*

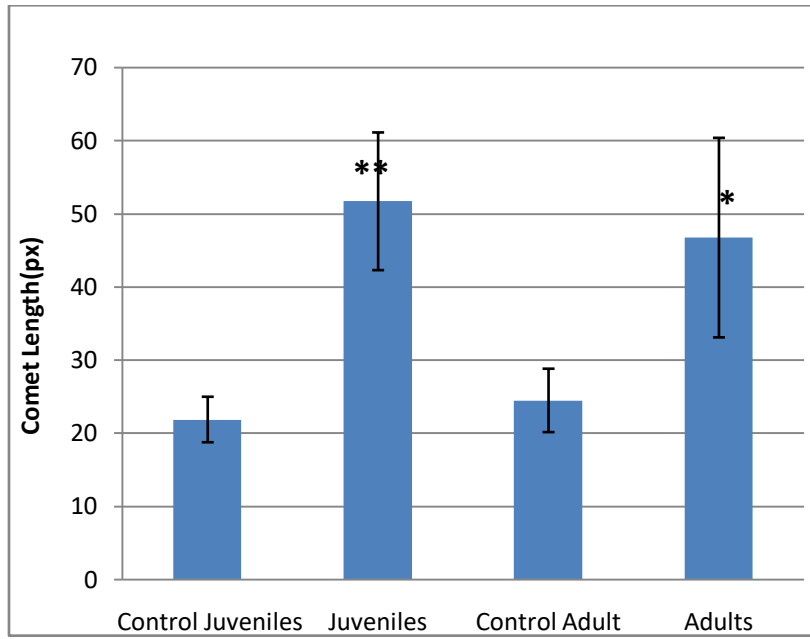


Fig. 2: Comet length in px(mean±SD) obtained in juvenile and adult test fish (** $p < 0.001$)

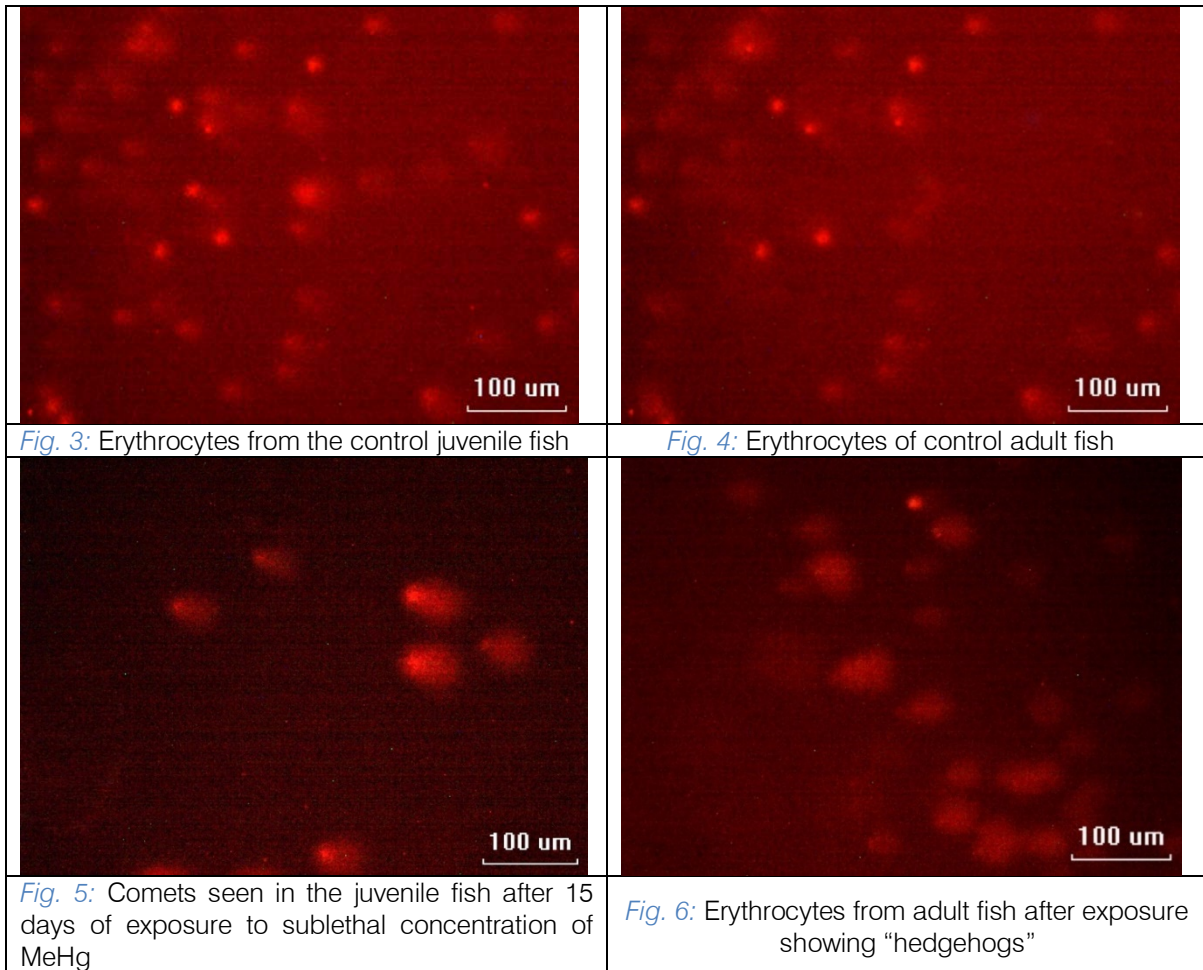


Fig. 3: Erythrocytes from the control juvenile fish

Fig. 4: Erythrocytes of control adult fish

Fig. 5: Comets seen in the juvenile fish after 15 days of exposure to sublethal concentration of MeHg

Fig. 6: Erythrocytes from adult fish after exposure showing "hedgehogs"

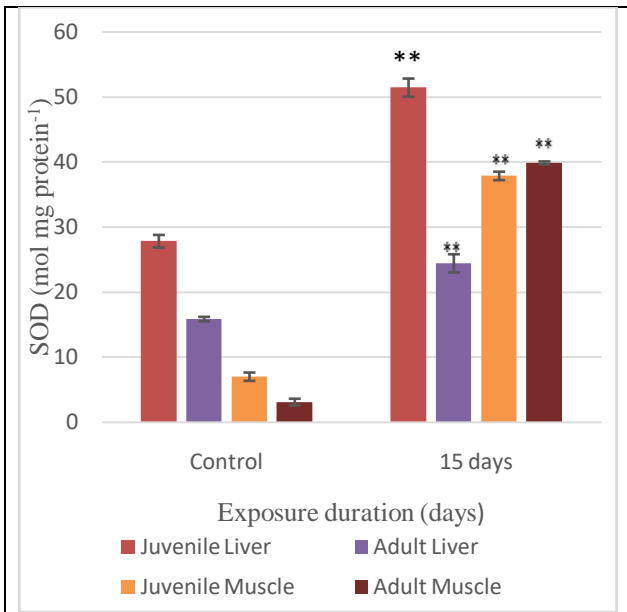


Fig. 7: SOD activity in the liver and muscles

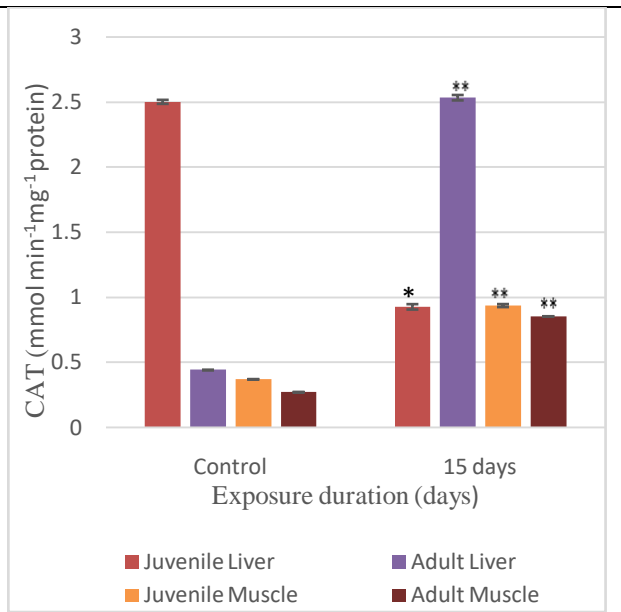


Fig. 8: CAT activity in the liver and muscle

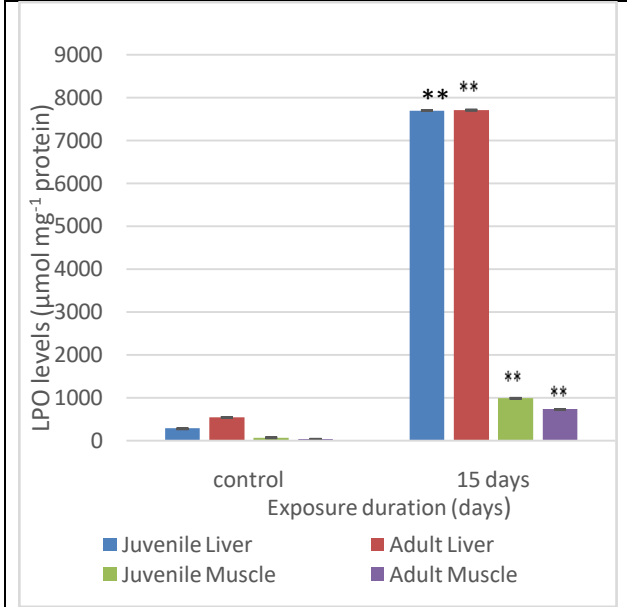


Fig. 9: MDA formed as a result of varying exposure duration of MeHg on the liver and muscle

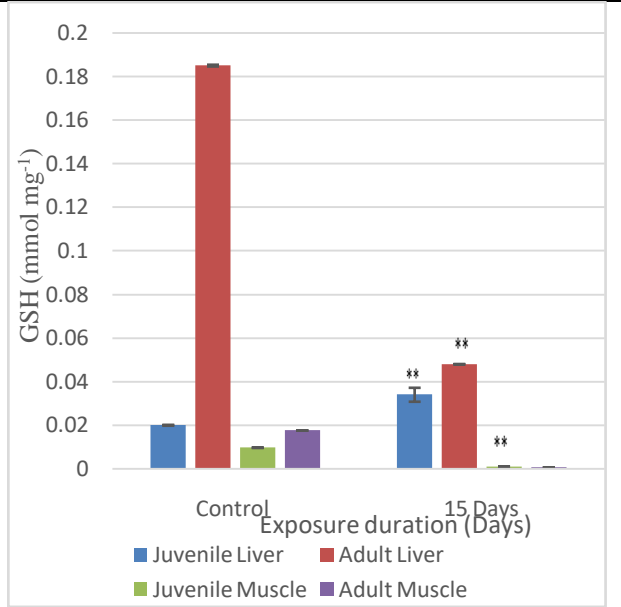


Fig. 10: Levels of GSH in the liver and muscle of juvenile and adult fish on MeHg exposure

IV. DISCUSSION

Exposure to toxins can affect the genes and alter DNA which is the carrier of information from generation to generation and the altered DNA will be inherited by the future progeny. According to Almeida *et al.*[24], mutations are any changes or damages in the DNA structure that can be inherited, create carcinogenesis or even cause cell death. Comet assay or SCGE is a rapid and sensitive method to analyse the DNA damage in any tissue [25]. Comet assay is the best procedure for the detection of the damaging effect of chemicals and pollutants under the laboratory and field conditions. It can detect genetic damage due to low levels of toxicant exposure, especially heavy metals

like mercury. The types of damages assessed are strand breaks, alkali-labile sites, DNA cross linking and erroneous as well as incomplete DNA excision repair sites. This method was used to assess the DNA damage in the lymphocytes exposed to mutagenic agents[26]. Metal salts create genotoxic effects by the generation of ROS that damage the DNA, and interfere with the DNA repair and replication processes. Creating damages like single and/or double strand breaks, DNA-DNA cross-links, DNA protein cross-links and base modifications[27]. Comet assay has been used to quantify DNA damage in single cells [28] under laboratory conditions [29].

The study conducted on the DNA damage caused due to the exposure of the juvenile and adult fish

to the toxicant reveals that MeHg is highly genotoxic. In this study, the lengths of the comets of the juveniles are seen to be significantly higher when compared to those of the controls; same is the case with the adults. Both the results are highly significant at $p < 0.01$. This result shows that MeHg exposure induces significantly higher instances of DNA damage in the erythrocytes of both juveniles and adults. Though more comets were formed in the juveniles than in adults, the statistical analyses do not show significant difference when compared to each other. It is considered that the longer the comet length, the more damaged the DNA is. The DNA damage as per the comet assay shows that the more affected are the juveniles, but the DNA in the adults seem to be highly fragmented creating more of ghosts than comets. Genotoxic agents produce DNA damage that can be either repaired or the damage is irreversible and continues, and such the damage can eventually lead to cell death. MeHg has the ability to damage cellular macromolecules like lipids and DNA by oxidative damage [30].

The sublethal concentration of MeHg in static conditions to which the *A. testudineus* were exposed to, was sufficient to induce significant alterations in SOD antioxidant enzymes such as and CAT inducing probable oxidative damages in lipids and proteins and, consequently, oxidative stress. SOD and CAT activities increased after 96 hours of exposure to mercuric chloride in all the tissues of *B. amazonicus* [12]. SOD catalyses the dismutation of O_2^- to water and hydrogen peroxide, which is detoxified by CAT. Due to the inhibitory effects on ROS formation, the SOD-CAT system provides the first defence-line against oxygen toxicity due to metals [31] and is usually used as a biomarker indicating ROS production [32; 16]. The induction of the SOD-CAT system indicates a fast adaptive response of the redox-defence system in the liver, gills, white muscle and heart of *B. amazonicus* after exposure to mercury [12]. On the exposure to effluents, CAT in channel catfish (*Ictalurus punctatus*) showed a significant increase. The increase or decrease in the activities of antioxidant enzymes depends on the intensity and duration of the exposure of the metals they are exposed to [33].

The most important and easily available redox balance buffer in the living body happens to be glutathione [34]. It is a scavenger of the oxyradical and is important in the antioxidant defence, and also is a very important measure of the detoxification capacity of an organism [35]. The GSH molecule can also scavenge other ROS directly and is involved in various processes vital for normal cellular function such as DNA and protein synthesis [36]. The cells of the organism in contact with metals usually expel them by coupling directly with GSH. Mercury binds to the sulfhydryl groups of glutathione stopping it from functioning as a free radical scavenger [37] and causes the collapse of the

antioxidant mechanisms in the cell. This can result in cell degeneration, loss of membrane integrity and eventually, cell necrosis [38], but various studies show that the GSH activities in fishes respond differently to the exposure to mercury. In *M. cephalus* and *L. aurata*, mercury exposure shows increase in the GSH content [39], whereas in *A. testudineus*, the GSH levels decrease on exposure to $0.166 \text{ mg L}^{-1} \text{ Hg}^{2+}$ for 24 hours [40]. The levels of GSH in snakeheads increase when exposed to subacute concentrations of mercury but decrease under longer exposure duration [41]. During long-term exposure, GSH could be involved in the formation of conjugates with Hg^{2+} leading to the formation of linear II covalent complexes [42]. The cause of the depletion in GSH could be the consumption during the phase II biotransformation (involving GSH-dependent enzymes) observed more in the muscles than in the liver and gill [43]. These complexes are excreted as mercapturic acid leading to the decrease in GSH in order to eliminate mercury [44]. Thus, the decrease in the GSH content could be due to oxidative stress, accumulation and even elimination process. The liver is the site of GSH synthesis and is exported to extrahepatic tissues like kidney, brain and muscle. White muscle has fewer number of mitochondria and are not as efficient at recharging GSH due to lowered transport from the liver [45]. Thus, the depletion of GSH in the muscle is a better indicator of pollutant-based oxidative stress than that in the liver. The levels of GSH normally tend to decrease with the increase in age of the organisms [46], which further decreases during oxidative stress. The increase in the juvenile liver GSH could be due to the increased replenishment of GSH by the uptake of amino acid substrates and the activities of biosynthetic enzymes in the liver. In the case of *B. amazonicus*, the GSH content in the white muscle decreased [12] similar to the observation in the present study. This decline in GSH content may be due to the ability of GSH to bind directly with mercury or due to the non-conversion of the thiol group back to GSH by GR. In most cases, if the initial increase in the free radicals is small, the antioxidant system is capable to neutralize the effects and readjust the balance between the ROS production and ROS scavenging capacities [47].

The present study revealed higher LPO levels in liver and muscle, a clear oxidative stress indication. LPO is a complex process resulting from reactions in biological membranes causing the formation of lipid hydroperoxides. These molecules are able to fragment the double bonds of unsaturated fatty acids and disintegrate the lipids of the cellular membrane [48]. The oxidative stress was confirmed by the increases in lipid peroxidation in liver, gills, white muscle and heart of *B. amazonicus* after exposure to mercuric chloride [12]. Similarly, after mercury exposure, increases in LPO levels were also detected in the brain and kidney of the Atlantic salmon [49] and in the liver of Russian sturgeon

Acipenser gueldenstaedti[50]. This study also emphasizes the capability of antioxidants such as CAT, SOD and GSH to be biomarkers of contaminant mediated oxidative stress in a variety of aquatic organisms, and the variations in their levels are a reflection of the response of the organism to pollutants[51].

V. CONCLUSION

This study showed that MeHg at very low concentration is capable of inducing various major damages to the genetic makeup as well as the antioxidant architecture of exposed fish. MeHg induced the damage at a concentration which is very much below the permissible limits put forth by health agencies and at a very short span of time. Such damage could be detrimental to the organism, and can even create possible defects in the future generations leading to change in the population structure and the genetic makeup of the species as well as the diversity of the ecosystem. This study also reports the formation of highly fragmented DNA beyond computer recognition (ghosts) as a drawback for the comet assay. Organic mercury even at sublethal concentrations and short-term exposure is potent enough to induce an oxidative stress in the case of the air-breathing fish that is otherwise capable of surviving under extreme conditions. These changes can impair DNA and lipid membrane functions, thus affecting the homeostasis. The protective mechanisms against the toxicant-induced stress fall short of maintaining the physiological integrity. The data obtained suggest that mercury at the concentrations that are irrelevant in terms of environmental and health points of view are capable of inducing negative impact on behaviour, health status, reproduction and overall success of *A. testudineus*, a native air-breathing fish of India, making its survival vulnerable.

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Informed Consent Statement

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Conflicts of Interest

The authors declare no conflict of interest.

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Fostering Governance at Remote Marine Protected Areas in Times of Blue Economy: Baseline for Stakeholders Composition

By Daniel Hauer Queiroz Telles & Caroline Malagutti Fassina

Abstract- The Blue Economy is an emerging concept that encourages better stewardship of the ocean and associated resources. Turning it into a global issue poses several challenges to ocean conservation effectiveness especially at Remote Marine Protected Areas (ReMPA). How to implement and manage the ReMPAs is still new to modern society, and the participation criteria in the decision-making processes is undermost in a legitimate perspective. The proposed framework highlights the importance of emerging studies to untangle Oceans territorialization and use(r)s, in order to establish composition parameters for shared and realistic management. The initial application is exemplified by taking two Brazilian ReMPA. The preliminary results seek to support the priorities of Scientific innovative methodological appropriation in Ocean decision-making, as envisioning new baselines of legitimacy for ReMPAS governance. Thus, the two asymmetries found represent baseline challenges towards a framework to be considered as a starting point for ReMPA participative governance guidelines. These asymmetries pose emerging questions about how will these territories be governed since the stakeholder's composition reflections to be considered in future scholars. The three highlights argue about the questions posted above and point preliminary conclusions.

Keywords: *remote marine protected areas; blue economy paradigm; power asymmetries; stakeholders.*

GJSFR-E Classification: FOR Code: 050205



FOSTER | NGGOVERNANCEAT REMOTEMARINEPROTECTEDAREAS | NT | MESDFBLUEECONOMYBASELINEFORSTAKEHOLDERSCOMPOSITION

Strictly as per the compliance and regulations of:



RESEARCH | DIVERSITY | ETHICS

Fostering Governance at Remote Marine Protected Areas in Times of Blue Economy: Baseline for Stakeholders Composition

Daniel Hauer Queiroz Telles ^α, PhD & Caroline Malagutti Fassina ^ο, PhD

Abstract- The Blue Economy is an emerging concept that encourages better stewardship of the ocean and associated resources. Turning it into a global issue poses several challenges to ocean conservation effectiveness especially at Remote Marine Protected Areas (ReMPA). How to implement and manage the ReMPAs is still new to modern society, and the participation criteria in the decision-making processes is undermost in a legitimate perspective. The proposed framework highlights the importance of emerging studies to untangle Oceans territorialization and use(r)s, in order to establish composition parameters for shared and realistic management. The initial application is exemplified by taking two Brazilian ReMPA. The preliminary results seek to support the priorities of Scientific innovative methodological appropriation in Ocean decision-making, as envisioning new baselines of legitimacy for ReMPAS governance. Thus, the two asymmetries found represent baseline challenges towards a framework to be considered as a starting point for ReMPA participative governance guidelines. These asymmetries pose emerging questions about how will these territories be governed since the stakeholder's composition reflections to be considered in future scholars. The three highlights argue about the questions posted above and point preliminary conclusions.

Keywords: remote marine protected areas; blue economy paradigm; power asymmetries; stakeholders.

I. INTRODUCTION

Turning the Blue Economy into a global reality poses several challenges to ocean conservation effectiveness especially at remote sites and areas beyond national jurisdictions [1]. This emerging concept encourages better stewardship of the ocean and associated resources, in order to conciliate the different kinds of uses and functions of the seas in its eighty percent of global trade volumes in the economic development[2,3]. Anthropocene production flows through the ocean and impacts it at global scale [4,5,6]. The current tangible global uses of the remote ocean are predominantly about flows of goods and fisheries, but many other functions and services are precedents and gradually recognized by the ecosystem-based perspective [7]. The evidence to corroborate the importance of marine habitats to Earth equilibria as well as to human wealth is enough known [8,9,10].

Blue Economy amplifies the legitimation for the existence and enforcement of Remote Marine Protected

Areas (ReMPA) [11,12,13]. How to implement and manage the ReMPAs is still new to modern society, which are now trying to find viable ways to organize this vast ocean territory [14,15,16]. Participation criteria in the decision-making processes of ReMPAs is undermost [17]. In a universal perspective of understanding and participatory action for a 'blue' management as a common process, comes up the question: who are the legitimate stakeholders in the vocational commitments of ReMPA? This essay proposes a conceptual framework to deal with the shared assumption of the Ocean as a space of related and overlapping uses to a primarily of universal interest, which brings its Common matters and relies on broad participation. The proposed framework highlights the importance of emerging studies to untangle Oceans territorialization and use(r)s, in order to establish composition parameters for shared and realistic management. The initial application is exemplified by taking the largest, remotest, and newest Brazilian ReMPA - São Pedro and São Paulo archipelagos and Trindade e Martin Vazarchipelagos.

The preliminary results seek to support the priorities of Scientific innovative methodological appropriation in Ocean decision-making, as envisioning new baselines of legitimacy for ReMPAS governance. Thus, the two asymmetries represent the challenges of the largest ReMPA management:(i) epistemological approach of Ocean territorial status and; (ii) governance attributions within MPA users and functions. These asymmetries pose emerging questions: alongside setting 'protected' territories in the Ocean, how will these territories be governed? A government of whom and for who? The three highlights argue about the questions posted above and point towards a framework to be considered as a starting point for ReMPA participative governance guidelines.

II. REMOTE MARINE PROTECTED AREAS (REMPAS) AS A TERRITORIAL FUNCTION ON A BLUE ECONOMY PARADIGM

The marine biome covers 71% of the Earth's surface, an area that has three hundred times more habitat for biodiversity than the terrestrial sites [18,19]. Nonetheless, this isn't humankind's natural habitat, and

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that natural reason underpins the political apparent delay over the marine space. Linking to policy, the vast majority of marine environment is still beyond national jurisdiction [20] and, whilst representing the largest environment on the planet, is the least understood and governed [21,22]. While some mechanisms exist for monitoring and environmental protection in the open ocean, including capacity building and technology transfer, and environmental impact assessments and area-based management tools as ReMPA, governance gaps are evident [23,24]. There is no overarching framework for the allocation of ReMPAs, standardized guidance for marine resource management and best practices to support both biodiversity research and governance in the Blue Economy emergence [25,26,27].

The Blue Economy approach recognizes and places renewed emphasis on the critical need for the international community to effectively address resources management in and beneath international waters or National ReMPA and not only treat the ocean as a new market. This pathway requires long-term collaboration across nation-states and the public-private sectors, on a scale and dimensions that have not been previously achieved, considering the vast marine areas and the unknown resources to be explored. It underpins the thinking behind the Commonwealth, taking the Ocean as part of the economic lives, envisioning equity and public participation in marine decision-making. Such widespread collaboration needs further development and refinement of international law and ocean governance mechanisms, but also theoretical inputs of contemporary comprehension of the complexity of the Ocean space and territory [28,29].

International concerns about access to marine resources and the need to establish MPAs have been addressed since the 1960s, from the discussions resulting in the UN Convention on the Law of the Sea, which set the baseline for promoting the Blue Economy concept worldwide. Later, the Aichi Targets define MPAs as the primary strategy for Ocean's governance, launching the goal of protecting the 10% of ocean surface, under sovereign coastal Nation's treaties or its Exclusive Economic Zone [30]. Making progress on international agendas, the current Ocean Decade throws light on marine conservation alongside the SDGs goals 14 and 16 [31]. All these universally built conceptions compose the current framework for the Blue Economy as a common vocation [32]. Problems of overexploitation of the global commons can be better managed when supported by international multilateral agreements setting global rules, regulations, and standards under which states change their behavior accordingly [33,34,35]. When these policy mechanisms are addressed to a ReMPA, advanced diagnoses are brought up as the multiple uses of the same areas that have reached a conservancy vocation: governance guidelines get that territory.

A territory is an act, a relationship, a movement, and a rhythm over which an amalgamation of controls is exercised [36], just as the diversity of scales of operation over it, whether in its understanding (science) or in its intervention (governance). The territory is a permanent state of cause and condition of the multiple dimensions of analysis on the social dynamics that configure it: political, economic and cultural, autonomous, and interdependent [37]. The tenuous distinction between space and territory has a brief relevance in the theoretical composition that refers to the various phenomena that materialize in the Seascape and marine ecosystems. The territory for the modern society is the practical categorization most considered for governance and political actions, and in turn, for conservation.

The intentionality of several parties is considered for understanding the territory dynamics and uses, whether as premises (genesis of movements, not yet materialized) or in the already consolidated perspectives, playing relations of possession, of appropriation, or belonging. Territories are not inert, but lived [38], practiced and managed [39], as complex entities. There is no territorial starting point that predates the relationship, from which the basic notion of conflicts, impacts, overlaps, and complementarities [40]. Such relationships form society as a whole, in its different and complementary instances; lived, perceived, and understood differently; so that the territory comes to be understood as a social and political fact [41]. Territory uses can frame the object of social analysis that all human being lives in [42]. A territory being used compounds the space and its historical results of different forces (cultural, economic, and political) and scales, that will conform to a permanent state of transformation and power asymmetries.

If we consider the approach of ocean spaces as territories [43,44], it is possible to see a strengthening in the relations between science and governance actions in these spaces. Under the United Nations Convention on the Law of the Sea, the discussions leading to an international legally binding mechanism started to address the advent of potential conflicts between the sovereign rights connected to the continental shelf beyond 200 nm and the protective measures applied in ReMPAs[45]. The concept of adaptive co-management arises from the integration between the proposed management of common-use resources with the adaptive management approach [46,47,48,49,50,51]. It pays explicit attention to learning (experiential and experimental) and collaboration (vertical and horizontal) between actors as the real users [52].

The marine ecosystem specificities lead to requirements conditions for management activities, expressed whether in protected or non-protected areas. The MPA history of creation and management is more recent compared to the history of terrestrial protected

areas, and this gets more emphasized referring to Remote areas [53]. As one argumentation of this essay, the users of ReMPAs are less legible in the landscape than the coast MPAs, and so less intelligible, however, are more fluid, transitional, or ephemeral. Thus, the distinct biophysical characteristics between terrestrial and aquatic ecosystems resonate with community characteristics and should be considered by governance strategies, as the example of watershed terrestrial experiences have already reached. In places where there is no consolidated communitarian instance, so the contemporary Common comprehension must be brought to light since Global Agenda to the State National Marine Policy and territorial complexity conceptualization.

III. POWER ASYMMETRIES OF THE OCEAN ON THE CONSOLIDATION CHALLENGE OF BRAZILIAN ReMPAs

The ReMPAs compose a strategy of oceans' appropriation as territory, with the prior function to order uses through legal rules set by the government, the provenance of ecosystems services, and envisioning local-spread out to regional and global sustainability as an unmistakable tool [54,55,56] harnessing the importance of the protagonist of coastal communities, especially in developing countries [57]. It is essential to consider that this debate is wide enough to be

deepened when it explores the governance relations between international and national agendas, specifically about environmental policies and geopolitics. The debate goes through national state paradoxes, integrating universal agendas of global commitments with state-nation sovereignty agenda, and are embedded by societal needs. Several legal debates are already in place as a result of regional alliances such as the European Union [58,59], but the extensive international law arena is beyond the scope of this analysis. The lack of understanding of the Ocean as territory and its governance beyond national jurisdiction has permeated the plans and discussions around the preparation for the UN Ocean Decade. The two proposals for power asymmetries are presented below with the Brazilian reality as a background in order to raise the emergence of such discussions.

To elucidate the two asymmetries, it is referred to the largest and remotest Brazilian MPAs: São Pedro and São Paulo archipelagos and Trindade e Martin Vaz archipelagos (Figure 1). It is not intended - nor excluded - to present them as absolute standards, but as examples of theoretical and empirical disconnections in preliminary approaches that can illustrate the background of MPAs consolidation challenges: legal status dilemma and emergent management.

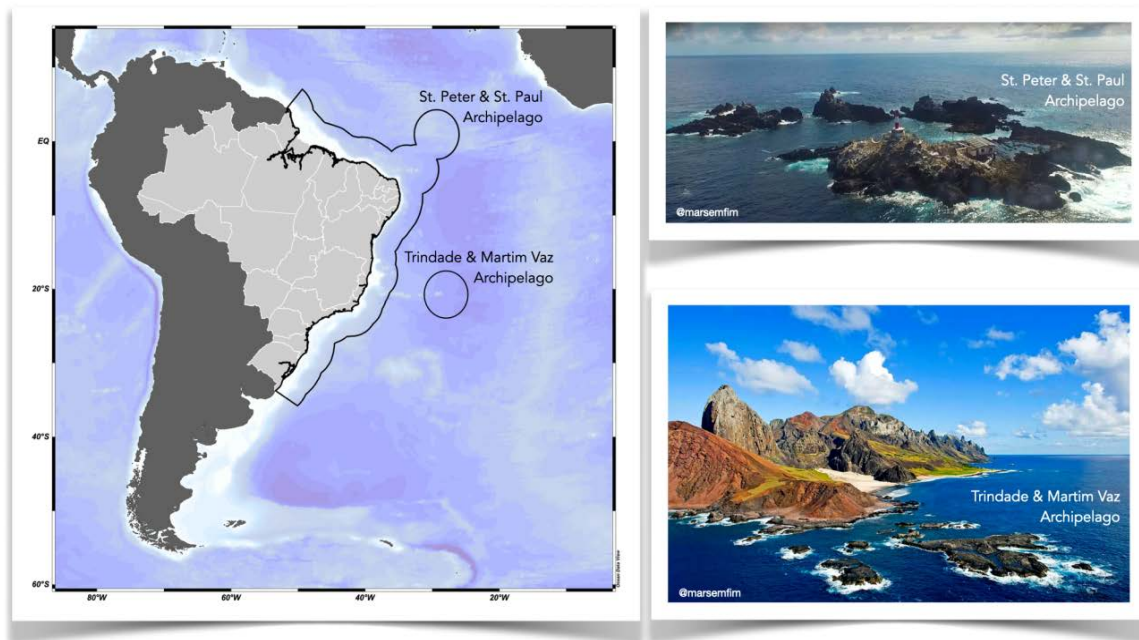


Figure 1: Remote Marine Protected Areas in Brazilian EEZ.

ImageCredit: Mar Sem Fim

Both the ReMPA covers a radius of 200 nautical miles corresponding to 40 million hectares around the archipelagos. Their objectives run around conserving marine ecosystems and biodiversity, as well as placing

nation rights over natural resources exploitation and management. The simple existence of these seamounts turned to MPA, expanded the Exclusive Economic Zone (EEZ) of Brazilian oceanic territory. Nonetheless, MPA

normative mentions the care for sustainable use, considering ordering fishing, navigation, tourism and other compatible economic activities “with environmental conservation that present themselves as strategic to the region” [60,61]. The ReMPA will be managed in a shared manner between the Navy and Conservancy institute. Although the conflict or overlapping between the two official institutions results from differences of attributions, the uncertainty of uses around those large and remote areas is previous, if considered the territory in its essence of relations, ephemerality, and amalgamation.

The first asymmetry derives from that epistemological seek for coherence in the conceptual abstraction of space. What constitutes a territory perspective of the open Ocean? Considering Brazil and its large and remote Southwest Atlantic MPAs, closely associated with naturalist formation and with no fixed communities, it is plausible to consider the reflection about a kind of 'space out there' which reflects the paradox of 'stabilization of the inherent instabilities' [62]. It is proper of modernity's territorialization by uses, but not by living places [63]. The absence of living places is counterbalanced, on the one hand, with the construction of universal values, codes, and legitimacy, when Aichi's goal requires numerical proportion for Ocean conservation. As this proportion increases, the vast and remoteness of the Ocean become contemplated by official decrees, boosting the original conservationist vocation which, in turn, is automatically aggregated with the critical chronic stage of widespread and increasing oceanic degradation whether by marine debris, chemical combustion, overfishing and big ships traffic outputs. "As a result, such conservation and sustainable use measures are currently implemented within a fragmented framework by regional and sectoral organizations with different management competencies" [64]. Thus, the first asymmetry lies in the global geographic debate between the comprehension of sectoral versus territorial categorization of uses and respective corporative versus universal interests. It is an asymmetry of theoretical narratives and their respective epistemological basis, but in the end, they should not be considered corporate epistemological incompatibilities, but interdisciplinary openings. That is the reason why Science is under the center of the hypothetical axiomatic resolution, even before state-nationalism or universal agendas.

The second asymmetry is about governance. Environmental protected areas in Brazil are commonly slow to mature in terms of their practical instruments: Management Plan, Zoning tools, and Committee's agenda. MPAs tend to get less public attention and/or investment than the ones on land and when there is public awareness, the quality of the debate is commonly questionable. In Brazil, the management plan corresponds to the master document of any protected

area. It is totally built on territorial grounds by considering the geographical analysis of the ecosystems and their biodiversity with minimum account for temporal variability and ocean change either by natural causes or human uses. The methodology for applying these instruments focuses on terrestrial biomes. However, experience has shown that huge method gaps when applied in MPAs, requiring adaptations mainly in the way of establishing the zoning and decision-making [65]. In view of the growing demand for ReMPAs, defined in various environmental policy instruments, such as Agenda 21 [66], the Convention of Biodiversity [67], and Brazilian Plan for Protected Areas [68], it is necessary to adapt the methodology of current MPA management instruments to the marine governance approach. So, the second asymmetry may be seen from a standpoint of epistemological-perspective or interdisciplinarity issue: a ReMPA requires more pragmatic executive agendas, due to lower demands for managing uses that are mostly indirect but a higher demand for monitoring of external impacts and 'invisible' uses. Although the asymmetries in MPA consolidation are much bigger when considering the remote maritime territories, the lack of better understanding of the singularities of uses at these places is supposed to be an opportunity for new models of the management plan, where figures the central debate of the present discussion [69].

But what are the real uses - and users - of such areas when fostering consolidation is its goal(s)? Here it is brought the perspective that both direct and indirect territorial uses must be scientifically based, technically diagnosed, and permanently monitored. Reaffirming that this refers to the situational reality of spaces that have been established as marine areas for the environment. Once this is officially defined, it moves towards a management agenda, where the technical term assumes a maritime spatiality for regionalization public policy [70]. Intended to operationalize this discussion, we present an outline of the ReMPA stakeholder composition criteria (Figure 2, next subitem).

The MPA is created, the polygonal oceanic area turns to a new role in territory perspective: an inventory of vocations defines zonation, within ReMPAs normative and polygonal creation. It is supposed to become a non-passive place then, also seen as the sea overcoming from the condition of orphan space, supported then by a specific legal framework [71]. Nonetheless, the obstacles in establishing a Marine Protected Area are priority related to the genesis of this newly created territoriality [72]. The MPA obstacles to governability appear mainly in the initial stage, when the idea is conceived, communicated, and discussed among the actors involved in a territory derived from quantitative and qualitative aspects of government interactions in the zero-step. If MPAs are not technical

management instruments, but above all, sociopolitical processes, there is a gap to be filled in the elucidation of who are the real users of that place.

IV. THE “USER-DECISION MAKER” STAKEHOLDER COMPOSITION

The finding of the two asymmetries elucidates stakeholder composition matter for MPA management. This challenge can be taken as a science gap around interdisciplinary openings and new methodologies for emergent studies. From this perspective, ReMPAs are

able to foster marine territorial management: either because of their spatial singularities or due to the state of the art within the incipient Blue Economy and the Commons principles evolved.

In order to illustrate an introductory path for logical comprehension over the process that connects the MPA existence in a remote ocean area with the parties involved as users and conservation functions, a detailed six-step framework for user-oriented analysis is presented in Figure 2.

MPA STAKEHOLDER COMPOSITION FRAMEWORK - BLUE ECONOMY PARADIGM

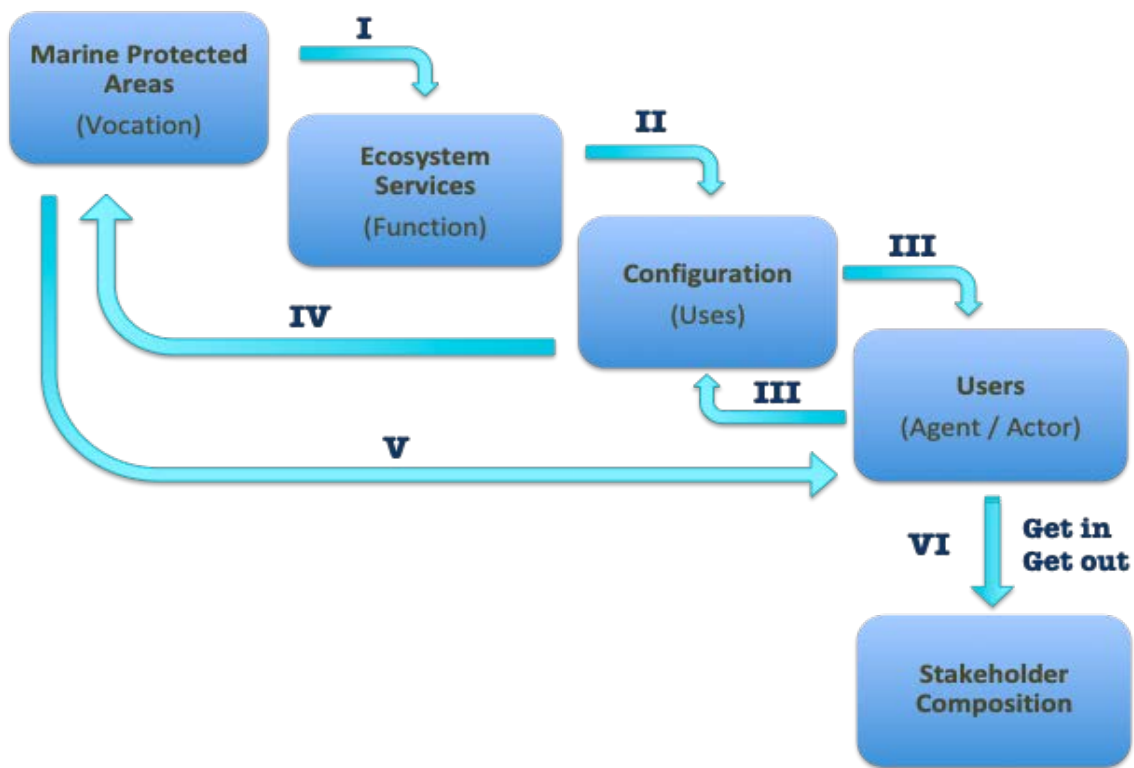


Figure 2: Framework of compatibility of ReMPA user decision-maker

Considering the challenges addressed, we visualize a sequential logic chain (Fig. 2) as a methodological baseline for ReMPAs as the Blue Economy advances. This schema connects inventory uses, vocation, and users of the ReMPA with its ecosystem services (Blue Economy demand), classify compatibility with ReMPA assets and compose the users that align with MPA function and blue economy goals for the decision-making process. Once the ReMPA vocation is defined and prioritized, ensuring its implementation, the users and their links with the environment should be mapped. In this process, it is relevant to define criteria for stakeholders' composition into management bodies, which in ReMPA opens a broad range of different sides of modern society. In the end, this logic chain should have answered the following queries: (I) What are the relations between MPA

vocation and its Ecosystems Services? (II) Are the Ecosystem Services mapped and user-defined? (IV) Is the ReMPA vocation in accordance with the proposed uses? Uses and the users by real agent promoters - which is possible to be understood in the territorial configuration by indicative data of impacts? Dialogically comparing territorial configuration and the existence of the MPA will provide a further typology. Then, select the users that fit their purposes on MPA functions and that align towards the blue economy paradigm, including the institutional composition by attribution.

V. CONCLUSION

Achieving Blue Economy's goals requires the active participation and inclusion of the societal groups into the management of marine territories. The overlying

view of the ocean by global trade organizations requires an initial scheme of user-oriented analysis for this open territory. Nevertheless, there are answers to be resolved in order to better qualify the decision-making process of planning and, mainly, managing those emblematic territories. Although it is expected from national States to exercise their sovereign rights to conservation actions, the contemporary global economy operation works sectorial and strategically on divergent paths to the universal precepts of Ocean's protection. That is the reason for a more appropriated comprehension of territory in the toughness design of a user protagonist in the decision-making process, but attended by a conceptual model that allows discerning the types of uses in relation to the functions originating from the existence of the protected area. The questions to be answered have to be better elaborated, but testing this preliminary purpose model can be one way to better fit the participative management of ReMPAs in the present days.

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ASSOCIATE OF SCIENCE FRONTIER RESEARCH COUNCIL is the membership of Global Journals awarded to individuals that the Open Association of Research Society judges to have made a 'substantial contribution to the improvement of computer science, technology, and electronics engineering.

The primary objective is to recognize the leaders in research and scientific fields of the current era with a global perspective and to create a channel between them and other researchers for better exposure and knowledge sharing. Members are most eminent scientists, engineers, and technologists from all across the world. Associate membership can later be promoted to Fellow Membership. Associates are elected for life through a peer review process on the basis of excellence in the respective domain. There is no limit on the number of new nominations made in any year. Each year, the Open Association of Research Society elect up to 12 new Associate Members.



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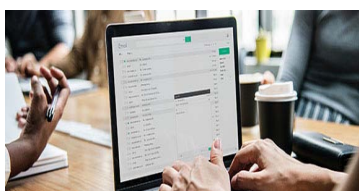
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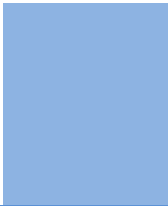
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3. Ensure corresponding author's email address and postal address are accurate and reachable.
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2. Drafting the paper and revising it critically regarding important academic content.
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Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

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Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.



Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

- a) A title which should be relevant to the theme of the paper.
- b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
- c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
- d) An introduction, giving fundamental background objectives.
- e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
- f) Results which should be presented concisely by well-designed tables and figures.
- g) Suitable statistical data should also be given.
- h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

- i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
- j) There should be brief acknowledgments.
- k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.



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It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

All manuscripts submitted to Global Journals should include:

Title

The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

Author details

The full postal address of any related author(s) must be specified.

Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, "What words would a source have to include to be truly valuable in a research paper?" Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

Abbreviations

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.



Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

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Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

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Techniques for writing a good quality Science Frontier Research paper:

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of science frontier then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

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6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.



20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium through which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

INFORMAL GUIDELINES OF RESEARCH PAPER WRITING

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.



Mistakes to avoid:

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- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.



The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.



Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

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- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

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Put figures and tables, appropriately numbered, in order at the end of the report.

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Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."



Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

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- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

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<i>References</i>	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring



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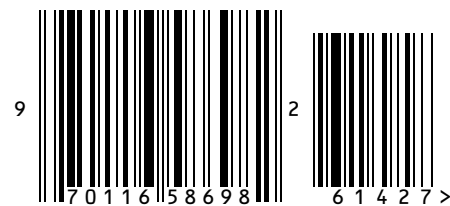
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