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Clarias gariepinus photo by Ivan Kwan

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Book:

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Chapter in book:

Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds) Tropical Forest Community Ecology. Wiley-Blackwell, New York.

Abstract:

Assaeed AM. 2007. Seed production and dispersal of *Rhazya stricta***.** 50th Annual Symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

Proceeding:

Alikodra HS. 2000. Biodiversity for development of local autonomous government. In: Setyawan AD, Sutarno (eds.) Toward Mount Lawu National Park; Proceeding of National Seminary and Workshop on Biodiversity Conservation to Protect and Save Germplasm in Java Island. Universitas Sebelas Maret, Surakarta, 17-20 July 2000. [Indonesian]

Thesis, Dissertation:

Sugiyarto. 2004. Soil Macro-invertebrates Diversity and Inter-Cropping Plants Productivity in Agroforestry System based on Sengon. [Dissertation]. Universitas Brawijaya, Malang. [Indonesian]

Information from internet:

Balagadde FK, Song H, Ozaki J, Collins CH, Barnet M, Arnold FH, Quake SR, You L. 2008. A synthetic *Escherichia coli* predator-prey ecosystem. Mol Syst Biol 4: 187. www.molecularsystemsbiology.com. DOI:10.1038/msb.2008.24

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Microbial quality of fish along with the Tilapia, African catfish and Sardinella artisanal value chains in Kpong and James Town, Ghana

EURYDICE ABOAGYE, KWAKU TANO-DEBRAH♥, ANGELA PARRY-HANSON KUNADU
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Manuscript received: 19 September 2019. Revision accepted: 4 January 2020.

Abstract. *Aboagye E, Tano-Debrah K, Kunadu APH. 2020. Microbial quality of fish along with the Tilapia, African catfish and Sardinella artisanal value chains in Kpong and James Town, Ghana. Bonorowo Wetlands 10: 1-17.* Fish from artisanal sources constitute the most critical animal protein in the Ghanaian diet. However, the availability and safety of fish on the Ghanaian market are unpredictable owing to potential rapid microbial growth, which results from high ambient temperatures and poor handling along the artisanal value chains. Little is known about the small-scale fish value chains and the key stakeholders' food safety knowledge and processing practices. This study aimed at mapping out the artisanal fish value chains of Tilapia (*Oreochromis niloticus*), African catfish (*Clarias gariepinus*), and sardinellas (*Sardinella aurita*), and assessing the food safety knowledge and handling practices of key stakeholders along the selected value chains. A survey using semi-structured questionnaires involving 93 fishers, 40 retailers, 40 processors, and 120 consumers was carried out to investigate stakeholders' knowledge and practices of food safety along the value chain. Samples of the selected fish species were taken along their respective value chains to test for the presence of safety indicators (*Salmonella, Vibrio,* and *Listeria* species), hygiene indicators (*Staphylococcus aureus* and *Escherichia coli*), and spoilage organisms (*Pseudomonas* spp. and *Proteus* spp.). The mean scores for food safety of retailers, processors, and consumers were generally insufficient at 55%, 43%, and 67.3%, respectively. The stakeholders also scored poorly in their handling practices, with mean scores of 41.2%, 63.0%, and 58.6% for fishers, processors, and consumers, respectively. Estimated fish losses were highest at the retailer and consumer stages of the value chain, with reported injuries as high as 35 to 100%—pathogens such as *Clostridium perfringens*, enteropathogenic *Escherichia coli, Staphylococcus aureus, Listeria* spp. And *Aeromonas sobria* were isolated from fresh and processed ready-to-eat fish samples. *Salmonella* spp. and *Vibrio* spp. were not detected on any samples tested. Mesophilic counts ranged from 7.96 ± 0.68 to 2.95 ± 0.23 log cfu/g reported from fresh fish samples, with similarly high fecal coliform counts averaging 3.11 log cfu/g. Processed fish samples had average total counts, fecal coliform counts, and yeasts and mold counts of 3.11, 2.27, and 2.45 log cfu/g, respectively. *Proteus vulgaris* and *Proteus mirabilis* were the predominant spoilage organisms present in almost all the fresh fish samples. This study provided much-needed insight into the unsatisfactory safety and quality of artisanal fish on the Ghanaian market and the specific microorganisms associated with them along the value chain. It also established the link between the food safety knowledge and handling practices of stakeholders within the value chain and the actual quality and safety of fish on the market.

Keywords: Microbial quality, fish, tilapia, African catfish, sardinella

INTRODUCTION

Fish contributes about 40-60% of the animal protein supply in the Ghanaian diet and is recognized as the most important source of animal protein in every part of the country (MoFAD 2011)). The cost of fish constitutes 22.4% of the food expenditure in all Ghanaian households and 25.7% in low-income families (BOG 2008). Fishing communities, often some of the poorest in the country, depend heavily on fish and related activities for their livelihoods (FAO 2013). As an agricultural commodity, fish is essential in ensuring food security, especially among the poorest in the country.

However, the importance of fish as a food security commodity in Ghana is affected by high post-harvest losses. In the high tropical temperatures of Ghana, fresh fish spoilage can be remarkably rapid after capture. Fish perishability is aggravated by its intrinsic properties, such as high water activity, near-neutral pH, and high digestible protein content, all of which provide conducive conditions for microbial proliferation (Ghaly 2010). Microbial activity alone accounts for the spoilage of 30% of landed fish worldwide (Bataringaya 2007). In Ghana, 10-30% of the artisanal catch sold for less than its actual worth due to quality deterioration (Akande and Diei-Ouadi 2010).

In terms of safety, fish has been implicated in several outbreaks of food-borne infections. It is a potential vehicle for food-borne diseases such as cholera, listeriosis, salmonellosis, and others (Popovic et al., 2010; Costa 2013; Akoachere et al. 2009). Many spoilage microorganisms, known to be opportunistic pathogens, including *Pseudomonas* spp. and *Proteus* spp., have also been associated with fish (Ikutegbe and Sikoki 2014; Popovic et al. 2010; Tryfinopoulou et al. 2007; Viji et al. 2014).

Therefore, poor fish quality has dire consequences, which transcend the loss of an important protein source. Substantial economic injuries are incurred annually because of losses in production volume, monies spent in treating foodborne infections, and human resources lost during the illness and the incidence of death (Akande and Dei-Ouadi 2010). With an already existing annual deficit of

320,000 Mt in Ghana's fish requirements, fish quality loss is a problem that requires urgent attention (BOG 2008).

The Artisanal fishery in Ghana contributes 70% to 80% of the total marine fish production and is the principal supplier of fish on the local market (Amador et al. 2006: FAO 2013). There is, however, scanty literature on the structure of the artisanal marine and freshwater fish value chains. The few reported studies do not clearly depict the key players in these value chains and how their knowledge and practices concerning food safety impact the final quality of the fish that reaches the consumer. The occurrence of key pathogens associated with fish and how they are affected by processing and handling along the value chain is also sparsely documented. Again, the specific spoilage organisms associated with fish sourced from Ghanaian waters and their occurrence along the value chain are unknown. These gaps in knowledge are of great concern given the severity of the consequences associated with fish spoilage, especially regarding food security.

Also of concern is the poor and unsanitary conditions prevailing in most fish landing sites in Ghana. It becomes even more necessary for key actors or stakeholders within the fish value chain to be aware of and consistently implement proper handling and storage of fish before processing and distribution to consumers. Therefore, it is vital to investigate the association between the food safety knowledge and practices of artisanal fish stakeholders and the actual microbiological quality of their fish. This would provide evidence and insight into how these stakeholders, through their cultural and food safety-related practices, impact the extent of fish losses and the microbiological quality of artisanal fish on the local market.

The objectives of this research were: (i) To map out and document the artisanal value chains of Tilapia (*Oreochromis niloticus*), African catfish (*Clarias gariepinus*), and sardinellas (*Sardinella aurita*) in Kpong and James Town. (ii) To test fish samples along the artisanal fish value chain for the presence of safety indicators (*Salmonella* spp., *Vibrio* spp. and *Listeria* spp.), hygiene indicators (*Staphylococcus aureus* and *Escherichia coli*), and spoilage organisms (*Pseudomonas* spp. and *Proteus* spp.). (iii) To establish the link between food safety knowledge and practices of stakeholders in the artisanal fish value chain and the micro-biological quality and safety of fish. (iv) To determine the potential implications of poor fish microbiology on food security availability and safety components.

MATERIALS AND METHODS

Study design

The study was in two parts. The first part was an initial cross-sectional survey that traced the local artisanal fish value chain. It also assessed the food safety knowledge and practices of key players or stakeholders within the identified value chain through questionnaire surveys. Participation in the study was voluntary, and anonymity and confidentiality of the response were ensured. Ethical clearance was obtained from the Noguchi Memorial Institute for Medical Research Institutional Review Board (NMIMR- IRB), University of Ghana.

The second phase was microbial analyses in the laboratory. The study examined the microbiological quality of three locally consumed fishes at different stages of their respective value chains. The Sardinella was used as a case study for marine fishes, while Tilapia and the African catfish were used for freshwater fishes. These fishes were selected based on their availability and popularity among different socio-economic groups within Ghana.

Sampling and data analysis for a cross-sectional survey

The fishers were the first point of contact fishers the individual fish value chains. They asked who supplied resources necessary for their expeditions and which groups of people they handed their catch to. The trail was then followed to identify the next group of stakeholders until the fish reached the final consumer. All the different kinds of processing the fish subjected to and the major fish markets, where fish were either retailed or wholesaled, were also identified in the process. Consumers were recruited because they had purchased raw, unprocessed fish from local informal markets at any time within the past six months.

Tilapia and the African catfish were traced from stakeholders in Kpong, Senchi, and Ayikpala, all of which were artisanal fishing communities located around the Volta Lake in the Eastern region of Ghana. Stakeholders in the Sardinella value chain were interviewed along with James Town and Choker's coastlines in Accra and the major fish markets, Salaga, Madina market, and Kaneshie in Accra and Tuesday market in Mamprobi, a suburb of Accra (Figure 1).

A total of 293 stakeholders in the value chain were interviewed with four semi-structured questionnaires. These included 93 fishers, 40 retailers, 40 processors, and 120 consumers. The interviewer entered all questionnaires were administered in the local language, and gave responses. All questionnaires were pre-tested before use. The questionnaire designed for fishers evaluated food safety practices based on four questions, while retailers/wholesalers, processors, and consumers were assessed based on eight items on the survey. Knowledge about food safety was also assessed based on seven items on the wholesaler, retailer, processor, and consumer survey.

In both the knowledge and practice sections of the surveys, categorical responses (yes/no/don't know) and open and more detailed responses were used. Each correct answer within the categorical responses carried a score of 1while correct responses in the open-ended questionnaires carried a score of 2. 'Wrong' or 'don't know answers' were given a score of zero. For each respondent, the questions' score was summed up and converted into percentages (0 to 100). A representative score of 70% and above was considered "sufficient knowledge/practice," while a score of <70% was considered "insufficient knowledge/practice." The scoring system applied here was adapted from similar studies by Osaili et al.*(*2013) and Zanin et al. (2015).

Figure 1. A map of towns and markets from which samples were obtained

Table 1. Study design for microbiological analyses

Stakeholders Raw fish	Product	Fish type (N) Tilapia	African catfish	Sardinella
Fishers	Freshly landed fish	1x2	1x2	1x2
Wholesalers	Fresh fish	1x2	1x2	1x2
Retailers	Fresh fish	1x2	N/A	1x2
Processors (fermented/salted)	Raw fish	1x2	1x2	N/A
Processors (Salted/dried)	Raw fish	1x2	N/A	N/A
Processors (Grilled)	Raw fish	1x2	N/A	N/A
Processors (Smoked)	Raw fish	1x2	1x2	1x2
Processed fish				
Processors	Fermented/dried	1x2	1x2	N/A
	Salted/dried	1x2	N/A	N/A
	Smoked	1x2	1x2	1x2
	Grilled	1x2	N/A	N/A
	Fried	1x2	1x2	N/A
Retailers	Smoked	1x2	1x2	1x2
	Salted	1x2	N/A	N/A
	Sun-dried	N/A	N/A	1x2

Note: N/A: Not applicable (samples were not typically found at that stage of the value chain)

Sampling for microbiological analyses

Sampling was done at different value chain stages for each type of fish. The samples were collected on at least two separate occasions and from different individuals provided they sourced their fish from areas in and around Kpong in the case of Tilapia and catfish, and James Town in the case of the sardinellas.

Samples were collected into sterile stomacher bags, appropriately labeled, and transported in thermos ice chests disinfected with 70% alcohol. All fresh and raw samples were transported on ice and analyzed in the laboratory within 4 hours of sampling. Processed fish samples were analyzed within 24 hours of collection. To prevent cross-contamination, processed fish were not sampled on the same day as the raw and fresh fishes.

Sampling of fish from Fishers

Sampling was done by convenience; sampling of the landed fish was done by asking the fishers to select the fish of interest from their catch randomly. Sampled fish was then transferred into a sterile stomacher bag handled by the gloved hands of the researcher. The bag was sealed and put into the thermos ice chest.

Sampling of fish from wholesalers and retailers

Fresh fish samples were purchased from wholesalers at the fish landing site and retailers at the informal fish markets in Salaga and Madina. The fishes obtained from individuals far removed each other in the market. The wholesaler or retailer was asked to select the fishes in the same manner randomly she would handle them when selling to a consumer or customer.

Sampling of fish from Processors:

Sampling here done at the processing site. Where it was available, samples of the raw fish intended for processing were also collected, and their storage temperature was measured with a thermocouple (Thermo scientific). However, such examples were collected and transported in a container separate from the processed fish samples. Processed fish in storage or ready to be served to consumers were randomly selected by the processor and sealed in sterile stomacher bags for transport on ice to the laboratory. The samples were stored in a cold room at 4$_o$C for no longer than 24 hours when they could work on immediately.

Microbiological analyses

Samples were analyzed for the total count or concentration of aerobic mesophiles, coliforms, staphylococci, yeasts and molds, and *Clostridium perfringens*. The fish samples were also analyzed for the presence of *Escherichia coli*, *Salmonella* spp., *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Staphylococcus aureus*, *Pseudomonas* spp., and *Listeria* spp.

Sample preparation

Ten grams of each sample was aseptically weighed into sterile stomacher bags with the addition of 90 mls peptone water. Because of their smaller sizes, juvenile sardinellas are considered the whole. In the case of tilapia and catfish, bits of the surface tissues, gills, gut, and muscle from the loin (thickest part of the fish muscle) were taken with sterile scissors and aseptically weighed to obtain the final mass. The weighed samples were then homogenized for up to 2 minutes in a Seward stomacher blender. Serial dilutions for the various microbial counts earlier mentioned were then carried out according to methods described by the International Commission for Microbiological Specifications for Foods (ICMSF, 1985).

Aerobic mesophilic count

In a method described by the (ICMSF 1985), homogenates of fish samples were serially diluted, and 0.1ml of three dilutions were pipetted into sterile disposable Petri-dishes. Twenty-milliliter portions of Plate Count Agar (PCA) (Biolab-Merck) were then poured on the inoculum using the pour plate technique. The set PCA dishes were incubated inverted at 20- 25 ˚C for 48±2 hours to account for psychrophiles present. Two replicates of at least two dilutions with 25-250 discrete colonies were enumerated following incubation. All counts were reported as the logarithm to base 10 colony-forming units per gram (log cfu/g).

Enumeration of total staphylococci and detection of S. aureus

Three serial decimal dilutions of the samples were plated using the pour plate technique on Baird-Parker agar (Oxoid CM275) supplemented with egg yolk tellurite emulsion (Oxoid). After incubation at 35-37°C for up to 48 hours, plates containing 20-200 typical staphylococcal colonies (black, circular) were counted. Up to 5 colonies on each plate subcultured on nutrient agar (Oxoid CM003), gram stain, and tested for catalase activity. All typical staphylococci were catalase-positive and were gram-positive stacked cocci. *Staphylococcus aureus* colonies were circular, convex, grey-black to jet-black with an off-white margin. They were also gram-positive cocci, catalase-positive, and coagulase-positive (APHA 2001).

Enumeration and detection of faecal coliforms and E. coli

Three serial decimal dilutions of the fish-peptone water homogenate were pour plated on Levine Eosin Methylene Blue (EMB) agar (Oxoid CM0069) and incubated at 35 ˚C for 24 hours. Plates with 20-200 Purple colonies were counted and subcultured on MacConkey agar (Oxoid). Faecal coliforms fermented the lactose (pale pink color on MacConkey) and were gram-negative rods.

Escherichia coli colonies appeared circular, dry, and flat with a metallic sheen. Five of such provinces subcultured on Sorbitol MacConkey (SMAC) agar (Merck), where they appeared as pinpoint, pale pink colonies. These suspect colonies were then purified on nutrient agar and transferred to Triple sugar iron agar (TSI) slants, Simon's citrate slants, and Sulphur-Indole-Motility (SIM) agar. *E. coli* colonies were indole positive, gas positive, H_2S negative, citrate negative, and fermented glucose and sucrose.

All presumptively identified *E. coli* colonies were confirmed with API 20E and serotyped using a serotyping kit. *Klebsiella* spp. often mimicked *E. coli* on EMB plates but had a moist appearance, with or without a metallic sheen, and was also slimy when touched with an inoculating loop. Suspect colonies were purified on nutrient agar and confirmed with API 20E (APHA 2001).

Clostridium perfringens *count*

Spread plates of serial dilutions were made using 0.1ml aliquots on TSC (tryptose-sulfite- cycloserine) agar (Oxoid CM0587) supplemented with egg-yolk and TSC supplement (Oxoid). After the agar had dried slightly, the surface was overlaid with 5ml of TSC agar and incubated upright in an anaerobic jar containing an aerobic gas generating kit (Oxoid anaerogen) and incubated at 35-37°C for 24 hours. Plates containing 20-200 black colonies with opaque halos were selected and counted. To confirm presumptive positive *Clostridium perfringens* colonies, 5 black colonies were selected and tested for motility in SIM agar and nitrate reduction in nitrate broth (Fluka 72548). *Clostridium perfringens* reduced nitrate and was nonmotile (APHA 2001).

Detection and enumeration of yeasts and moulds

The homogenate's serial decimal dilutions were pour plated in Malt Extract Agar (MEA)- Oxoid CM0059,

supplemented with 10% lactic acid and incubated at 25˚C for up to 5days. Plates containing 20-200 colonies were counted.

Detection of Pseudomonas *species:*

In a method described by Tryfinopoulou et al. (2001), dilutions of the homogenate were pour plated in Pseudomonas agar (Oxoid CM0559) with 5ml of glycerol and a vial of pseudomonas CFC (Cephaloridine-fucidine-ccetrimide supplement (Oxoid. They incubated at 25◦C for 24 hours and 48 hours. *Pseudomonas aeruginosa* appeared as straw-colored colonies with green pigmentation. Presumptive positive colonies were subcultured on nutrient agar and tested for oxidase activity. *Pseudomonas* spp. were Gram-stained (gram-negative rods) and confirmed with API 20E.

Detection of Vibrio *species*

Twenty-five grams of fish samples were weighed and homogenized in 225ml of alkaline peptone water. The homogenate was aseptically dispensed as 10ml aliquots in lightly capped test tubes and incubated for 18-24 hours at 35oC. The pre-enriched samples were vortexed, after which a 3 mm loop (about 0.1ml) was aseptically taken and streaked unto well-dried Oxoid CM0333 Thiosulphate Citrate and Bile salts Sucrose (TCBS) agar plates. The plates were incubated (inverted) at 35-37˚C for 18 to 24hours or until satisfactory growth.

Suspect *Vibrio cholerae* colonies on TCBS agar were large, smooth, flat, and yellow, while *Vibrio parahaemolyticus* were smaller, green, and round. *Aeromonas* spp. could mimic both appearances on the TCBS agar. All suspect colonies were purified on nutrient agar and tested for oxidase activity. Colonies found to be oxidase positive were purified further on nutrient agar and confirmed with API 20E (APHA 2001).

Detection of Salmonella *species*

Twenty-five grams of fish samples were homogenized in 225ml of selenite broth, dispensed into loosely capped sterile test tubes as 10ml aliquots, and incubated for 18-24 hours at 35oC to recover injured cells. Three drops of the pre-enriched culture were evenly inoculated on plates of Modified Semi-solid Rappaport Vassiliadis medium (MSRV, Oxoid CM1112). A loop full of the pre-enriched culture was also streaked on dried plates of *Salmonella-Shigella* agar (SSA, Park scientific M0240). Growth on both media was examined after 18-24 hours at an incubation temperature of 35oC.

Presumptive positive *Salmonella* colonies appeared on SSA as straw-colored with or without black centers. *Proteus* spp. often swarmed the SSA plates, appeared as black colonies, and had a very foul smell. Suspect *Salmonella* colonies were isolated and purified on nutrient agar for biochemical testing.

Suspect *Salmonella* growing on the MSRV were greyish and appeared motile. A growth sample was streaked onto a dried SSA plate with a sterile loop and incubated overnight. *Salmonella*-like colonies observed were streaked unto Nutrient agar for purification. All purified colonies were tested for urease reactions on Urea agar slants and TSI agar.

Gram-negative rods with adverse urea reactions were confirmed using the API 20E kit (APHA 2001). All identified *Proteus* species were tested for indole reaction to differentiate *P. mirabilis* or *P. penneri* from *P. vulgaris*.

Detection of *Listeria* species*Twenty-five-gram* fish samples homogenized in 225ml of Merck Listeria Enrichment Broth (LEB) were distributed into loosely caped test tubes as 10 ml aliquots. After 24 hours of incubation at 35°C, 1ml of the LEB-fish homogenate was septically transferred into 9ml of Fraser broth (Oxoid CM0895). This was incubated at 35±2°C for another 24 hours. A loop full of the enriched homogenate was then streaked unto well-dried plates of Chromogenic Listeria agar (LCA, Oxoid CM1017), incubated at 35±2C for 24 hours or until satisfactory growth was satisfactory. Suspect *Listeria* colonies appeared as blue-green colonies surrounded by an opaque halo.

Selected colonies on the LCA plates were purified on 5% sheep blood agar (Oxoid). Presumptive *Listeria* colonies appeared whitish on blood agar and displayed β-hemolytic activity. Discrete colonies from the blood agar were also tested for motility on SIM agar, catalase activity, Gram's reaction. Presumptive positive *Listeria* were catalase-positive, gram-positive short rods and displayed umbrella motility in SIM agar (APHA 2001).

Physical and chemical analysis of intrinsic properties of fish

Temperature

The fish (both fresh and processed) temperature was measured with a thermocouple (Hanna Instruments) calibrated in hot water at 100°C and ice water at 0°C on each sampling day. The thermocouple probe was first disinfected with 70% ethanol before measuring the temperature at the midsection and tail regions. The average of the three readings was recorded and reported as mean± Standard deviation.

pH

The moisture content of the fish sample was first determined using the standard method described in I.S 14950: 2001 fish dry and dry salted. If the fish was, for example, found to contain 20% moisture, it implied that every 10g of the sample weighed had 8g of dry matter. To obtain 10g of dry fish matter, 12.5g of the sample was considered and homogenized in 87.5g of deionized water. The pH of the homogenate was then measured with a glass electrode pH meter. The average of three readings was recorded and corrected for temperature differences (I.S 14950: 2001).

Determination of risk factors along the fish value chain

To establish the link between the food safety knowledge and practices of stakeholders and the actual quality and safety of the fish, a flow chart was designed based on evidence from the survey and the laboratory microbiological analyses.

The risk of poor handling is determined from the food safety knowledge scores and handling practices determined

from the survey. Also, the risk of fish spoilage and pathogenic contamination was established by the presence of spoilage organisms and pathogens, respectively, from fish sampled at the various stages of the value chain. Finally, temperature abuse and poor hygiene were determined by recorded fish temperatures and counts of hygiene indicator microbes, respectively.

Statistical analysis

Using their demographic characteristics as covariates, a binary logistic regression was used to determine the possible predictors of stakeholders' food safety knowledge and handling practices. Descriptive statistics such as means, standard deviations, and frequencies were used to analyze microbial counts. The means were data from three independent experiments for fresh and processed fish microbial counts. Analyses of variance, ANOVA (one-way) were used to assess the significance of differences between counts of microbes obtained from different stakeholders and between the counts sourced from the different value chains (marine and freshwater).

The percentage prevalence of isolated microorganisms was determined using Cross tabulations. Pearson's chi-square was used to test the association between food safety knowledge and practices and the estimated fish losses. All statistical analyses were done using IBM SPSS version 21, Minitab version 14, and Microsoft Excel (2010).

Assumptions and limitations

Fishers were generally harder to track and interview because they often landed at different times of the day and were constantly busy negotiating, sorting, or selling their catch at the time of the interviews. To reduce the time required in interviewing them, fishers were not assessed on their food safety knowledge. Instead, it was assumed that their practices were more important, given that they typically handed over their catch immediately after landing. The study also assumed that the reported practices of the stakeholders were their actual practices.

RESULTS AND DISCUSSION

Demographic characteristics of stakeholders in the Kpong and James Town artisanal fish value chain

Every stage in the artisanal fish value chain was dominated by women, except the fishers, who were all male. Wholesalers and fresh and processed fish retailers were predominantly female (87.5%). Processors were also mostly female, recording 97.5% in all areas surveyed in this study. The demographic characteristics of the stakeholders are depicted in Table 2.

It was interesting to note that most stakeholders were above 40 years of age, except consumers. This, as explained by the stakeholders, was primarily because a significant amount of capital and social connections were required to enter the fish business. As many as 32% of fishers had never received a formal education. Similarly, 17% of wholesalers and retailers, and 27% of processors, also had no formal training.

Fishers in James Town were predominantly ethnic "Ga" while those in Kpong mainly were of "Ewe" and "Ga-Dangbe" descent.

Table 2. Demographic characteristics and profile of stakeholders within the fish value chain

Biodata		% Stakeholder (N)			
		F'man (N=93)	Whol/Ret (N=40)	Procs (N=40)	Cons (120)
Sex	Male	100 (93)	12.5 (5)	2.5 (1)	9.2 (11)
	Female	0 (0)	87.5 (35)	97.5 (39)	90.8 (109)
Age	18-24 yrs	22.6 (21)	25 (10)	15 (6)	42.5(51)
	30-39 yrs	26.9 (25)	35 (14)	30 (12)	32.5(39)
	>40 yrs	50.5 (47)	40 (16)	55 (22)	25 (30)
Ethnicity	Ada	24.7 (23)	17.5 (7)	10 (4)	2.5 (3)
	Krobo	0 (0)	0 (0)	15 (6)	4.2 (5)
	Ewe	39.8 (37)	47.5 (19)	32.5 (13)	15.8 (19)
	Akan	3.2 (30)	7.5 (3)	2.5 (1)	53.3 (64)
	Ga	32.3 (30)	27.5 (11)	40 (16)	15.8 (19)
	Northerner	0 (0)	0 (0)	0 (0)	8.3 (10)
Religion	Christian	74.2 (69)	92.5 (37)	92.5 (37)	94.2 (113)
	Muslim	2.2 (2)	5 (2)	0 (0)	5.8 (7)
	Traditional African	10.8 (10)	2.5 (1)	5 (2)	0 (0)
	None	12.9 (12)	0 (0)	2.5 (1)	0 (0)
Education	None	32.3 (30)	17.5 (7)	27.5 (11)	19.1 (56)
	Pri/Midsch/ SHS	65.6 (61)	80 (32)	79.5 (29)	65.9 (193)
	Tertiary	2.2 (2)	2.5 (1)	0 (0)	14.7 (43)
Longevity in fisheries and related activities	1-5yrs	8.6 (8)	25.0 (10)	15.0 (6)	N/A
	6-10yrs	11.8 (11)	12.5 (5)	12.5 (5)	N/A
	11-15yrs	11.8 (11)	12.5 (5)	17.5 (7)	N/A
	16-20yrs	20.4(19)	15.0 (6)	17.5 (7)	N/A
	>20yrs	47.3 (44)	35.0 (14)	37.5 (15)	N/A

Note: N/A: not applicable; F'man: fishers; Whol/Ret: wholesaler/retailer; Procs: processor; Cons: consumer

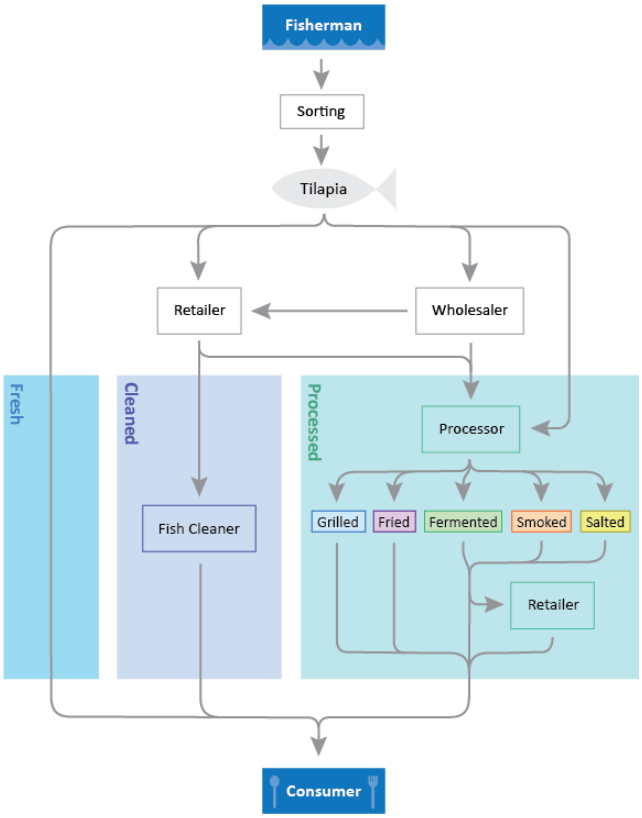

Figure 2. Artisanal value chain for Tilapia sourced from Kpong, Eastern region, Ghana

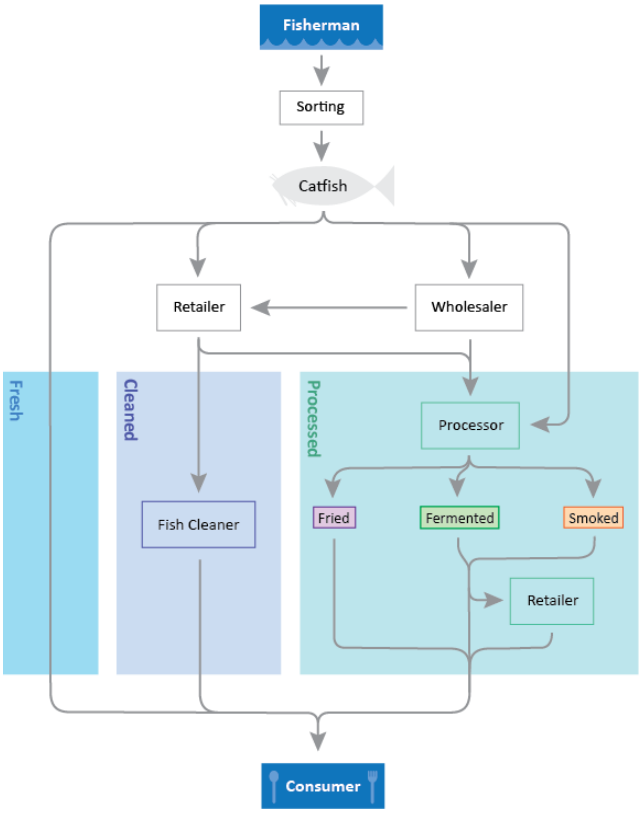

Figure 3. The value chain of the African catfish sourced from artisanal fishers in Kpong, Eastern Region, Ghana

Mapping out the artisanal fish value chains in Kpong and James Town

The value chains of tilapia and the African catfish, traced from the artisanal fishers in Kpong, are depicted in Figures 2-3, respectively. The value chain began with fishes, most of whom own their canoes and a few who rented the canoe daily or weekly. Tilapia and the African catfish (freshwater fishes) were often sold at landing by fishers to women who in turn sold the fish either on wholesale or retail to consumers, processors, and other small-scale retailers. Many of these small-scale retailers sold the fresh fish in vehicular traffic to travelers on the Kpong-tema roads. At the same time, some also sold the fresh fish in distant markets like the Madina and the Makola markets in Accra and markets in Tema. Also, at retail, a secondary group of stakeholders was depicted in Figures 2-3 as "Fish cleaners." This group of people, who included both men and women, would often hang around the landing site and render the service of gutting, scaling, and sizing of the tilapia and catfish bought by consumers. This is important because these individuals increased the number of handlers along the value chain and were a potential source of recontamination.

Tilapia was typically processed by smoking, salting ("Koobi"), grilling, frying, and fermenting ("Momone"). The African catfish was also mainly processed by smoking, frying, and fermenting. Fried tilapia and catfish were retailed directly by processors who hawked in traffic on the Kpong-Tema Highway. The Grilling of Tilapia was mainly carried out at night and retailed directly by the processors as street food. Smoked and salted tilapia often sold wholesale to retailers who sold the fish to consumers previously mentioned local markets. However, it was still possible to purchase salted and smoked tilapia directly from the processors. This also applied to smoked and fermented catfish. To keep smoked tilapia or catfish from rapid deterioration, processors and retailers reported that they re-smoked or reheated the fish daily until it sold to consumers. Nonetheless, most tilapia and catfish were reportedly bought by consumers in their raw or unprocessed state. Most consumers, however, reported buying sardinellas already processed. The absence of a frozen fish value chain, the Tilapia value chain, and the African catfish's value chain was significant. Cold store operators in and around the study sites reported having obtained their fish from inland fish farms rather than artisanal fishers. If any of the artisanal catch, therefore, very little ended up in frozen storage or cold stores before reaching the consumer.

The value chain of the sardinella, which is sourced from marine habitat, was markedly more complicated than the freshwater fishes. This value chain is depicted in Figure 4. This value chain also began with the fishers but had a unique and exciting group of stakeholders, the fish queens known locally as "*Lonye."* These women appeared to wield great influence throughout the sardinella value chain, and in fact, the value chains of most fishes landed at the James Town fishing harbor.

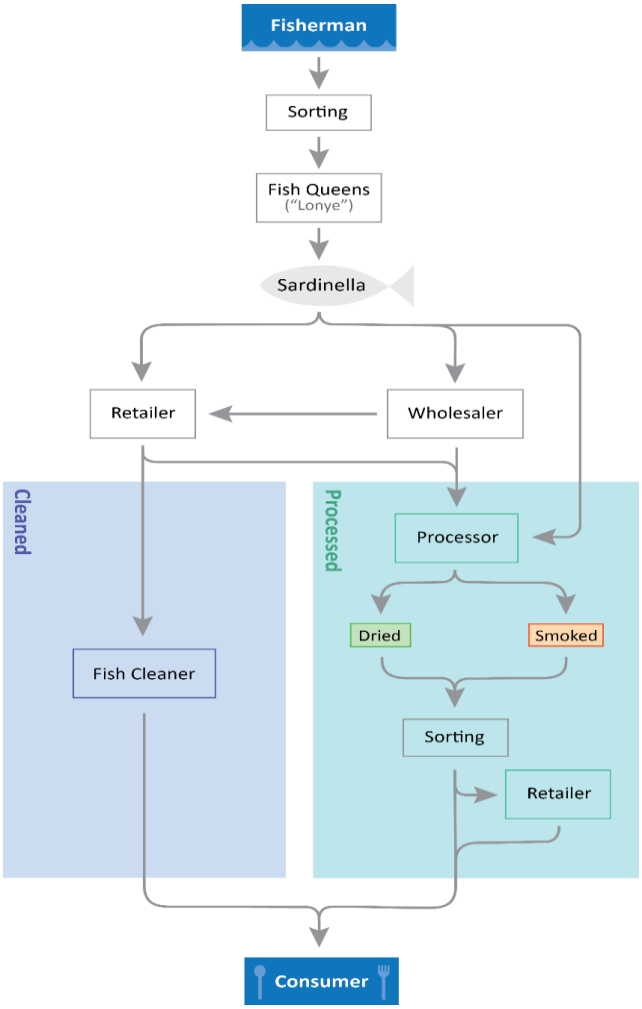

Figure 4. The value chain of the sardinella sourced from artisanal fishers in James Town, Accra, Ghana

The Fish Queens were typically the captain's wives of the fishing canoes, and in many cases, these women also owned the boats. They also reportedly pre-financed some fishing expeditions by providing fishing gears and pre-mix fuel for the outboard motors. When the fish queen held the canoe or pre-financed the operation, she handed the most significant percentage of the catch or the entire catch. The fish queens reportedly had arrangements with several fishers on different canoes and were able to sell large quantities of fish pooled from different fishers. A similar system has reported in the Lake Victoria fisheries bordering Uganda (FAO 2013). Many fishers also owned their canoes or worked for other men who financed their expedition, much as the fish queen did. They, however, also sold their fish to the fish queens and scarcely engaged themselves in selling the fish directly to consumers or other stakeholders. The roles of these women have also been described by Akrofi (2002), who referred to them as "fish mummies."

Sardinellas were typically hot smoked, sun-dried, and occasionally fried. Smoking was, however, the primary mode of processing. Smoked sardinellas were retailed on local markets like Madina, Kaneshi, and Makola, all in Accra and distant markets such as Kumasi in the middle belt of the country and the Northern regions as well.

Fish smokers, who often processed their fish in large quantities to make efficient fuel and labor, typically purchased their raw fish from the fish queens. With the order placed, the fish was carried in cane baskets on young boys' heads to the processing site or by public transport if the site was too far off. The sardinellas were thus ready to be smoked within a few hours after landing. It should be noted that very little smoking was carried out on the beach of James Town. Most of the sardinellas landed in James Town were smoked on the beach of "Chorkor," a fishing settlement about 50 minutes' walk (4km) from James Town. Many of the fishers in James Town were also reportedly resident in "Chorkor." Chorkor was thus the primary hub of fish smoking; many wholesalers and retailers from different parts of the country were reported to purchase smoked fish in this town for resale in local and international markets.

When sufficient quantities could not be obtained from artisanal sources during lean seasons, fish smokers mainly reported buying frozen sardinellas from cold stores. Like tilapia and catfish, sardinellas in frozen storage are not obtained from artisanal sources. Cold store operators bought their sardinellas from industrial fishing trawlers at the Tema Port.

Contrary to the practice in most developed countries, wholesale and retail of fresh artisanal sardinella, tilapia or catfish was rarely measured out in costs per weight of fish. At the wholesale level, fish is sold per basket or crate. The baskets were filled at the discretion of the fishers or wholesalers and not weighed. Retail was done by

counts or numbers in the case of tilapia and catfish, while sardinellas retailed in handfuls. This practice invariably led to frequent handling of the fish by both retailers and consumers, thus introducing opportunities for contamination. It also led to variations in prices between different retailers, resulting in longer bargaining times.

In the case of Tilapia and the African catfish, the price could vary considerably depending on the size of the fish in the crate. The fishes were therefore sorted by size and species before the sale. Stakeholders also used sorting in the marine fish value chain. However, smaller fish species in the marine fish value chains were often sold without sorting. This was particularly true for sardinellas mixed with Anchovies at the landing and the wholesale level, mainly because Anchovies were similar in size and could be utilized in place of sardinellas in most recipes. Processors, therefore, smoked these two species together but separated them after smoking. Once smoked, the sardinellas and anchovies were sorted out and sold separately to consumers and other retailers, mainly because sardinellas fetched a higher price. However, this sorting step after processing increases handling and could introduce post-processing contamination.

Food safety knowledge and practices among stakeholders

The mean knowledge score for all stakeholders was found to be 6.0±2.3 (60%), while the mean practice score was 5.64 ±2.8 (56%), generally suggesting insufficient levels of food safety knowledge and practice. The exception was with the retailers who had a mean practice score of 7.85±1.64 (79%), which suggested sufficient levels of food safety practices.

Fishers, processors, and consumers had mean practice scores of 4.12±2.99 (41%), 6.30±2.04 (63%), and 5.86± 2.42 (59%), respectively. Mean knowledge scores of retailers, processors, and consumers were found to be 5.50±1.99 (55%), 4.30±2.05 (43%), and 6.73±2.02 (67%), respectively. Table 3 displays the responses on the food safety knowledge of stakeholders along the value chains of the fishes used in this study.

Table 3 generally revealed good knowledge among all stakeholders regarding indications of fish spoilage, proper ways of handling fish and preventing fish spoilage. Table 4 also displays the questions which assessed fish handling practices and showed that the stakeholders reported practices that supported this knowledge.

However, it can be argued that this knowledge is fundamental to their trade and essential to prevent economic losses. It is traditional knowledge passed down through generations, as evidenced by the fact that 87% of processors and retailers reported that their skills are were acquired through family traditions. Therefore, it can be deduced that they had no genuine knowledge of the actual causes of fish spoilage. That is, they practiced what they had been taught to do traditionally without genuinely understanding the basis of their actions. Table 5 displays the actual responses given by the stakeholders when asked about the cause of fish spoilage.

Table 5. Stakeholders gave responses on the cause of fish spoilage

What causes fish spoilage (responses)	% Stakeholder (n)		
	Whole/ret	Processor	Consumer
Microorganisms	2.5 (1)	0 (0)	4.2 (5)
Lack of cold storage	65.0 (26)	17.5 (7)	74.2 (89)
Flies and other insects	15.0 (6)	7.5 (3)	6.7 (8)
Prolonged storage	2.5 (1)	15.0 (6)	5.0 (6)
Under-processing	5.0 (2)	20.0 (8)	0.8 (1)
Chemical treatment of fish	0 (0)	0 (0)	1.7 (2)
Unhygienic handling	0 (0)	0 (0)	1.7 (2)
Do not know	10.0 (4)	0 (0)	3.3 (4)

Table 3. Responses to questions on food safety knowledge

Questions	% Stakeholders who gave correct or wrong responses					
	Ret/whole (40)		Processors (40)		Consumers (120)	
	Correct	Wrong	Correct	Wrong	Correct	Wrong
Are you able to identify spoilt fish?	87.5	12.5	100.0	0.0	92.5	7.5
What are the indications of fish spoilage?	69.7	30.3	82.5	17.5	75.8	24.2
What causes fish spoilage?	67.5	32.5	40.0	60.0	87.5	12.5
Could handling contribute to spoilage?	77.5	22.5	87.5	12.5	83.3	16.7
What are some of the bad handling practices	77.5	22.5	60.0	40.0	83.3	16.7
Describe the condition of infected fishes	32.5	67.5	17.5	82.5	5.5	94.5
Can eating a diseased fish cause illness	22.5	77.5	20	80	52	48
What illnesses are caused by eating spoilt fish?	2.5	97.5	20	80	49.7	51.3
How is fish spoilage prevented?	95.0	5.0	77.5	22.5	93.4	6.6
Average scores	**5.50±1.99**		**4.30±2.05**		**6.73±2.02**	

Table 4. Responses to questions on food safety practices

Questions	% Stakeholders							
	Fishers (93)		Whole/retail (40)		Processors (40)		Consumers (120)	
	Correct	Wrong	Correct	Wrong	Correct	Wrong	Correct	Wrong
Do you inspect raw fish before purchasing/selling?	N/A	N/A	85.0	15.0	80.0	20.0	81.0	18.2
What indications of spoilage do you look out for?	N/A	N/A	85.0	15.0	75.0	25	75.8	24.2
How do you transport raw fish?	52.7	47.3	85.0	15.0	45.0	55.0	20.8	79.2
How do you prevent spoilage?	52.5	47.5	95.0	5.0	81.5	22.5	N/A	N/A
How do your store your fish?	48.0	50.0	82.5	17.5	97.5	2.5	93.3	6.7
What do you do with spoilt fish?	N/A	N/A	5.0	95.0	32.5	67.5	90.8	9.2
Average scores	**4.12±2.99**		**7.85±1.64**		**6.30±2.04**		**5.86±2.42**	

The majority of wholesalers, retailers, and consumers pointed to the lack of cold storage as the cause of fish spoilage. At the same time, processors attributed spoilage to insufficient processing and high moisture after processing. These and other responses, such as insect infestation, were scored as correct responses because they pointed to conditions that favored microbial growth. Only six out of the respondents looked correctly at microorganisms, while none mentioned autolysis. It should also be noted that respondents were assessed based on their responses in the questionnaire survey and not their actual observed practices regarding fish handling and food safety practices.

The gender, age, ethnicity, longevity in the fish business, religion, level of education, and the type of stakeholder within the value chain were used to predict the probability that a stakeholder within the fish value chain would have sufficient food safety knowledge and practices. The binary logistic regression results to assess this association is displayed in Table 6.

Based on responses from the questionnaire survey, the model predicted that retailers were more likely to have sufficient food safety practices than other stakeholders within the value chain. Individuals who were Ewe's, Krobo's, and Ga Dangbe (predominantly within the freshwater value chain) were also more likely to engage in good fish handling and food safety practices. Therefore, this suggests that stakeholders within the freshwater value chain were significantly more likely to engage in good fish handling practices than those in the marine fish value chain. However, the odds of this livelihood were notably very small (Odds ratio = 0.03, on average).

Figure 5 compares the percentage of stakeholders who were found to have sufficient knowledge of food safety and fish handling practices to establish a relationship between reported knowledge and practices. It can be observed from Figure 5 that, while almost 90% of retailers in the value chain sufficiently practiced food safety rules concerning fish handling, only 30% of these individuals had sufficient knowledge about food safety. A similar trend was apparent among the processors. An insignificant correlation (rho=0.008, p-value= 0.856 at 95% CI) between knowledge and practices supports the argument that many stakeholders practiced good handling practices without necessarily understanding the importance of their actions.

However, most other studies assessing food safety knowledge and practice reported a different trend, where good knowledge did not always translate to good practices. This examines amplified by the findings of Omemu and Aderoju (2008). Their study on the food safety knowledge and practices of street food vendors in Nigeria revealed that good knowledge of the importance of handwashing did not translate into improved quality handling practices. However, the situation among stakeholders in the Ghanaian fish value is less concerned. The lack of understanding of the bases of their good food safety practices may lead them to use those practices nonchalantly.

Table 6. Logistic regression predicting the level of knowledge and self-reported food safety practices from stakeholders' demographic characteristics

Predictor	B		Wald chi-square		p-value		Odds ratio	
	Know	Prac	Know	Prac	Know	Prac	Know	Prac
Gender	-1.36	-2.42	1.12	7.55	0.29	0.86	0.26	0.09
Age	0.682	0.00	1.61	0.00	0.21	0.99	1.98	1.00
Ethnicity	-	-	4.76	27.5	0.41	*0.00	-	-
• Ada	-1.90	-3.48	1.74	19.05	1.87	*0.00	0.15	0.03
• Krobo	17.68	-3.14	0.00	6.42	0.99	*0.01	47.3	0.04
• Ewe	-2.57	-3.60	3.95	24.71	0.05	*0.00	0.08	0.03
Longevity	-1.79	0.33	0.45	3.02	0.50	0.08	0.84	1.39
Religion	-1.04	-1.12	4.03	0.28	0.05	0.60	0.35	0.09
Education	-1.29	0.35	0.03	0.84	0.87	0.36	0.89	1.42
Stakeholder (Retailer)	-1.65	-2.74	3.18	10.34	0.074	*0.001	1.192	0.065

Note: *significant at p-value <0.05; Know= food safety knowledge; Prac= fish handling practices

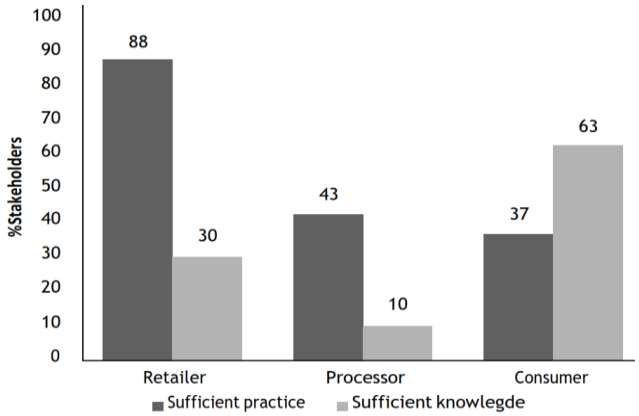

Figure 5. Relationship between the knowledge and practices of some stakeholders in the fish value chain

Fish losses due to spoilage in the artisanal fish value chain

Stakeholders in both the marine and freshwater value chains reported that they never really considered fish spoilt, especially with tilapia, catfish, and sardinellas. According to most, fish only loses its freshness and is useful as food even when it looks rotten. Tilapia and catfish were, for example, salted and sun-dried into "momone" or "*lonshala*." *Lonshal* fetches a much lower price than in the fresh state. Again, *lonshala* is primarily intended for flavoring and used in tiny quantities during cooking. The fish, therefore, ceased to be a primary source of protein in the diet once it lost its freshness before processing. Stakeholders thus estimated their losses based on fish they had to devalue because they were no longer considered fresh by consumers and customers.

Figure 6 depicts the levels of fish losses due to spoilage experienced by the different stakeholders along the value chain.

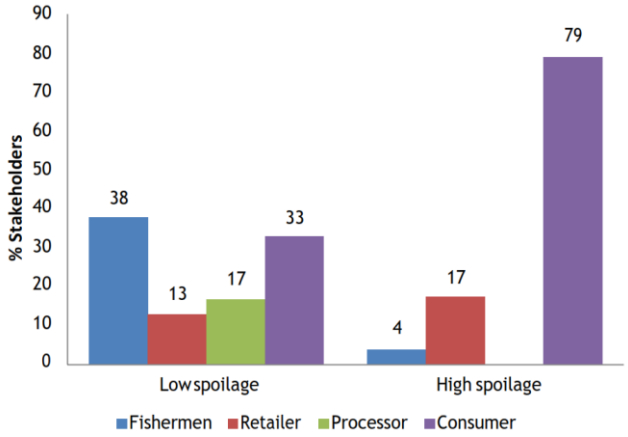

Figure 6. Estimated fish losses along the fish value chain

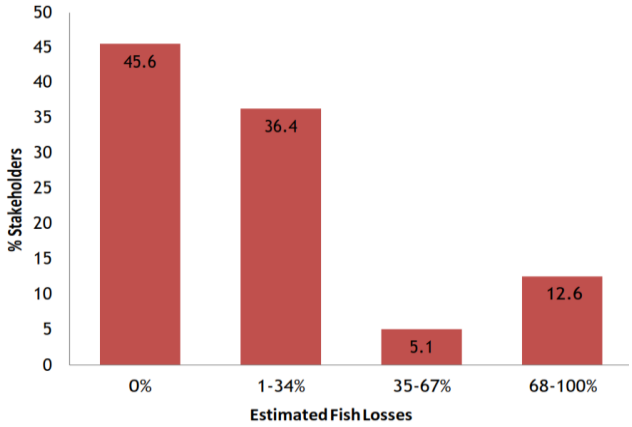

Figure 7. Stakeholder estimates of fish downgraded, devalued, or discarded due to spoilage

Table 7. Stakeholders' responses to using fish no longer considered fresh

Use of "spoilt" fish	Number of stakeholders (%)		
	Wholesaler/ retailer	Processor	Consumer
Discarded	1 (1.4)	13 (17.8)	59 (80.8)
Modified to *lonshala/momone*	18 (66.7)	0 (0)	9 (33.3)
Animal feed	1 (14.3)	0 (0)	6 (85.7)
Sold to consumers as "fresh."	1 (100)	0 (0)	0 (0)
Processed as originally intended	0 (0)	7 (87.5)	1 (12.5)
Returned to purchase point	0 (0)	0 (0)	2 (100)
Consumed Regardless	0 (0)	0 (0)	2 (100)
Never experience spoilage	19 (23.8)	20 (25.0)	41 (51.3)

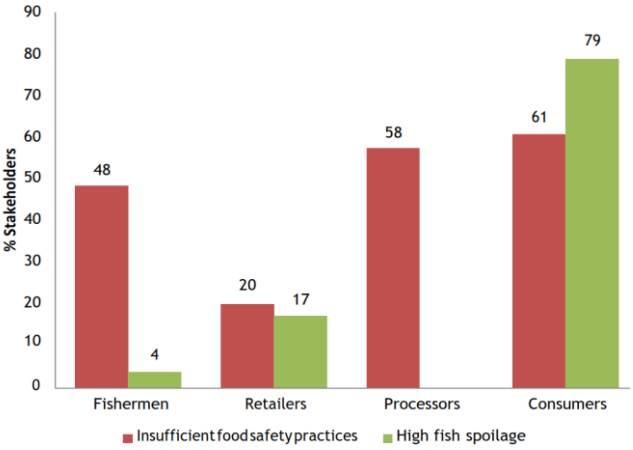

Figure 8. Relationship between insufficient food safety practices and fish losses among stakeholders

Spoilage was classified in this study as low when the losses ranged between zero and 34%. The losses were considered high when as much as 35 to 100% of the fish were lost. Consumers understandably suffered the greatest amounts of losses, sometimes losing up to 100 % of all fish bought, owing to the fact that they were at the very end of the value chain. They also attributed these high losses to Ghana's reliable electrical power supply. For consumers, the cause of action for fish that had lost their freshness was to discard or use the spoilt fish as animal feed (refer to Table 7). Fish, therefore, intended for use as the primary protein in meals were lost, and significant economic losses had to be incurred to replace the spoilt fish.

Processors, wholesalers, and retailers rarely experienced high losses due to their ability to transform and add value to fish that had lost their freshness. Processors, for example, reported that they could still process spoilt fish as intended initially (without downgrading it to *momone*) because they believed their processing methods could render the spoilt fish safe for consumption. Fishers experienced minimal losses comparatively, most likely because they reported that they always had a ready market at landing.

Akande and Dei-Ouadi (2010) reported that between ten and thirty percent of the artisanal catches in Ghana were downgraded due to quality deterioration. Similar findings from this study, displayed in Figure 7, showed up to 34 % losses among 37 % of all stakeholders and between 68 to100 % losses among 13 % of stakeholders.

The stakeholders' food safety knowledge and practices within the fish value chain also appeared to play a role in the reported extent of fish losses. A strong association (Pearson chi-square = 16.137, p-value 0.00) was found between the level of knowledge and the estimated fish losses. This suggested that the level of food safety knowledge of a stakeholder can reliably predict the extent of their fish losses.

Presented in Figure 8. is a graph illustrating the relationship between the extent of fish losses and food safety practice.

Microbiological quality and safety of fish at different stages of the artisanal fish value chain

Salmonella spp., *Vibrio parahaemolyticus*, and *Vibrio cholerae* were not detected in any of the samples tested. Ikutegbe and Sikoki (2014) also reported the absence of

this organism, although their study focused only on dried-smoked fish samples. All the suspect *Vibrio* colonies were confirmed as *Aeromonas sobria* by API 20E, with a prevalence rate of 4.8%. Table 8 lists the incidence of some of the pathogenic and spoilage organisms detected in raw and processed fish samples.

The absence of *Salmonella* spp., *Vibrio parahaemolyticus,* and *Vibrio cholerae* on the tested fish samples is of key importance given the significant public health threats they are capable of. However, the presence of Aeromonas sobria on both raw and processed fish samples is very troubling. This organism is a pathogen that can cause foodborne gastroenteritis in humans and extraintestinal symptoms such as septicaemia, meningitis and endocarditis, and osteomyelitis. It is especially dangerous in immuno-compromised individuals. This organism had been previously isolated from fish by Boari et al. (2008) and Ashiru et al. (2011), who isolated the organism on catfish and tilapia even at refrigerated temperatures. *Aeromonas* spp. have also been implicated in the spoilage of fish (Gram and Dalgaard 2002) because of their ability to produce enzymes such as lipases and proteases.

Proteus spp. was detected at a prevalence of 55% in all the raw samples and up to 66.7% in the processed samples. This organism is capable of decarboxylating histidine into histamine, produces H2S, and is therefore capable of causing fish spoilage. It is also an opportunistic pathogen that can infect immune-compromised individuals. Several studies have implicated pseudomonas species as the dominant bacterium in fish spoilage (Tryfinopoulou et al., 2007; Popovic et al., 2010; Ikutegbe and Sikoki 2014; Viji et al. 2014). However, in this study, the dominant spoilage organisms detected in all fishes at different value chain stages appeared to be *Proteus mirabilis* and *Proteus vulgaris* (Tables 9-10). Akoachere et al. (2009) also isolated *Proteus vulgaris* and *Proteus penneri* in fish sourced from the coastal waters of Cameroun, a country with similar climatic and socio-economic conditions as Ghana.

The total mesophilic counts determined for fresh catfish from fishers and wholesalers were consistently below the maximum allowable limit of 7 Log cfu/g set by the International Commission on Microbial Specifications for Foods (ICMSF), which initially suggested good overall hygienic quality. However, raw tilapia intended for grilling was found to contain unacceptable counts of 7.96±0.68 Log cfu/g consistently. However, this value did not significantly vary from other fresh tilapia and fresh sardinella samples, as presented in Table 9. Erkan and Ozden (2008) assessed the quality of sardines stored on ice in Turkey reported mesophilic bacteria counts of 3.8 to 4 logcfu/g on the first day of storage and up to 6 log cfu/g after nine days of storage. These results were consistent with the findings of this study.

The microbiological quality of the processed fish samples also appeared initially to be good, given the generally low counts of total mesophilic bacteria and total counts of yeasts and molds. A study by Ikutegbe and Sikoki (2014) followed the counts of heterotrophic fungi on smoked fish sourced from retail markets in Nigeria also reported counts of up to 3.40 log cfu/g in smoked fish stored for three weeks or less. Retailers in Ghana kept smoked fish for more extended periods, up to 6months. Counts for fungi (yeasts and molds) on dried fish samples in this study were generally lower in comparison.

Tables 9-10, however, present evidence to suggest issues with the safety of fish on the Ghanaian market. Essential quality and safety indicator pathogeare ns are detected at different value chain stages. To begin with, *Listeria* spp. They are discovered at a prevalence of 11% in raw tilapia and catfish and up to 66.7% in fermented fish samples. This is of great concern given the public health threat posed by this organism (Bomfeh et al., 2015). The microorganism was also present in salted-dry fish ("koobi") and smoked-dry fish.

Table 8. Prevalence of some pathogenic and spoilage organisms on raw and processed Tilapia, catfish and sardinella

Microorganisms	**% Prevalence (n/N)**						
	Fish product						
	Raw	**Smoked**	**Salted**	**Fried**	**Dried**	**Grilled**	**Fermented**
Salmonella spp.	0 (0/27)	0 (0/17)	0 (0/6)	0 (0/5)	0 (0/3)	0 (0/3)	0 (0/3)
Vibrio spp.	0 (0/27)	0 (0/17)	0 (0/6)	0 (0/5)	0 (0/3)	0 (0/3)	0 (0/3)
Listeria spp.	11 (3/27)	23.5 (4/17)	33.3 (2/6)	0 (0/5)	0 (0/3)	0 (0/3)	66.7 (2/3)
Proteus spp.	55.5 (15/27)	52.9 (9/17)	33.3 (2/6)	40 (2/5)	66.7 (2/3)	0 (0/3)	66.7 (2/3)
Staphilococcus aureus	29.6 (8/27)	17.6 (3/17)	16.7 (1/6)	0 (0/5)	0 (0/3)	0 (0/3)	0 (0/3)
Klebsiella spp.	48.1 (13/27)	11.8 (2/17)	16.7 (1/6)	0 (0/5)	0 (0/5)	0 (0/3)	0 (0/3)
Aeromonas spp.	7.4 (2/27)	5.9 (1/17)	0 (0/6)	0 (0/5)	0 (0/3)	0 (0/3)	0 (0/3)
Pseudomonas spp.	11.1 (3/27)	0 (0/17)	0 (0/6)	0 (0/5)	0 (0/3)	0 (0/3)	0 (0/3)
Escherichia coli	7.4 (2/27)	0 (0/17)	0 (0/6)	0 (0/6)	33.3 (1/3)	0 (0/3)	33.3 (1/3)
Clostridium perfringens	77.8 (21/27)	0 (0/17)	33.3 (2/6)	50.0 (3/6)	100.0 (3/3)	0 (0/3)	0 (0/3)

Table 9. Microbial quality of raw, unprocessed fish at different stages of the artisanal fish value chain

Stakeholder	Fish species	Product	n	Food safety indicators			Food spoilage indicator	Food hygiene indicators			Overall quality indicator	Physico-chemical properties	
				List.	Kleb.	Cl perf. Log cfu/g	Proteus	F. coli	E. coli	S. aur.	TMC Log cfu/g	Temp (°C)	pH
Fishers	Tilapia	Fresh fish	3	A	P	3.71 ±2.68b	Pm	2.98 ±0.57a	A	P	5.53 ±0.25ab	29.32±0.37a	7.26±0.17a
Fishers	Catfish	Fresh fish	2	A	P	2.83 ±0.22ab	Pm	3.70 ±0.95ab	P	P	3.18 ±3.08a	29.25±0.21a	7.60±0.19a
Fishers	Sardinella	Fresh fish	3	A	P	2.70 ±0.11ab	Pmv	2.12 ±0.28a	A	A	5.89 ±0.09ab	26.68±3.09a	7.42±0.15a
Wholesaler	Tilapia	Fresh fish	3	A	A	2.31 ±1.28ab	Pm	2.31 ±1.28a	A	P	5.52 ±2.80ab	19.23±7.89a	6.50±0.13c
Wholesaler	Catfish	Fresh fish	2	A	P	ND	Pm	1.99 ±0.13a	A	A	2.95 ±0.23a	27.70±0.45a	7.16±0.54a
Retailer	Tilapia	Fresh fish	3	P	P	2.83 ±0.22ab	Pv	5.54 ±2.09b	A	P	6.28 ±0.20ab	15.00±12.45a	-
Retailer	Sardinella	Fresh fish	3	A	P	2.43 ±2.02ab	Pmv	2.32 ±1.19a	A	A	5.46 ±0.36ab	26.00±2.00a	-
Processor (Salted)	Tilapia	Raw fish	1	P	A	ND	Pm	3.43 ±0.00ab	A	A	4.67 ±0.00ab	32.30±3.50a	7.79±0.16ab
Processor (Fermented)	Tilapia	Raw fish	2	A	A	2.96 ±0.39ab	Pv	3.52 ±0.27ab	A	A	4.96 ±0.90ab	30.24±3.53	7.79±0.16ab
Processor (Fermented)	Catfish	Raw fish	2	P	A	2.71 ±1.14ab	Pv	2.9 ±0.68ab	P	A	5.40 ±1.91ab	29.95±3.98a	8.09±0.10b
Processor (Grilled)	Tilapia	Raw fish	3	A	A	3.36 ±0.14b	A	3.38 ±0.16ab	A	A	7.96 ±0.68b	23.03±3.21a	-

Note: Values in the same column with different superscripts are significantly different at α=0.05. Abbreviations: A= absent; P=present; Pm= *Proteus mirabilis*; Pv= *Proteus vulgaris* ND=not detected; Temp: temperature. Lis: *Listeria* spp.; Kle: *Klebsiella* spp.; Cl perf: *Clostridium perfringens*; F. coli: faecal coliforms; S.aur: *Staphylococcus aureus*; TMC: Total mesophilic count

Table 10. Microbiological quality of processed fish at different stages of the artisanal fish value chain

Stakeholder	Fish species	Product	n	Food safety indicators			Food spoilage indicator	Food hygiene indicators			Overall quality indicator Log cfu/g		Physico-chemical properties	
				Lis	Kle	Cl. Perf Log cfu/g	Proteus	F coli. Log cfu/g	E. col	S. au	TMC	YM	Temp (°C)	pH
Processor	Tilapia	Fermented	2	A	A	2.08±1.14b	Pm	2.46±0.00b	A	A	4.61±0.00b	2.36±0.00ab	31.95±4.88a	-
Processor	Catfish	Fermented	2	P	A	1.54±0.09b	Pv	1.75±1.06b	P	A	2.97±0.01b	ND	35.33±2.00a	7.51±0.10a
Processor	Tilapia	Grilled	3	A	A	ND	A	1.73±0.70b	A	A	5.37±3.79	2.33±0.90ab	37.46±14.23b	-
Processor	Tilapia	Smoked	2	A	A	ND	A	ND	A	A	1.50±0.71b	2.11±1.57ab	50.65±21.85b	-
Retailer	Tilapia	Smoked	3	P	A	ND	Pmv	ND	A	A	3.20±1.25b	ND	34.50±4.88a	-
Processor	Catfish	Smoked	2	P	P	ND	Pm	2.39±1.96b	A	P	1.93±0.21b	2.24±1.75ab	82.56±2.00b	-
Retailer	Catfish	Smoked	3	P	A	ND	Pv	ND	A	A	2.73±0.40b	ND	28.27±2.00a	-
Processor	Sardinella	Smoked	3	A	A	ND	Pmv	ND	A	A	2.92±0.87b	1.51±0.88a	28.73±2.00a	6.46±0.03c
Retailer	Sardinella	Smoked	3	A	A	ND	Pm	1.85±0.85b	A	A	4.07±1.63b	2.38±1.32ab	31.57±2.61a	6.76±0.35ac
Processor	Tilapia	Fried	2	A	A	ND	Pm	ND	A	A	1.23±0.40b	1.51±0.88a	34.27±3.033a	7.38±0.10a
Processor	Catfish	Fried	3	A	A	ND	Pm	ND	A	A	ND	1.93±1.31ab	34.27±3.033a	7.58±0.10a
Processor	Tilapia	Salted	2	A	A	1.75±1.29b	A	2.17±1.65b	A	A	2.66±1.53b	5.17±3.64b	32.68±2.85a	7.47±0.10a
Ret	Tilapia	Salted	3	P	A	ND	A	ND	A	A	3.14±0.13b	ND	28.600±2.00a	7.05±0.00a
Processor	Sardinella	Dried	3	A	A	3.88±2.54b	Pv	3.56±2.93b	P	A	4.10±0.37b	1.93±1.61a	31.90±2.00a	7.21±0.10a

Note: Values in the same column with different superscripts are significantly different at α=0.05. Abbreviations: A= absent; P=present; ND: not detected: Pm= *Proteus mirabilis*; Pv= *Proteus vulgaris;* Temp: Temperature. Lis: *Listeria* spp.; Kle: *Klebsiella* spp.; Cl perf: *Clostridium perfringens*; F.coli: faecal coliforms; S.au : *Staphylococcus aureus*; TMC: Total mesophilic count

A study by Tano-Debrah et al. (2011) attributed the occurrence of Listeria on fermented fish, mainly to post-process contamination. Samples in that study were also collected in James Town and some informal fish markets in Accra, and detectable Listeria monocytogenes on salted-dry and sun-dried tilapia and herrings were reported. Evidence presented also suggested that salting and drying methods used by processors could not adequately control the organism.

However, the high incidence of Listeria on fermented fish in this study was more likely attributable to the opportunities available for proliferation from landing until the start of fermentation. These included consistently high storage temperatures (Table 9) and poor hygienic conditions and handling practices reported from landing until the commencement of fermentation. Unlike the other processing methods, Fermentation was typically carried out as a means to salvage fish that has lost its freshness or fish that may be considered spoiled by consumers. A review of studies on African fermented fishes by El Sheikah et al. (2014) similarly reported safety issues related to *Clostridium, Salmonella*, and aflatoxin contamination in *momone* and other fermented fish products.

It was also noteworthy that the aerobic mesophilic counts of raw tilapia sampled from the grill- processors were not significantly reduced in the final grilled tilapia samples. A similar trend was observed between fresh samples of tilapia and catfish intended for fermentation and salting and their flast processed equivalents. There was also a remarkable persistence of other pathogenic microorganisms like *Staphylococcus aureus* and *Klebsiella pneumonia* on smoked and salted fish and the presence of *Clostridium perfringens* on salted tilapia fried tilapia and sun-dried sardinella samples. It was more plausible to attribute this observation to post-processing contamination rather than the inadequacy of processing methods, owing to poor hygienic conditions of storage and handling reported among stakeholders within the fish value chain. However, questions about the adequacy of processes such as sun drying, salting, and smoking in ensuring safety could not be discounted entirely. Poor hygienic conditions prevailing at most processing sites, in addition to evidence from studies reporting on the poor quality of salt, wash water, and drying temperatures, could very well account for the high incidence of pathogenic and spoilage organisms observed in this study (El Sheikah et al. 2014). Some of the hygiene issues observed at processing sites included open defecation close to processing areas, presence of livestock, drying of fish directly on the ground or close to the ground, and exposure of fish to the elements, especially during sun drying.

Also of significance was detecting *Clostridium perfringens* in all the raw fish samples tested. *Clostridium perfringens* has been implicated as the etiological agent in many food poisoning outbreaks, and its presence on food samples indicates sewerage contamination (Lalitha and Surendran 2003). Therefore, it was not surprising that there were also high counts of facal coliforms on both the fresh and processed fish samples. The shores of James Town have long been a dumping site of sewerage for the city of Accra, and poor fishing communities along the Volta lake have also been known to dispose of fecal matter into the Lake (Awuah and Abrokwa 2008). The high loads of *Clostridium perfringens* and fecal coliforms on landed fish from these sources, coupled with the poor handling practices documented in this study, may very likely ensure survival and proliferation of the microorganism even under the dehydrating conditions of processing.

In a similar study, Lalitha and Surendran (2003) reported a 22% incidence rate of *C. perfringens* in fish and shellfish sampled in Kerala, India. Their study reported the detection of C. *perfringens* at every stage of the value chain from which the samples were sourced. This was especially the case of the tilapia samples in their study- The microorganism was detected in 94% of all unprocessed raw samples tested (17 out of 18), with counts significantly higher in the raw fish at landing. El-Shorbagy, Reda, and Mona (2012) also reported a prevalence of 57.1% in 56 processed samples and 59% in 57 samples. These researchers also found the microbe in three types of salted sardines but found none in canned fish.

Another important fish quality and safety indicator detected in samples from this study was *Escherichia coli*. Two diarrhoeagenic *E. coli* (*E. coli (2) 0146* and *E. coli (4) 027}* were isolated from fresh catfish from fishers and fermented catfish from processors, respectively. The former is an enteropathogenic (EPEC) strain, while the latter is an enterotoxigenic (ETEC) strain of E. coli. Both have been implicated in acute and persistent watery diarrhea in children primarily between the ages of 6months to 3years (Warrell et al., 2003). ETEC can particularly cause diarrhea in all ages because of its ability to mimic clinical symptoms of diarrhea. Costa (2013) concluded that the presence of these organisms on the fish samples could indicate the existence of other enteric pathogens and recommended adequate processing before consumption.

Escherichia coli (4) 0148 detected on all the sun-dried sardinella samples were also ETEC strains. This finding is of particular importance because, more often than not, dried sardinellas are consumed without further heat processing in Ghana. They are usually made into powder and used to increase the protein content of complementary feed given to children. This is of obvious concern given the public health implications of ETEC. The survival of ETEC in the otherwise unfriendly conditions of low water activity in the dry fish could be the result of the drying methods employed in processing the sardinellas.

Klebsiella pneumoniae was another fish safety indicator pathogen detected in some samples. It is an opportunistic pathogen that can cause nosocomial infections of the respiratory tract, urinary tract, and blood, especially in children immuno-compromised by a diarrhoeal infection.

Also, the different stakeholders' temperatures are used in fish storage in all three value chains. The temperatures were less than ideal and may very well account for the incidence of the spoilage and pathogenic organisms observed. The temperature at the landing of all the fish species ranged from 26oC to 29oC. These values did not vary significantly at the next stage of the fresh fish value chain, which involved retailers and wholesalers. The high

average temperatures recorded also appeared to contradict reports by the majority (85%) of wholesalers and retailers who claimed to keep their fish on ice during the sale period.

The disparity between the reported use of ice to reduce the temperature of the fish and the actual recorded temperatures could, however, be explained by the fact that the amount of ice used was insufficient to affect cooling to desirable temperatures. Again, the Styrofoam containers used by stakeholders in the fresh fish value chain, and the wooden boxes with sack lining used in the marine fish value chain, did not provide enough insulation to maintain cold temperatures. The recorded temperatures for the processed fish samples, especially in the freshwater fish value chain, were exceptionally high (50 to 85oC) and significantly different from other fresh and processed fish temperatures because they were sampled a few minutes after processing.

However, the pH of the fish measured at the landing and different stages of the value chain appeared to conform to trends reported in similar studies. The general pH of fresh tropical fish muscle reported by Susanto et al. (2011) ranged from 6.0 to 7.3 on the first day of capture and from 6.8 to 8.2 by the second day when kept at ambient temperature. In this study, the pH at landing (from fishers) ranged from 7.2 to 7.8 for the freshwater fishes and between 7.3 and 7.6 for the marine fish. These values significantly decreased at the wholesale stage in the tilapia value chain. Because the same batch of fish was not followed from fishers to wholesalers, it was impossible to accurately assume that the observed significant increase in acidity from one stage in the value chain to the next was due to microbial or autolytic activity. High glycogen levels in the fish before capture may account for the increased acidity because of the resultant accumulation of lactic acid.

A significant rise in alkalinity in raw catfish and tilapia intended for fermentation and salting was observed from samples sourced from processors. Therefore, the stakeholders considered such fish no longer fresh and transformed into “Momone” and “Koobi.” A rise in fish muscle alkalinity with increasing storage time was also observed in a study by Erkan and Ozden (2008). These researchers attributed the pH increases to the accumulation of alkaline compounds such as ammonia, mainly derived from microbial action. Viji et al. (2014) also supported this theory but warned that pH was a poor quality indicator of fish quality.

Compared to the freshwater value chain, fewer pathogens were isolated from the marine fish value chain. Sardinellas, which were to be sold fresh to consumers, were also potentially stored for up to two weeks by retailers. However, this did not imply that the quality of the marine fish was better than freshwater fishes, evidenced by the high unacceptable total mesophilic counts observed in raw tilapia samples.

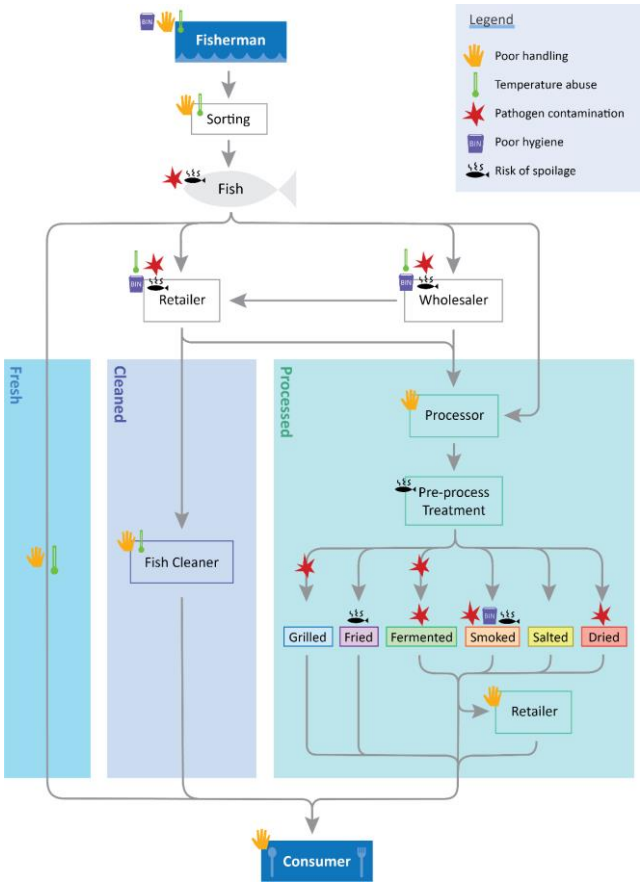

Figure 9. Association between food safety practices and the actual microbial quality of Artisanal fish

Association between stakeholder knowledge and practices and the actual microbial quality of artisanal fish

It is evident from Figure 9 that pathogens and spoilage organisms could be found at almost every stage of the value chain where poor handling practices by stakeholders were reported. The exception was with wholesalers and retailers of fresh, unprocessed fish, who generally said sufficient handling practices but still had sick quality fish. This deviation from the trend was clearly a result of insufficient cooling temperatures and temperature abuse during storage. Temperature abuse was observed throughout the value chain and is, without question, a significant reason for the poor quality of fish found in this study.

Another possible contributor to the poor quality of fish observed at the retail and wholesale stage of the fresh fish value chain could be the existing traditional laws that place certain restrictions on the uninhibited fish flow on the artisanal value chain. Fishing in James Town on Tuesdays, for example, was strictly taboo. Consequently, the women who retailed fresh or raw fish in the local fish market were also not allowed to sell their fish on Tuesdays. Although crucial in ensuring the sustainability of fisheries, this cultural practice also implied that fresh fish remained longer (up to two weeks) at the retail stage of the value

chain. This represented a classic case in which a stakeholder practice that was not directly related to food safety may have implications for the poor quality of fish observed at the retail stage.

Figure 9 also shows potential opportunities for recontamination of processed fish in the artisanal fish value chain. Here again, poor handling practices by processors themselves and retailers of processed fish may cause the poor quality of processed fish to reach the consumer. This is of particular concern given that fish at these stages of the value chain (processor and retailer) led directly to the consumer, who may, in all likelihood, consume this fish without further processing.

The high reported fish losses due to spoilage were also corroborated by detecting various spoilage microorganisms, particularly *Proteus* spp. At different stages of the value chain. Figure 9 indicates the risk of fish spoilage, especially with fish reaching wholesalers and retailers from fishers, at the actual retail and wholesale point, and during processing before entering the consumer, the highest incidence of spoilage reported by wholesalers and retailers. The few processors who reported losses said that waste occurred mainly before processing during pre-process operations such as spicing before grilling or sun-drying before hot-smoking. This is again confirmed during microbiological testing as spoilage organisms isolated at these exact points in the value chain.

The link between the food safety knowledge and practices of stakeholders and the actual microbiological quality of the fish thus becomes visible. Poor food safety knowledge and handling practices of stakeholders have clear implications on the final microbiological quality of fishes handled by these stakeholders.

Food security implications

Given the findings of this study, a valid argument can be made about a potential threat to food security. To begin with, the significant economic implications of high reported fish losses are undeniable. Although one may argue that much of this loss is salvaged through fermentation processing, economic losses are still incurred because fermented fish fetches a considerably lower price than fresh fish. It no longer serves its purpose as a primary protein in a meal. Fermented fish is typically consumed in very small quantities as a condiment for soups and sauces and is therefore not consumed in sufficient quantities to represent a significant source of protein. Ikutegbe and Sikoki (2014) also reported a decline in fish protein levels, increasing microbial load and storage time. Again, the high quantities of salt used in fermentation render the fish quite unhealthy when frequently used in meals (Table 11).

The majority (61%) of consumers in this study reported that they used meat as an alternate source of protein when fish was unavailable. They, however, stated that meat was more expensive than fish and therefore had to spend more money on food when fish was unavailable. Up to 80% of consumers also chose fish as their primary source of protein because they believed it was healthier than other protein sources. The majority (76%) also trusted the safety of the fish on the local market, a matter of concern, given the probability that most of these consumers may not take the necessary precautions in processing their fish because they trust it to be safe.

Regarding safety, the poor microbiological quality of fish from artisanal sources could contribute to the high disease burden among the most vulnerable groups. Fish has been implicated in several outbreaks of food-borne infections. It is a potential vehicle for food-borne illnesses such as cholera, listeriosis, salmonellosis, and others (Popovic et al., 2010; Costa 2013; Akoachere et al. 2009). In Ghana, diarrhoeal diseases are among the top three causes of death among children under five. According to a WHO/UNICEF report, 55000 children under five died from diarrhea in 2008 alone (Wardlaw et al., 2010). Diarrhoeagenic microorganisms such as *E. coli. Aeromonas sobria* and *Clostridium perfringens* detected on ready-to-eat fish given as complementary feed to children may very well contribute to the high incidence of diarrhea in Ghanaian children under five.

From the nutritional, safety, and an economic point of view, the food security of the many Ghanaians who depend on artisanal fish as a primary source of protein and livelihood may be under reasonable threat.

Table 11. Consumer responses to questions on food security about fish availability and safety

Tested components of food security	Consumer questions	% Responses of consumers		
		Yes	No	Do not know
Afford-ability	Are you able to afford fish every time?	73	27	-
	What are your alternate sources of protein when fish is unavailable?			
	Meat	61	-	-
	Eggs	23	-	-
	Legumes	3	-	-
	Mushrooms	8	-	-
	Crab/snails	2.5	-	-
	Vegetables	2.5	-	-
	Is meat more expensive than fish?	55	40	5
Availability	Are you able to get fish every time you want it (all year round?	81	19	-
Health/ nutrition	What is your reason for choosing fish as your primary source of protein?			
	Health	42	-	-
	Fish is more nutritious	38	-	-
	Fish is cheaper	8	-	-
	Taste	12	-	-
	Are there adverse health effects associated with eating unwholesome fish?	48	12	40
	Do you think fish sold on the local market is safe for human consumption?	76	22	2

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Structure and biomass accumulation of natural mangrove forest at Gazi Bay, Kenya

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Abstract. *Githaiga MN, Kotut K, Kariuki F, Kairo JG. 2020. Structure and biomass accumulation of natural mangrove forest at Gazi Bay, Kenya. Bonorowo Wetlands 10: 18-32.* This study aimed to determine the forest structure and estimate biomass accumulation above and below ground in the mangrove forest of Gazi Bay. The Gazi Bay mangrove forest's western, middle, and eastern forest blocks were investigated for forest structure, whereas the western forest block was determined for biomass accumulation. To calculate below-ground biomass accumulation, in-growth cores of 80 cm long, 20 cm broad, and 60 cm deep were employed. Above-ground biomass accumulation was calculated using tree height and stem diameter data at breast height (DBH_{-130}). Tagging shoots observed leaf phenology. At the start, environmental variables were measured every four months for a year across four mangrove species zones. The linear regeneration sampling approach determined natural regeneration's composition and distribution pattern (LRS). Salinity revealed a strong negative connection with above-ground biomass accumulation among the soil environment characteristics studied. Sonneratia alba had the highest biomass accretion rate of 10.5 1.9 t ha-1 yr-1 among the four forest zones. *Rhizophora mucronata* (8.5 0.8 t ha^{-1} yr^{-1}), *Avicennia marina* (5.2 1.8 t ha^{-1} yr^{-1}), and *Ceriops tagal* (2.6 1.5 t ha^{-1} yr^{-1}) were the next most abundant species. Above-ground and below-ground biomass accumulation differed significantly among zones ($F_{(3, 8)} = 5.42$, $p = 0.025$) and ($F_{(3, 8)} = 16.03$, $p = 0$ 001), respectively. There was a significant difference in total biomass accumulation across zones ($F_{(3, 8)} = 15.56$, $p = 0.001$). A root: shoot biomass accumulation ratio of 2: 5 was calculated for the entire forest. This study's findings provide more accurate estimates of mangrove carbon capture and storage, which can be used in carbon credit discussions in the emerging carbon market.

Keywords: Biomass accumulation, Gazi Bay, Kenya, mangrove forest

INTRODUCTION

Mangroves are salt-tolerant plants and shrubs that grow along with the intertidal areas of tropical and subtropical coastlines. Millions of people in the tropics rely on keystone coastal ecosystems for economic, ecological, and environmental reasons. Mangroves provide essential habitats and feeding grounds for various benthic and pelagic marine animals and bird species and commercial fisheries resources and nursery grounds for coastal fisheries (Costanza et al. 1997; Saenger 2002; FAO 2007a; FAO 2007b) (). Among the tropics, up to 75 % of commercial fish species spend part of their life cycle in mangroves (Mumby et al., 2008). Climate regulation, nutrient cycling, habitat provisioning, coastline protection, and the provision of building materials and fuelwood are all significant functions of mangroves. The global value of mangrove goods and services is estimated to be $ 1.6 billion per year (FAO 2007b).

The world's mangroves cover 1.5 million square kilometers (Giri et al., 2011). This equates to around 0.4 percent of tropical forests or ~12% of the total land area on the planet (Komiyama et al., 2002). Despite their modest size, they play an important part in the carbon cycle (Bouillon et al., 2008). Mangroves are one of the most prolific ecosystems on the planet, storing more than 1,000 Mg C ha^{-1} on average. Carbon is stored in both living and dead wood, with sediment serving as the primary storage location (Donato et al., 2011).

Scientists are interested in understanding the potential function of mangroves in carbon capture and storage due to observed trends in global warming and the need for climate change mitigation. Reduced emissions from deforestation and degradation (or REDD+) are worldwide policies that focus on decreasing emissions and increasing carbon stocks by addressing deforestation, forest degradation, forest conservation, and sustainable forest management. Countries willing and able to reduce deforestation and forest degradation emissions will be reimbursed under the REDD+ program. Kenya has submitted a proposal to the World Bank's Forest Carbon Partnership Facility for REDD Readiness Preparation (R-RPP) (FCPF). This proposal lays out a plan for REDD preparatory operations, including how the work would be structured and managed and the financial resources required. Kenya intends to increase its forest cover to 10% with authorization, which will help mitigate climate change while also enhancing the livelihoods of forest-dependent populations through several advantages such as carbon credits.

The study's precise goals were to: (i) to analyze the forest structure across the three blocks of mangrove forest in Gazi Bay, (ii) to figure out how certain physicochemical parameters affect biomass accumulation in the Gazi mangrove forest, (iii) to determine the link between above-

ground and below-ground biomass accumulation in each of the Gazi mangrove forest's species zones, (iv) to determine the variation in total biomass accumulation and AGB: BGB ratios of mangrove species in the Gazi mangrove forest's distinct zones.

MATERIALS AND METHODS

Study site

The study was carried out on the southern coast of Kenya, on a place called Gazi Bay (4º25'S and 39º30'E), about 55 km away from Mombasa City (Figure 1).

The embayment has a surface area of 18 km^2. The presence of the Chale peninsula to the east and a surrounding coral reef to the south protects it from severe waves. Gazi's mangrove area is estimated to be 615 hectares (Kairo et al. 2001). The Gazi Bay mangrove forest area is characterized by a sloping topography and a tidal amplitude of roughly 3.8 m with a maximum of 4.1 m (Kenya Ports Authority tide tables for Kilindini, Mombasa) (Matthijs et al. 1999). Two tidal creeks run through it. The western creek continues inland as the river Kidogoweni, while the eastern brook does not receive any freshwater. The Gazi mangrove forest was classified into three forest blocks based on the two streams, utilizing indices such as tree DBH, height, canopy cover, stand density quality of trees, and regeneration rates. The forest west of the Kidogoweni (western) creek at Gazi village is the western block, while the forest east of the Kinondo (eastern) creek near Kinondo village is the eastern block. Between the two creeks and near Makongeni village is the middle forest block. Soil physicochemical variables, biomass accumulation, and leaf phenology were analyzed on the western block.

Study design

A stratified random research design was utilized, with four zones representing the leading mangrove species in Gazi spread throughout the topographic gradient of the western forest block. *Sonneratia alba* represented the seaward zone, whereas *Rhizophora mucronata*, *Ceriops tagal*, and *Avicennia marina* represented the second, third, and upper zones, respectively. Two sites were chosen at distinct locations for *R. mucronata* and *A. marina* zones based on the growth and structural properties of the vegetation. In this study, A. marina in site "A" was in an area with dwarf mangroves near the landward side, but *A. marina* in site "B" was on the seaward side. In this study, *R. mucronate* in site “A” was at a lower tidal level, had a closed canopy, and had decreased regeneration, whereas *R. mucronata* in site "B" had an open canopy and high regeneration. The *C. tagal* stand consisted of stunted mature trees that stood no more than 2.0 meters tall and had a closed canopy. In each site, three plots of 10 m x 10 m were marked at a distance of 30 m, for a total of 18 plots (Figure 2).

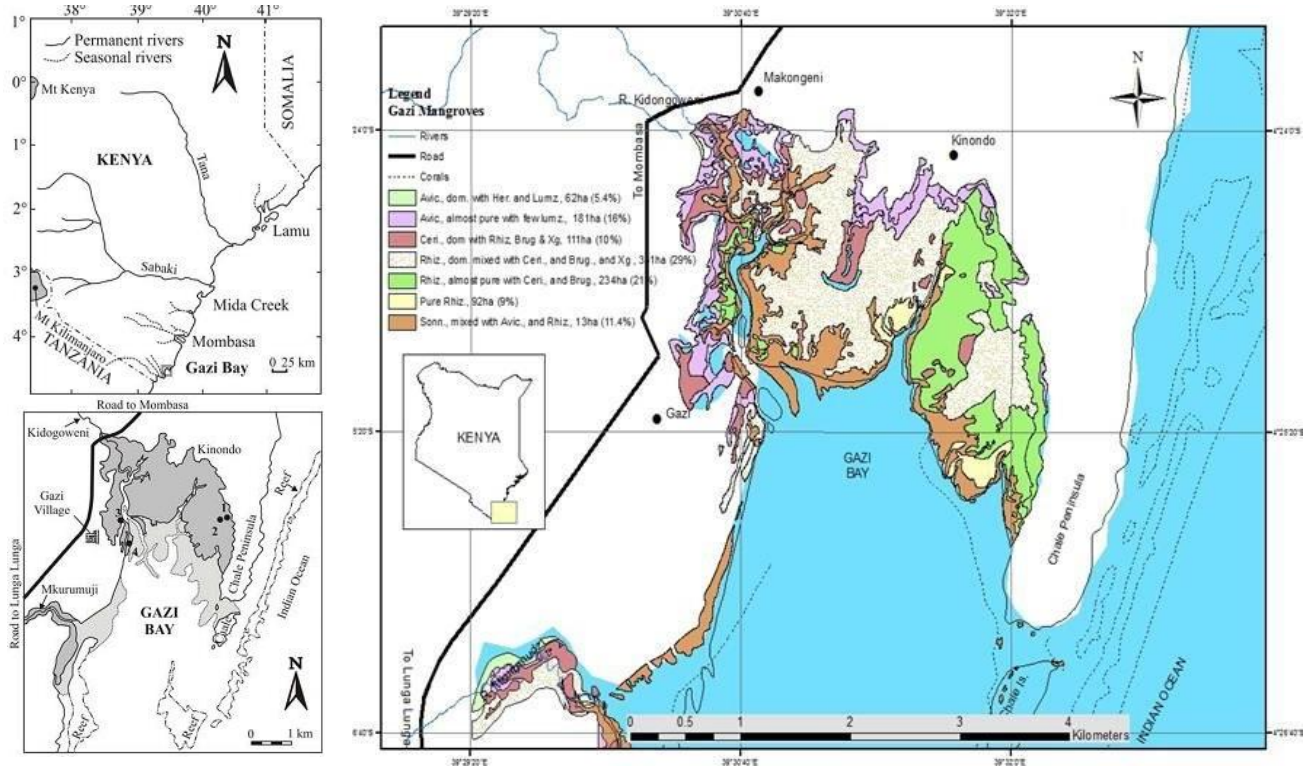

Figure 1. Map of the study site in the mangrove forest of Gazi Bay on the Kenyan Coast. (Source: Bosire et al. 2003)

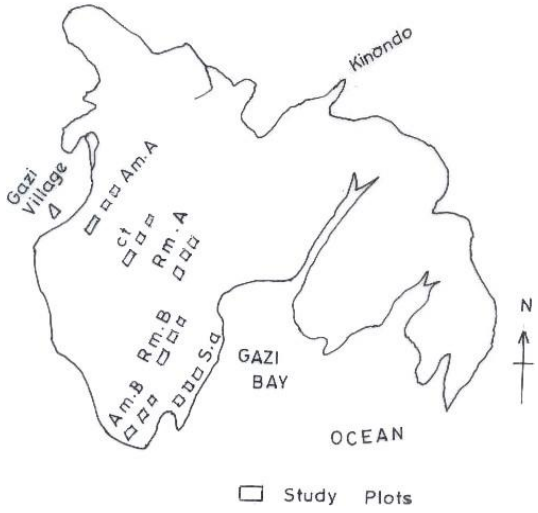

Figure 2. Plot layout for the present study at Gazi mangrove forest. (Am, Ct. Rm and Sa refer to *Avicennia marina, Ceriops tagal, Rhizophora mucronate,* and *Sonneratia alba* zones, respectively)

Physicochemical factors

Measurement of height above datum

The day's highest tidal level (Kilindini tides table 2011) was indicated as the benchmark or beginning reference point for subsequent observations to determine height above datum (HAD). The height above the datum of this point was calculated using the Kilindini tides table's daily estimate. A water-filled transparent hose pipe was placed on the ground, with one end of the pipe at the reference location.

The pipe was gently lifted to the next plot seawards until there was no water flow. The height from the ground was measured with a ruler held vertically to the watermark. The relative height of the plot was calculated by subtracting this height from the height of the reference point. This was done several times, passing through the plots towards the sea.

Measurement of redox potential and pH

Sediment was collected monthly from four randomly chosen points inside the plot at depths of 10 cm and 40 cm to determine physicochemical soil properties. A multimeter was used to detect the sediment redox potential and pH. (HANNA HI 8424 microcomputer pH meter).

Sediment surface water salinity and temperature

Sediment samples for salinity measurement were obtained from four random locations in the same plots, mixed, and transported in sample bags. The samples were centrifuged in the lab to extract the interstitial water. Salinity was measured using a hand-held refractometer (ATAGO, Tokyo, Japan). Temperatures of sediment surface water were measured in situ at four different locations throughout the plot.

Sediment grain size analysis

For granulometric analysis, surface scrapes of sediment samples weighing around 50 grams were obtained from four random points in each plot. The sediment samples were weighed and oven-dried at 80°C for 24 hours in the laboratory, after which they were reweighed to determine the percentage moisture content. For grain size analysis, approximately 25 grams of each sample's dry sediment was treated with 10 ml of aqueous sodium hexametaphosphate and passed through a series of sieves with varied mesh apertures ranging from 63 to 500 m mesh size. The relative percentage of each particle in the sediment was calculated.

Assessment of mangrove forest structure

Structural attributes of mangroves

Mangrove trees with a Diameter at Breast Height (DBH) of less than 5.0 cm were located and their locations documented. For *A. marina* and *S. alba*, the following parameters were measured: tree height (m), crown cover (%), and stem diameter (DBH) (cm) at 130 cm above the ground (D130). Due to the low height of the trees in *C. tagal*, stem diameter (DBH) was measured at 100 cm above ground, but stem diameter in *R. mucronata* trees was measured 30 cm above the highest prop root. The sum of the cross-sectional areas (CSA) of all trees in the species (m2 ha-1) at breast height was used to compute the basal area (BA) of each species. (See Equation (i) below) The sum of the number of stems per plot was divided by the area of the plot in m2 multiplied by 10,000 to get the stem density. (See Equation (ii) for further information.) By adding the relative density, relative frequency, and relative dominance of each species, the importance value index (IV) (a metric that shows the relative contribution of a plant species to the structure of a stand) was calculated (Cintron and Schaeffer-Novelli, 1984). (See Equation (iii) for further information).

The product of number of species, basal area (BA) (m^2 ha^{-1}), maximum tree height (m), and stem density (D, ha^{-1}) x 10^{-5} was used to calculate the complexity index (Ic) of each forest zone (a measure of how complex or structurally developed a vegetation stand is) (Holdridge et al. 1971). The following are the equations: (i) BA (m^2 ha^{-1}) = CSA/plot area (m^2) x 10,000. (ii) Stem density (Stems ha^{-1}) = number of stems in plot/area of plot (m^2) x 10,000. (iii) Relative density + relative frequency + relative dominance. (iv) *Ic* = Number of species × BA (m^2 ha^{-1}) x max. tree height (m) x density (ha^{-1}) x 10^{-5}. The product of number of species, basal area (BA) (m^2 ha^{-1}), maximum tree height (m), and stem density (D, ha^{-1}) x 10^{-5} was used to calculate the complexity index (*Ic*) of each forest zone (a measure of how complex or structurally developed a vegetation stand is) (Holdridge et al. 1971). The following are the equations: (i) BA (m^2 ha^{-1}) = CSA/plot area (m^2) x 10,000. (ii) Stem density (Stems ha^{-1}) = number of stems in plot/area of plot (m^2) x 10,000. (ii) IV = Relative density + relative frequency + relative dominance (iv) *Ic* = number of species x BA (m^2 ha^{-1}) x max. tree height (m) x density (ha^{-1}) x 10^{-5}.

Quality of the mangrove poles

Trees were classified into three form classes, 1, 2, and 3, depending on the usefulness of the main stem in construction, to measure wood quality. Form 1 was assigned to trees with straight poles, whereas form 2 was

assigned to trees with intermediate poles that required little adjustment before construction. Form class 3 was allocated to crooked poles that were inappropriate for construction.

Natural regeneration

The composition and pattern of natural regeneration were assessed using linear regeneration sampling (LRS) (Sukardjo 1987; FAO 1994; Kairo et al. 2002a). The occurrence of juveniles of various species was recorded and grouped according to their height classes and arbitrarily designated, Regeneration Classes (RC) I, II, or III, inside 5 x 5 m^2 subplots (within the main (10 x 10) m^2 quadrats). The ratio of RCI: II: III was used to evaluate the effectiveness of spontaneous regeneration (FAO 1994). Seedlings less than 40 cm height were designated as regeneration class 1 seedlings (RCI). RCII denoted saplings with a height of 40 to 150 cm, whereas RCIII denoted small trees with a height of more than 1.5 m but a DBH of less than 2.5 cm.

Biomass accumulation estimates

Above-ground biomass accumulation

Twelve trees were randomly selected for monitoring increment in stem diameter and height in each of the 18 plots measuring 10 m x 10 m. By measuring tree height (m) and stem diameter, above-ground biomass accumulation was estimated once every four months for a year. Cohen (2011) employed a general allometric equation derived for the Kenyan coast mangrove forests (In biomass =-2.29711+ (ln DBH x 2.54528) (R^2 = 0.90) to determine above-ground biomass. Although various allometric formulas for the mangrove species in Gazi Bay exist, e.g., Slim et al. (1996), Kirui et al. (2006), Kairo et al. (2009), these were not utilized in this work to compute the above-ground biomass for several reasons. For starters, they were species-specific, and earlier equations were based on a small data set (n = 8-55 trees vs. n = 337 trees in Cohen (2011)). Furthermore, when evaluated, some of the equations were found to overestimate biomass at low DBH; for example, Kirui et al. (2006) lowered biomass by a factor of 1.8 at low DBH but by a factor of 1.5 when DBH was increased.

Below ground biomass increment

The root in-growth core approach described by Vogt et al. was used to estimate below-ground biomass increment (1998). In the areas between the trees, two rectangular cores measuring 80 cm long by 20 cm wide and 60 cm deep were made in each of the 18 plots. The silt was removed from the cores, and the coarse roots (diameter > 3mm) were sorted out. The roots were macerated into minute bits to restore nutrients, mixed with the sediment, and returned to the core. Plastic pipes pegged at each corner of the cores were used to mark the positions of the cores. After a year, the cores were extracted, and new roots were divided into size classes of 3 mm, 3-5 mm, 5-10 mm, 10-20 mm, and >20 mm. To calculate the wet: dry weight ratio, the roots were weighed and oven-dried at 80°C to a constant dry weight, then reweighed.

Total biomass and ratio of BGB: ABG: accumulation

The sum of above-ground and below-ground biomass accumulation was used to compute total biomass accumulation. The BGB to AGB ratio was determined by dividing the below-ground biomass accumulation by the above-ground biomass accumulation.

Leaf phenology

Six twigs dispersed throughout the crown canopy of each of the 12 randomly selected trees per plot were tagged for phenological shot observations. The leaves on the chosen twig were numbered sequentially on the adaxial surface with a xylene-free permanent marker, with care made to avoid damaging the leaf epidermis. Any unnumbered leaves in the higher sections of the twig were classified as newly emerging in subsequent sampling. There was a loss of numbered leaves that was noted. The same twigs were examined monthly for a year's reproductive structures (buds, flowers, and fruits). Phenology research was crucial in determining when plants grew quickly, and seeds were ready for propagation.

Data analysis

Microsoft ® Excel spreadsheet 2007, MINITAB, or Statistical packages analyzed the data. All data were checked for normality and, if necessary, adjusted for parametric testing. Single classification ANOVA was used to examine the significance of mean values collected from two different Avicennia and Rhizophora sites. The data from the two sites were merged before being subjected to additional analysis because they were not statistically different for all of the cases studied. The mean biomass accumulation among the species was compared using ANOVA testing.

RESULTS AND DISCUSSION

Soil physicochemical factors

Height above datum of the four mangrove zones

Each of the four mangrove species has a different height above datum (HAD) than the others. *S. alba* was found on the seaward margin's lower tidal zone, with a mean HAD of 1.70 ± 0.02. The *R. mucronata* zone was adjacent with a mean HAD of 2.53 ± 0.01m. *C. tagal* was found in the intermediate intertidal zone with a HAD of 2.86 ± 0.01 m, whereas *A. marina* was found on the landward margin with a HAD of 3.47 ± 0.2 m (Means ±1 S.E.). Figure 3.

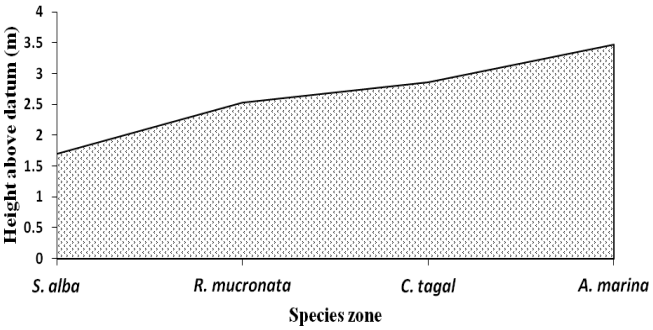

Figure 3. Species zone from the sea to the landward margin

Sediment physicochemical factors

Salinity values in the *S. alba* species zone ranged from 32.0 ± 0.2% during the dry season (January-April) to 30.0 ± 0.6% during the wet season (May-September). During the dry season, the redox potential was as high as 56.0 ± 10 mV, while during the wet season, it was as low as-158.0 ± 19 mV. During the dry season (January-April), pH ranged from 6.2 to 5.8, while during the rainy season (May-September), pH ranged from 6.2 to 5.8. Temperatures ranged from 32.0 ± 0.2 °C in the dry season (January to April) to 30.0 ± 0.4 °C in the wet season (May to October) (May to September). Salinity values in the *R. mucronata* species zone ranged from 32.5 ± 0.2% during the dry season (January-April) to 29.5 ± 0.6% during the wet season (May-September). The redox potential was as high as-115.0 26 mV during the dry season, while during the rainy season, it was as low as-215.0 ± 9.3 mV. During both seasons, the pH range stayed consistent at 6 0. Temperatures ranged from 30.0 ± 0.4 °C in the dry season (January to April) to 39.9 ± 0.2 °C in the wet season (May to October) (May to September).

In the *C. tagal* species zone, salinity levels ranged from 33.0 ± 0.2% during the dry season (January-April) to 32.0 ± 0.2% during the wet season (May-September). During the dry season, redox potential ranged from-112.0 ± 58 mV to-197.0 ± 62 mV, while during the rainy season, it ranged from-112.0 ± 58 mV to-197.0 ± 62 mV. During the dry season (January-April), pH ranged from 6.1 to 5.2, while during the rainy season (May-October), pH ranged from 6.1 to 5.2. (May-September). Temperatures ranged from 38.0 ± 0.2 °C in the dry season (January to April) to 32.0 ± 0.2 °C in the wet season (May to October) (May to September). Salinity levels in the *A. marina* species zone ranged from 48.0 ± 0.5 during the dry season (January-April) to 32.0 ± 0.1 during the wet season (May-September). During the dry season, the redox potential was as high as-85.0 ± 6.0 mV, whereas it was as low as-112.0 ± 58 mV during the wet season. During the dry season (January-April), pH ranged from 6.1 to 4.7, while during the rainy season (May-September), pH ranged from 6.1 to 4.7. Temperatures ranged from 35.5 ± 0.2 °C in the dry season (January to April) to 32.0 ± 0.2 °C in the wet season (May to October) (May to September). When environmental parameters were compared across the zones, it was discovered that substrate salinity levels ranged from 30% in the *Sonneratia* zone to 48% in the *Avicennia* zone. During the dry season, salinity was higher than during the wet season. During the dry season ($F_{(3, 8)}$ = 13.13; p = 0.002) and the rainy season ($F_{(3, 8)}$ = 25.87; p = 0.000), there was a substantial difference in salinity levels throughout the zones. Among the environmental factors studied, redox potential was the most variable. The *Sonneratia* zone had a high of 56.0 mV, while the *Rhizophora* zone had a low of-215.0 mV. During the wet season, redox potential was significantly varied throughout the zones ($F_{(3, 8)}$ = 27.38; p = 0.000). During the dry season, however, the difference was not significant ($F_{(3, 8)}$ = 5.70; p = 0. 0.22). The pH was slightly acidic in all zones, ranging from 4.7-6.5. During the dry season, pH values were significantly lower. In the four zones studied, the temperature of the substrate surface water ranged from a low of 30.0 °C to a high of 39.9 °C. During the dry season, the temperature of the substrate did not differ substantially across the zones ($F_{(3, 8)}$ = 1.87; p = 0.214), but it did during the wet season ($F_{(3, 8)}$ = 8.08; p = 0.008).

Sediment grain sizes

Fine sand, coarse sand, and silt clay grain sizes accounted for 64.4 %, 21 %, and 14.0 % of sediments in the *S. alba* species zone, respectively (Figure 4). Fine sand, coarse sand, and silt clay accounted for 50 %, 38.3 %, and 11.8 % of the sediments in the *Rhizophora* species zone, respectively. The proportion of sediments belonging to distinct grain size classes in *C. tagal* species zones was 57.3 %, 35 %, and 7.7% for silt clay, fine sand, and coarse sand, respectively. The percentage of sediments corresponding to different grain sizes in the *A. marina* species zone were 64 %, 20.5 %, and 12.9 % for fine sand, coarse sand, and silt clay, respectively.

Mangrove forest structure

Importance value

The primary mangrove species' importance values (IV) in the research site were determined as the sum of relative derivatives of frequency, dominance, and density ranging from 3.7 for *S. alba* to 191.3 for *R. mucronata* (Table 1). In the western, middle, and eastern forest blocks, *R. mucronata* had the highest IV values of 87.2, 146.9, and 191.3, respectively. *C. tagal* came in second, with importance values of 67.7, 48.7, and 23.3, respectively, for the western, middle, and eastern blocks. For the western, middle, and eastern forest blocks, *A. marina* rated importance values of 65.1, 46.6, and 21.5, respectively. *Bruguiera gymnorrhiza* had importance values of 7.4, 46.6, and 52.4 in the western, middle, and eastern forest blocks, respectively, while *Xylocarpus granatum* had importance values of 59.2, 7.6, and 4.4 in the western, middle, and eastern forest blocks. For the western, middle, and eastern forest blocks, *S. alba* exhibited the lowest IV values of 13.0, 3.7, and 7.0, respectively (Table 1).

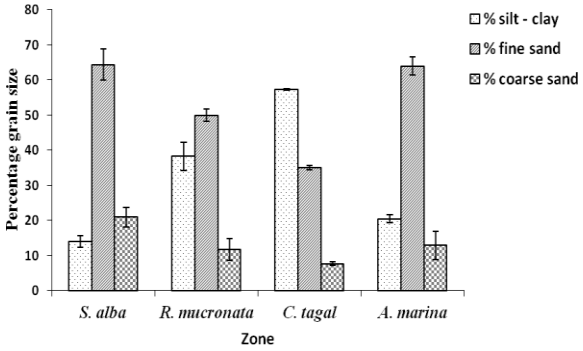

Figure 4. Percentage contribution of different grain sizes to the total sediment weight in different forest zones of Gazi mangrove forest

Table 1. Structural attributes of the western, middle, and eastern forest blocks of Gazi mangroves, Kenya

Forest block	Species	Stems density ha^{-1}	Basal area (m^2 ha^{-1})	Relative (%)			IV
				Density	Dominance	Frequency	
Western (Gazi)	*A. marina*	323.0	3.43	24.69	25.04	15.38	65.1
	B. gymnorrhiza	15.0	0.16	1.15	1.17	5.13	7.4
	C. tagal	377.0	1.46	28.82	10.66	28.21	67.7
	R. mucronata	308.0	4.50	23.55	32.85	30.77	87.2
	S. alba	46.0	0.64	3.52	4.67	5.13	13.3
	X. granatum	238.0	3.51	18.2	25.62	15.38	59.2
	Total	1307	13.70	99.92	100	100	300
Middle (Makongeni)	*A. marina*	153.0	6.37	11.85	26.13	8.57	46.6
	B. gymnorrhiza	133.0	3.97	10.3	16.28	20.0	46.6
	C. tagal	243.0	1.72	18.82	7.05	22.86	48.7
	R. mucronata	739.0	12.10	57.24	49.63	40	146.9
	S. alba	6.0	0.09	0.46	0.37	2.86	3.7
	X. granatum	18.0	0.13	1.39	0.53	5.71	7.6
	Total	1292	24.38	100	100	100	300
Eastern (Kinondo)	*A. marina*	122.0	1.43	10.45	7.49	3.57	21.5
	B. gymnorrhiza	156.0	3.36	13.37	17.63	21.43	52.4
	C. tagal	72.0	0.55	6.17	2.88	14.29	23.3
	R. mucronata	794.0	13.31	68.04	69.72	53.57	191.3
	S. alba	17.0	0.37	1.46	1.94	3.57	7.0
	X. granatum	6.0	0.06	0.51	0.31	3.57	4.4
	Total	1167	19.89	100	100	100	300

Table 2. Summary of structural characteristics of Gazi mangroves, Kenya

Station	Western			Middle			Eastern		
Diameter class (cm)	**5.1-10.0**	**10.1-15.0**	**> 15.0**	**5.1-10.0**	**10.1-15.0**	**> 15.0**	**5.1-10.0**	**10.1-15.0**	**>15.0**
No of species	6	5	5	5	6	4	5	5	5
Stem density (ha^{-1})	1056	350	281	1520	1024	1024	1195	436	501
Mean height (m)	3.70	5.20	6.90	4.80	5.68	6.90	5.33	13.40	23.0
Basal area (m ha^{-1})	69.4	25.1	41.9	40.22	48.2	16.5	47.9	21.6	101.0
Complexity index*	16.02	2.28	4.06	11.98	16.80	4.66	11.50	6.30	58.20

Note: * The complexity index C.I. equals the product of (1), (2), (3), and (4) divided by 10^5

Complexity index

The middle block was the most complex (10.81), while the western block was the least complicated (5.01), according to the structural complexity index (CI, which is derived as the product of the number of species, basal area, stand density, and mean height x 10^{-5}). (Table 2). Tree height and diameter at breast height (DBH) variation across the zones

There were significant variances in stem diameter and tree height within and across the different mangrove blocks studied. In the western block, 57% of the trees had a DBH between 5.1 and 9.0 cm, 20% had a DBH between 9.1 and 13.0 cm, 13% had a DBH between 13.1 and 17.0 cm, 6% had a DBH between 17.1 and 20.0 cm, and 5% had a DBH more than 20.0 cm. In the same block, 58 % were between the height of 2.0 and 4.5 meters, 11% were between the height of 4.6 and 5.0 meters, and 30 % were taller than 5 meters. In the middle block, 37% of the trees had a DBH between 5.1 and 9.0 cm, 25% had a DBH between 9.1 and 13.0 cm, 18% had a DBH between 13.1 and 17.0 cm, 8% had a DBH between 17.1 and 20.0 cm, and 11% had a DBH more than 20.0 cm. In the same block, 35% had a height of 2.0-4.5 m, 25% had a height of 4.6-5.0 m, and 40% had a height of > 5 m. In the eastern block, 49% of the trees had a DBH of 5.1-9 0 cm, 20% had a DBH of 9 1-13.0 cm, 13% had a DBH of 13.1-17 0 cm, 5% had a DBH of 17.1-20.0 cm, and 12% had a DBH more than 20 0 cm. In the same block, 21% had a height of 2.0-4.5 m, 30% had a height of 4.6-5.0 m, and 49% had a height of > 5 m. (Figure 5). The connection between stem density and DBH size class distribution in all forest blocks showed a reverse-J curve (Figure 6).

Stem density

In the three forest blocks, mangrove densities were 1167, 1292, and 1307 stems ha^{-1} in the eastern, middle, and western blocks, respectively, with a mean of 1255±44 stems ha^{-1}. The proportions of species present in the western block to its overall density were 29% (*C. tagal*), 25% (*A. marina*), 24% (*R. mucronata*), 18% (*X. granatum*), 4% (*S. alba*), and 1% (*B. gymnorrhiza*).

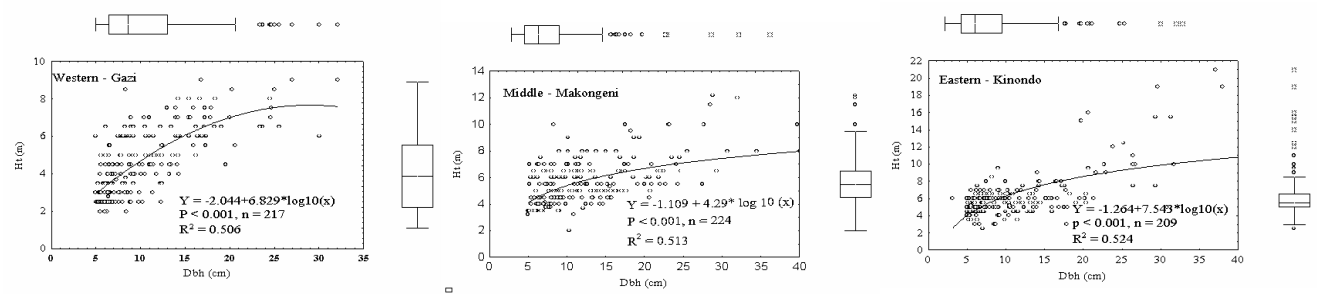

Figure 5. Height-diameter relationship in the three forest blocks of Gazi mangrove forest. The box plots display the percentile distribution of the DBH and heights in the forest blocks. The extremities of the plot correspond to the maximum and minimum observations in the data set. The ends of the boxes are positioned at the 25% and 75% percentile of the data.

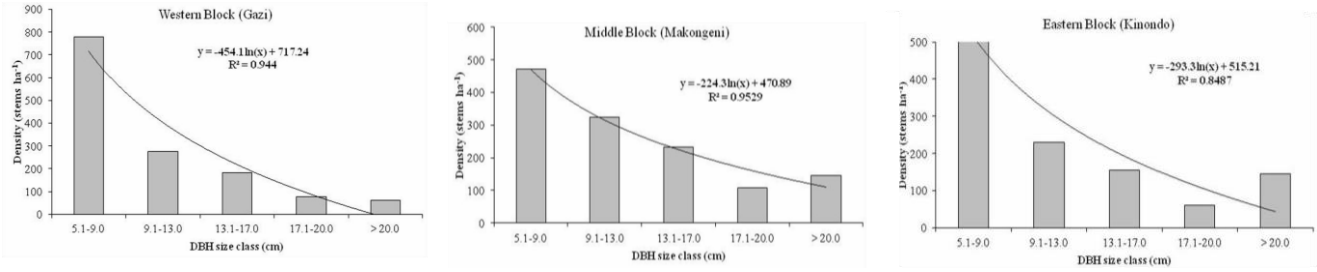

Figure 6. Stem density and DBH size class relations at Gazi Bay mangrove forest, Kenya

The relative contributions to total density in the middle block were 57% (*R. mucronata*), 19% (*C. tagal*), 12.0% (*A. marina*), 10% (*B. gymnorrhiza*), 1.5% (*X. granatum*) and 0.5%. (*S. alba*). In the eastern block, contributions to total density were 68% (*R. mucronata*), 13% (*B. gymnorrhiza*),10% (*A. marina*),6% (*C. tagal*), 1.5% (*S. alba*) and 0.5% (*X. granatum*).

Quality classes of the stems in the mangrove forest

The western Gazi forest block had the lowest percentage of form class 1 poles (2.4%), compared to 5.9% in the middle block and 10% in the eastern block, in terms of the quality of the poles. The western block had 34.1 % in the form 2 size class category, compared to 53 % and 54.9 % in the middle and eastern blocks, respectively. In contrast, the western block had 63.6 % in the form 3 class category, compared to 41.2 % and 35.2 % in the middle and eastern blocks, respectively (Table 3).

Natural regeneration

Across all of Gazi's mangrove forest blocks, the density and composition of natural regeneration classes varied considerably (Table 4). In the western block, the regeneration class one (RCI) density was 10,423 saplings ha^{-1}, accounting for 48.4% of the overall juvenile density. The RCII were 3,579 seedlings per hectare, while the RCIII were 7,564 seedlings per hectare, representing 16.6% and 35%, respectively (Table 4). RCI had a density of 47,618 seedlings ha^{-1} in the middle block, accounting for 59.6% of the total saplings. In comparison, established seedlings RCII and RCIII had densities of 13,614 and 18,608 seedlings ha^{-1}, respectively, accounting for 17% and 23.3 % of the total seedlings.

Table 3. Quality classes of the poles in the three forest blocks of Gazi mangrove forest, Kenya

Forest block	Species	Quality classes			Total
		1	2	3	
Western (Gazi)	*A. marina*	0	69	234	323
	B. gymnorrhiza	0	8	8	16
	C. tagal	8	177	192	377
	R. mucronata	15	115	177	307
	S. alba	8	23	15	46
	X. granatum	0	54	185	239
	Total	31	446	831	1307
	Proportion (%)	2.4	34.1	63.6	
Middle (Makongeni)	*A. marina*	5	92	60	157
	B. gymnorrhiza	11	65	54	130
	C. tagal	16	157	65	238
	R. mucronata	43	352	347	742
	S. alba	0	5	0	5
	X. granatum	0	11	5	16
	Total	76	683	531	1288
	Proportion (%)	5.9	53.0	41.2	
Eastern (Kinondo)	*A. marina*	0	67	56	123
	B. gymnorrhiza	33	39	83	155
	C. tagal	6	56	11	73
	R. mucronata	78	461	256	795
	S. alba	0	17	0	17
	X. granatum	0	0	6	6
	Total	177	640	412	1169
	Proportion	10.0	54.7	35.2	

Table 4. Juvenile densities (saplings ha^{-1}) in the three forest blocks of Gazi mangrove forest, Kenya

Site	Species	Regeneration classes			Total
		RCI	RCII	III	(ha^{-1})
Western (Gazi)	*A. marina*	8	42	58	108
	B. gymnorrhiza	23	8	46	77
	C. tagal	5000	17	4 008	9026
	R. mucronata	5385	3454	3377	12216
	S. alba	0	8	0	8
	X. granatum	17	50	75	142
	Total	10433	3579	7564	21576
Middle	*A. marina*	0	0	0	0
(Makongeni)	*B. gymnorrhiza*	783	94	117	994
	C. tagal	41169	6424	7191	54784
	R. mucronata	5666	7092	11300	24058
	S. alba	0	0	0	0
	X. granatum		4	0	4
	Total	47618	13614	18608	79836
Eastern	*A. marina*	22	0	6	28
(Kinondo)	*B. gymnorrhiza*	144	161	1489	1794
	C. tagal	2872	1094	961	4927
	R. mucronata	12928	6883	5950	25761
	S. alba	0	0	0	0
	X. granatum	0	0	6	6
	Total	15966	8138	8412	32516

RCI had a density of 15,966 saplings ha-1 in the eastern block, accounting for 49.1% of the total saplings. In contrast, established saplings RCII and RCIII had densities of 8,138 and 8,412 saplings ha^{-1}, accounting for 25% and 25.9% of the total saplings, respectively. The difference in mean sapling density in the three mangrove forest blocks was not significant (F (2,6) = 1,163; p = 0.284), according to the one-way ANOVA test. The majority of the juveniles belonged to *Rhizophora* (56.9%) and *Ceriops* (41%), respectively, with the remaining saplings split between *Bruguiera* (1.9%), *Avicennia* (0.1%), and *Xylocarpus* (0.1%). In the western, middle, and eastern forest blocks, the regeneration ratios for RCI, RCII, and RCIII were 3:1:2, 3:1:1, and 2:1:1, respectively.

Biomass accumulation estimates

Above-ground biomass accumulation

In the study plots, above-ground biomass accumulation was highest in the *Rhizophora* zone, with a mean of 7.2 ± 0.4 t ha^{-1} yr^{-1} (range: 6.3-7.8 t ha^{-1} yr^{-1}). The *Sonneratia* zone came next with a mean of 6.0 ± 1.6 t ha^{-1} yr^{-1} (range: 2.9-7.7 t ha^{-1} yr^{-1}). The *Avicennia* zone had the third-highest mean biomass accumulation with 4.2 ± 0.8 t ha^{-1} yr^{-1} (range: 3.0-5.6 t ha^{-1} yr-^{1}), while the *Ceriops* zone had the lowest mean biomass accumulation with 2.0 ± 0.7 t ha^{-1} yr^{-1} (range: 0.8-3.4 t ha^{-1} yr-^{1}) (Figure 7). The difference in mean above-ground biomass accumulation of the four species zones was determined to be significant ($F_{(3, 8)}$, = 5.42; p = 0.025) using a one-way ANOVA test.

Below ground biomass accumulation

The distribution of root weights into the four diameter size classes (less than 3 mm, 3-5 mm, 5-10 mm, and more than 10 mm) revealed that the lower size class category (less than 3 mm) had higher root weights in all zones than the higher size class categories (Figure 8).

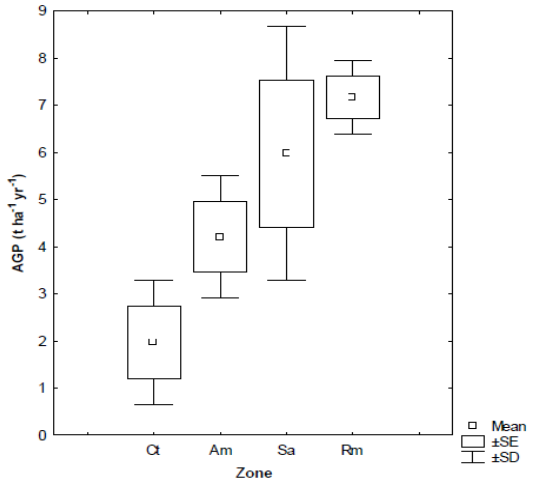

Figure 7. Above-ground biomass (AGB) accumulation rates at different vegetation zones of Gazi mangrove forest, Kenya**.** (*C*t: *Ceriops*, Am: *Avicennia*, Sa: *Sonneratia* and Rm: *Rhizophora*)

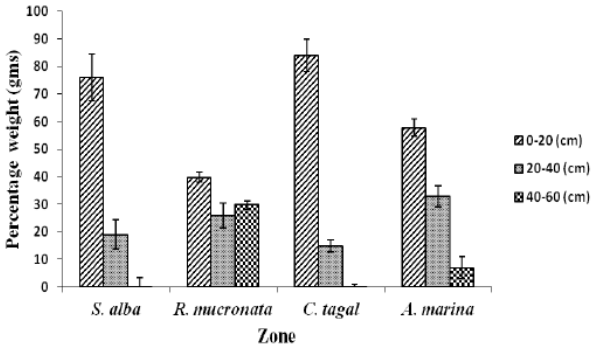

Figure 8. Percentage root weight distribution by depth in each zone at the Gazi mangrove forest

In the *S. alba*, *R. mucronata*, *C. tagal*, and *A. marina* zones, the smaller size class group of less than 3 mm produced root mean weights of 8, 3, 7, and 3 kg ha^{-1} yr^{-1}, respectively. *S. alba*, *R. mucronata*, *C. tagal*, and *A. marina* zones had mean root weights of 4, 1, 2, and 3 kg ha^{-1} yr^{-1}, respectively, in the 3-5 mm size class. *S. alba*, *R. mucronata*, *C. tagal*, and *A. marina* zones had root weights of 15, 1, 5, and 3 kg ha^{-1} yr^{-1}, respectively, in the 5-10 mm size class. The root weights for the *S. alba*, *R. mucronata*, *C. tagal*, and *A. marina* zones were 18, 1, 4, and 5 kg ha^{-1} yr^{-1}, respectively, in the larger size class category of more than 10 mm.

The *Sonneratia* zone exhibited the largest biomass accumulation, with a mean of 4.5 ± 0.9 t ha^{-1} yr^{-1} (range: 3.9-5.5 t ha^{-1} yr^{-1}) followed by the *Avicennia* zone with a mean of 1.7 ± 0.6 t ha^{-1} yr^{-1} (range: 1.3-2.5 t ha^{-1} yr^{-1}). The *Rhizophora* zone had a mean below-ground biomass accumulation of 1.3 ± 0.1 t ha^{-1} yr^{-1} (range: 1.2-1.4 t ha^{-1} yr^{-1}) while the *Ceriops* zone had a mean of 0.6 ± 0.4 t ha^{-1} yr^{-1} (range: 0.3-1.0 t ha^{-1} yr^{-1}) (Figure 9). The mean below-ground biomass accumulation in the zones studied differed significantly ($F_{(3, 8)}$ = 27.83; p = 0.001), according to a one-

way ANOVA test. Total biomass accumulation and the ratio of AGB: BGB

The total biomass accumulation of each species was calculated by combining its above-and below-ground biomass accumulation. *S. alba* zone had the largest accumulation rate, averaging 10.5 ± 1.9 t ha^{-1} yr^{-1} (range: 8.4-11.9 t ha^{-1} yr^{-1}), followed by *R. mucronata* zone (8.5 ± 0.8 t ha^{-1} yr^{-1}) (range 7.7-9.2 t ha^{-1} yr^{-1}), *A. marina* zone (5.2 ± 1.8 t ha^{-1} yr^{-1}) (range: 3.3) (Figure 10). The overall biomass accumulation was significantly different among the mangrove zones, according to a one-way ANOVA test ($F_{(3, 8)}$ = 15.56; p = 0.001). The ratio of below-ground (BG) to above ground (AG) biomass accumulation (BG: AG) in the *S. alba* zone was about 1:1, with below-ground biomass accumulation accounting for around 43% of total biomass accumulation. The BG: AG ratio in the *A. marina* zone was roughly 1:2, with below-ground biomass accumulation accounting for 33% of total biomass. The BG: AG ratio in the *C. tagal* zone was 1:3, with below-ground biomass accounting for 25% of total biomass. The lowest BG: AG ratio was found in the *Rhizophora* zone, which had a BG: AG ratio of 1:6 and accumulated 14 % of the total biomass below ground. For the entire forest, a mean below ground to above-ground biomass accumulation ratio (BG: AG) of 2:5 was calculated.

The distribution of root weights into the four diameter size classes (less than 3 mm, 3-5 mm, 5-10 mm, and more than 10 mm) revealed that the lower size class category (less than 3 mm) had higher root weights in all zones than the higher size class categories. In the *S. alba*, *R. mucronata*, *C. tagal*, and *A. marina* zones, the smaller size class group of less than 3 mm produced root mean weights of 8, 3, 7, and 3 kg ha^{-1} yr^{-1}, respectively. *S. alba*, *R. mucronata*, *C. tagal*, and *A. marina* zones had mean root weights of 4, 1, 2, and 3 kg ha^{-1} yr^{-1}, respectively, in the 3-5 mm size class. *S. alba*, *R. mucronata*, *C. tagal*, and *A. marina* zones had root weights of 15, 1, 5, and 3 kg ha^{-1} yr^{-1}, respectively, in the 5-10 mm size class. The root weights for the *S. alba*, *R. mucronata*, *C. tagal*, and *A. marina* zones were 18, 1, 4, and 5 kg ha^{-1} yr^{-1}, respectively, in the larger size class category of more than 10 mm.

The *Sonneratia* zone exhibited the largest biomass accumulation, with a mean of 4.5 ± 0.9 t ha^{-1} yr^{-1} (range: 3.9-5.5 t ha^{-1} yr^{-1}) followed by the *Avicennia* zone with a mean of 1.7 ± 0.6 t ha^{-1} yr^{-1} (range: 1.3-2.5 t ha^{-1} yr^{-1}). The *Rhizophora* zone had a mean below-ground biomass accumulation of 1.3 ± 0.1 t ha^{-1} yr^{-1} (range: 1.2-1.4 t ha^{-1} yr^{-1}) while the *Ceriops* zone had a mean of 0.6 ± 0.4 t ha^{-1} yr^{-1} (range: 0.3-1.0 t ha^{-1} yr^{-1}) (Figure 9). The mean below-ground biomass accumulation in the zones studied differed significantly ($F_{(3, 8)}$ = 27.83; p = 0.001), according to a one-way ANOVA test. Total biomass accumulation and the ratio of AGB: BGB.

The total biomass accumulation of each species was calculated by combining its above-and below-ground biomass accumulation. *S. alba* zone had the largest accumulation rate, averaging 10.5 ± 1.9 t ha^{-1} yr^{-1} (range: 8.4-11.9 t ha^{-1} yr^{-1}), followed by *R. mucronata* zone (8.5 ± 0.8 t ha^{-1} yr^{-1}) (range 7.7-9.2 t ha^{-1} yr^{-1}), *A. marina* zone (5.2 ± 1.8 t ha^{-1} yr^{-1}) (range: 3.3) (Figure 10). The overall biomass accumulation was significantly different among the mangrove zones, according to a one-way ANOVA test ($F_{(3, 8)}$ = 15.56; p = 0.001). The ratio of below-ground (BG) to above ground (AG) biomass accumulation (BG: AG) in the *S. alba* zone was about 1:1, with below-ground biomass accumulation accounting for around 43% of total biomass accumulation. The BG: AG ratio in the *A. marina* zone was roughly 1:2, with below-ground biomass accumulation accounting for 33% of total biomass. The BG: AG ratio in the *C. tagal* zone was 1:3, with below-ground biomass accounting for 25% of total biomass. The lowest BG: AG ratio was found in the *Rhizophora* zone, which had a BG: AG ratio of 1:6 and accumulated 14 % of the total biomass below ground. For the entire forest, a mean below ground to above-ground biomass accumulation ratio (BG: AG) of 2:5 was calculated.

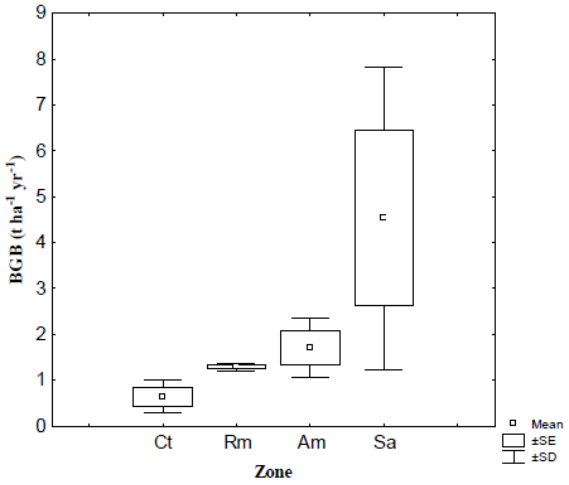

Figure 9. Below ground biomass accumulation at different vegetation zones of Gazi mangrove forest, Kenya. (*C*t: *Ceriops*, Am: *Avicennia*, Sa: *Sonneratia* and Rm: *Rhizophora*)

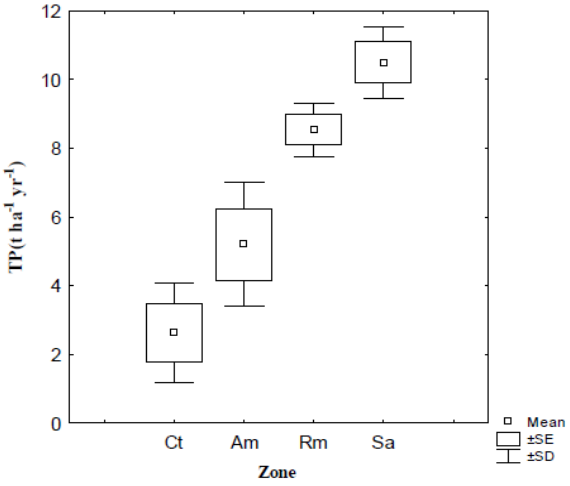

Figure 10. Total biomass accumulation at different vegetation zones at Gazi mangrove forest, Kenya. (Ct: *Ceriops*, Am: *Avicennia*, Sa:*Sonneratia* and Rm: *Rhizophora*)

Leaf phenology

For the four studied species, leaf emergence ranged from two to four leaves per month (Figure 11). *Sonneratia* had the highest monthly leaf emergence count of 3.9 ± 2 leaves per twig, which peaked in October-November and gradually decreased during the observation period. The same species experienced a mean leaf loss of 3.2 ± 2 leaves per twig each month. As a result, in the *S. alba* zone, there was a net growth of 0.7 1 leaf per shoot per month. The average leaf emergence and loss in the *R. mucronata* zone were 2.8 ± 2 (range: one to five) and 1.4 1 (range: one to two), respectively, resulting in a net gain of 1.4 ± 2 leaves (range 1-3). Higher leaf emergence was seen at two peak times that coincided with the wet seasons (October to December and May to July), while low leaf emerging was seen during the dry season (March to May). Leaf emergence and loss per twig per month in the *Avicennia* zone ranged from one to seven (mean 2.3 ± 2) and one to two (mean 1.2 ± 2) leaves per twig per month, resulting in a mean net gain of 1.2 ± 2. During the dry months (December to February), leaf emergence was reduced, while leaf productivity peaked immediately after the arrival of rains at the end of April. Throughout the observation period, leaf loss was minimal and nearly consistent (Figure 11). Ceriops leaf emergence averaged 3.3 ± 2 leaves per twig per month, with a monthly loss of 1.1 ± 1 leaves per shoot resulting in a net gain of 2.2 ± 2 leaves per twig per month. Leaf emergence in *C. tagal* was bimodal, with peaks occurring between short rains (October-November) and long rains (December-January) (May-July). Between January and March, leaf productivity was low (Figure 11).

Relationships

According to the soil parameters studied, aboveground biomass accumulation was adversely linked with the mean salinity of the soil throughout both the dry and wet seasons. The relationship between temperature and above-ground biomass accumulation, on the other hand, was only significant during the wet season (Table 5). The following regression equations can be used to express the functional relationship between above ground and below-ground biomass accumulation for each zone:

S. alba zone: BGB = 0.592 ln (AGB) + 2.351, $r^2 = 0.026$
R. mucronata zone: BGB =-1.49 In (AGB) + 3.1622, $r^2 = 0.112$
C. tagal zone: BGB = 0.239In (AGB) + 0.6935, $r^2 = 0.092$
A. marina zone: BGB = 0.493In (AGB) + 1.555, $r^2 = 0.66$

All of the other species zones had low r2 values, except the *Avicennia* species zone; thus, this equation for the *Avicennia* species zone is useful in estimating the below-ground biomass accumulation from the above-ground biomass accumulation, whereas those for *S. alba*, *R. mucronata*, and *C. tagal* species zone are of limited value.

Discussion

Mangrove forest structure

Importance value (IV) of mangrove species

The leading species in the Gazi Bay mangrove forest was *R. mucronata*, followed by *C. tagal*, *A. marina*, *B. gymnorrhiza*, *X. granatum*, and *S. alba*, according to the structural composition of species defined by the importance value. Other mangrove forests in Kenya, such as Kiunga, Kairo et al. (2002b), and Vanga on the south coast, have a similar structural composition (Kairo et al. 2012). Macnae (1968) found it in different West Indian Ocean (WIO) mangrove formations, as well as in the mangroves of Puttalam Lagoon and Dutch Bay in Sri Lanka (Amarasinghe and Balasubramaniam 1992).

Height above datum contributed to species zonation at Gazi Bay mangrove forest, as evidenced by a considerable variance in height above datum for the four zones. Due to its inability to endure large salinity fluctuations, S. alba occupied the seaward side in inundation class I of Watson (1928). The *Sonneratia* trees could survive the floods due to their well-developed pneumatophores. The *R. mucronata* zone, which is inundation class II, is located next to the *S. alba* zone. *Rhizophora* trees' well-developed prop roots allow them to survive strong tide velocities and trap sediments. The C. tagal zone is found in the mid-tide zone, classified as inundation class III. *Ceriops* is one of the most challenging mangrove tree species, with an extensive salinity tolerance range. *Ceriops* grow to a shrub in high saline environments, such as those found in Gazi, but in fewer saline environments, such as those found in Mida Creek, the species can reach a height of more than 25 meters (Kairo et al. 2012). *A. marina*, found on the landward edge, falls under inundation class IV. *A. marina*, like *Ceriops*, can survive a wide range of salinity, which it controls using a secretion mechanism (Schmitz et al., 2007). Double zonation was seen in *Avicennia* in Gazi (Wang'ondu et al., 2010). Avicennia grows as huge trees with high DBH in low salinity areas on the seaward side but as scrub with low production in greater salinity areas on the landward side.

Table 5. Pearson correlation coefficient between above-ground biomass (AGB) accumulation and selected soil environment properties during the dry season (DS) and the rainy season (WS) at Gazi mangrove forest.

Zone		Salinity		Redox		Temperature		pH		n
		DS	WS	DS	WS	DS	WS	DS	WS	
S. alba	r =	-0.990	-0.999	0.663	0.707	0.431	0.287	0.006	0.428	3
	p =	0.023	0.029	0.539	0.501	0.716	0.640	0.950	0.546	
R. mucronata	r =	-0.852	-0.932	0.854	0.813	-0.859	0.135	0.316	0.905	6
	p =	0.031	0.007	0.031	0.049	0.028	0.799	0.620	0.013	
C. tagal	r =	-0.328	-0.960	0.143	0.306	0.135	0.303	0.376	0.370	3
	p =	0.787	0.029	0.629	0.627	0.799	0.804	0.580	0.759	
A. marina	r =	-0.970	-0.973	0.354	0.309	-0.409	0.019	-0.398	0.287	6
	p =	0.001	0.001	0.491	0.551	0.420	0.912	0.434	0.581	

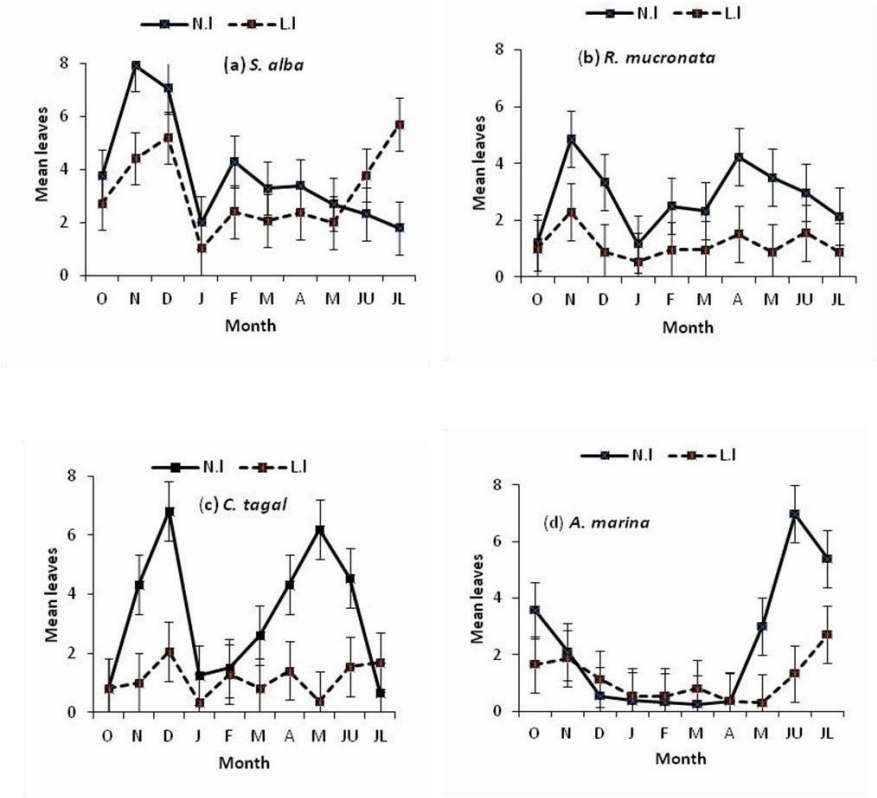

Figure 11. Mean leaf emergence and loss at different zones of the Gazi mangrove forest (*Means* ±.1 *S.E*). N.l = new leaves, L.l = lost leaves)

Complexity index

The high complexity index of the middle forest block suggested that the trees had a larger basal area and canopy height than the western and eastern blocks (Table 2), which could be due to less harvesting. The middle and eastern forest blocks at Gazi were substantially more complex than the western forest block. This is to be expected, given that the western forest block borders the human settlement of Gazi village and that previous removal of wood products has lowered the quality of mangroves in the area (Kairo 2001). According to the current study, 63.5 % of trees in the western block were of quality class 3, whereas tree quality in the middle and eastern blocks was primarily of class 1 and 2 (Table 3). Human impacts on the wood quality decline were generally greater in Gazi than in Mida Creek, where a considerable percentage of the poles were of quality class 1. In contrast to the former, Mida Creek is a conservation area where tree removal is strictly controlled (Kairo et al. 2002a).

Tree height and DBH relationship across zones

The proximity to human settlements can be linked to differences in stem diameter and height observed in the three analyzed blocks. The western forest block has the highest percentage (57%) of low-size class trees (Table 3). The western block was the most accessible and close to Gazi village. As a result, this forest block is subjected to more extraction pressure than the others.

Stem density

In the Gazi mangrove forest, the average stem density was 1255 ± 44, which is comparable to other mangrove habitats in Kenya. Mohamed et al. (2009) found a density of 1264 stems ha^{-1} in the peri-urban mangroves of Mombasa. A density of 1585 and 1197 stems ha^{-1} have been recorded in Uyombo and Kirepwe of Mida Creek, respectively (Kairo et al. 2002a). Kairo et al. (2002a) found a density of 2077 stems ha^{-1} in the Kiunga mangroves and 1934 trees ha^{-1} in the Vanga mangroves (Kairo et al. 2012). As a result, the stem density of natural mangrove stands was lower than that of the replanted mangrove forest of Gazi, which had a density of about 2500 stems ha-1 (Kairo 2001). This was attributed to the natural stands' poles being harvested regularly instead of the replanted stands, which were not. The observed "J" distribution curves (Figure 6) are characteristic of natural mangrove forests and show that poles were cut selectively. Mangrove forests in Kenya have been poorly maintained, as seen by low stem density in natural forest stands. As a result, a management plan for mangrove forests, similar to other upland forests, is required to ensure the supply of needed goods and services.

Natural regeneration

The recruitment of juveniles in the western forest block (21,576 juveniles), middle forest block (79,836 juveniles), and eastern forest block (32,516 juveniles) may be deemed appropriate, as FAO (1994) defines adequate regeneration density as more than 2500 saplings ha^{-1}. Bosire et al. (2008) found a 50-61 % mortality rate by the end of year two in a two-year study of sapling survival and structural development in natural and reforested mangroves of Gazi. The survival rate ranged from 1230 to 5277 seedlings ha^{-1} in the same study. When the results of this study are compared to previous data, it appears that this forest can

recover. On the other hand, reforestation modifies local hydrodynamics and other physical-chemical variables, resulting in higher regeneration in reforested areas (Bosire et al., 2003).

In the *S. alba* zone, there was very little understory. A combination of limited propagule influx, washing by wave activity, and shading impacts could have resulted in seedling recruitment failure in the *S. alba* zone. Seedling growth and survival in mangrove habitats are hampered by less light beneath closed canopies (Clarke and Kerrigan 2002). Natural mangrove regeneration is aided by canopy gaps (Sherman et al. 2000). Reduced resource competition (notably greater light availability) and crab predation are the reasons. Gradual sediment movement seen in the *S. alba* zone may be altering substrate conditions, making seedling establishment unfavorable.

Quality of the stems in the mangrove forest of Gazi

Most of the good quality poles (class 1) in the Gazi mangrove forest have been cut, leaving only 2.4 %, 5.9 %, and 10% in the western, middle, and eastern blocks, respectively. The forest, on the other hand, has a higher percentage of class 2 and 3 in the forest blocks (class 2: 34.1%, 52.9 %, and 54.8 %; class 3: 63.5 %, 41.2 %, and 35.2 % at the western, middle and eastern blocks respectively). Higher percentages of class 2 and 3 poles indicate degraded forest, and the Gazi mangrove forest can thus be classified as degraded. The western forest block appears to be more deteriorated than the other two forest blocks based on relative percentages for the three-class groups. Trees with crooked poles of low commercial value were left out after straight poles were cut down in the Gazi mangrove forest (Kairo et al. 2010). This has long-term consequences because this forest may no longer be capable of providing building poles, a valuable ecosystem function to coastal populations. At the same time, biomass stocks will be depleted. Disparities in human pressure can be blamed for the differences in forest quality between the three locations. The proximity of human settlements to the mangroves of the western block and the fact that people from across the creek come here to cut trees leads to increased consumptive wood extraction from the forest, resulting in fewer mangrove poles. Mangrove poles in Kenya are classified and sold according to their diameter classes, which include Fito/Pau (6.0 cm), Mazio (8.0-11.0 cm), Boriti (11.5-13.5 cm), and Banaa (20.1-35 cm). More stems in the bigger diameter classes, taller vegetation, and a higher total tree density have resulted from less exploitation in the middle block.

Biomass accumulation estimates

Above-ground biomass accumulation

According to this study, the accumulation of above-ground biomass in the four mangrove species zones differed significantly. This could be due to various reasons, including age, structural characteristics of the species, substrate conditions, and management strategies. The structural elements of the *R. mucronata* zone account for the substantial above-ground biomass accumulation. *Rhizophora*, unlike other species, has prop roots that can be regarded as part of the above-ground biomass. Because *R. mucronata* produces better poles, the older trees have been cut in the past, leaving younger trees to thrive quicker. The ancient age of the trees and the saline substrate stresses the trees, resulting in low aboveground biomass accumulation in *C. tagal*. Above-ground biomass accumulation in natural mangrove stands has ranged from a low of 2.02 t ha^{-1} yr^{-1} in *Avicennia germinas*, a dominated stand in Mexico (Day et al. 1996), to a high of 26.7 t ha^{-1} yr^{-1} in *Rhizophora apiculata* in southern Thailand (Christensen 1978). In Kenya, a 12-year replanted of *R. mucronata* increased above-ground biomass of 8.89 t ha^{-1} yr^{-1} (Kairo et al. 2008). Changes in aboveground biomass accumulation could be caused by differences in environmental circumstances or plant traits.

Below ground biomass accumulation

Sonneratia alba zone had a high biomass accumulation of 4.5 0.9 t ha^{-1} yr^{-1}, while *Ceriops* had biomass of 0.6 0.4 t ha^{-1} yr^{-1}. The differences in BGB accumulation within the zones could be ascribed to both species-specific features and site factors. *Sonneratia* grows faster than *C. tagal* in general. Kairo et al. (2002a) found a growth rate of 1.81 m yr^{-1} for *Sonneratia* compared to 0.3 m yr^{-1} for *Ceriops* in plantation establishment. Higher salinity fluctuations in the *A. marina* zone may have needed additional root investment for nutrient uptake in hypersaline circumstances, as seen by the Avicennia zone's high root: shoot ratio. Because most of its roots are above ground in prop roots, *R. mucronata* accumulated less biomass below. The upper layer (0-20) cm of all mangrove zones has a higher percentage of root growth, demonstrating that most mangroves are shallow-rooted, with biomass accumulation decreasing with depth. Other studies throughout the world have shown similar findings. Castaneda et al. (2011) found that most of the roots (62-85 %) were distributed in the shallow root zone of the Everglade mangroves in America. In a top/root biomass ratio study in mangroves in Southeast Asia, Komiyama et al. (2000) found that root biomass decreased with depth. According to most research, fine roots, which are essential for aeration and nutrient acquisition, produce the maximum biomass and are located near the surface (Tamooh et al., 2008). Because of the anoxic nature of the mangrove ecosystem, shallow roots improve gaseous exchange at the rooting zone and nutrient uptake from tides and runoff.

Compared to terrestrial forests, mangroves often collect substantial amounts of biomass in their below-ground roots, resulting in a significantly higher root: shoot ratio (Komiyama et al., 2000). In terrestrial forests, below-ground biomass accounts for less than 30% of total biomass, while, in mangroves, root biomass accounts for 40-60% of total biomass (Saenger 1982; Lugo 1990). On the other hand, R. mucronata, C. tagal, and A. marina zones exhibited lower below-ground biomass accumulation of 15%, 23%, and 33%, respectively. The considerable investment in below-ground biomass found in the *S. alba* zone provides stability against strong tidal velocities and increases surface area for gaseous exchange.

Some species, such as *A. marina*, tend to make significant investments in root biomass, which may reflect the difficulties of growing in nutrient-depleted, hypoxic, and unstable soils. The current study found a below-ground biomass accumulation rate of 1.3 (range: 1.2-1.4 t ha^{-1} yr^{-1}) for the *R. mucronata* zone, significantly greater than the 0.2 t ha^{-1} yr^{-1} for *Rhizophora apiculata* in the Hinchinbrook channel, Australia (Clough, 1998). Similarly, the contribution of below-ground biomass to total biomass accumulation was 14 percent, which was slightly greater than the 8.5 percent recorded for the *Rhizophora* species in Malaysia (Ong et al. 1995). Gong and Ong (1990) reported a below-ground biomass accumulation rate of 1.2 to 3.6 t ha^{-1} yr^{-1} for *A. marina*, which was similar to the results of this study (range: 1.3-2.5 t ha^{-1} yr^{-1}). A biomass accumulation of 1.5 t ha^{-1} yr^{-1} is estimated in the neotropical mangroves of Florida, United States (McKee and Faulkner, 2000). The estimated lower ground biomass increases of the *Rhizophora* species in Honduras by Cahoon et al. (2003) are 0.27 t ha^{-1} yr^{-1}.

Total biomass and ratio of AGB: BGB

According to the findings of this study, zones on the seaward edge generated total biomass than those on the landward edge (Figure 10). Differences in total biomass accumulation rates could be primarily attributable to salinity differences. Salinity does not vary significantly because the S. alba and R. mucronata zones get daily tidal floods. The *A. marina* and *C. tagal* zones, on the other hand, only receive tidal inundation at spring tide, exposing the plants to a wider range of salinity fluctuation and, as a result, higher salinity stress that inhibits growth. The existence of spatial changes in environmental conditions across the forest complex is confirmed by a considerable difference in biomass accumulation between the seaward *S. alba*, *R. mucronata* zones, and the landward *A. marina* and *C. tagal* zones.

The total biomass accumulation for *R. mucronata* (8.5 0.8 t ha-1) in this study is larger than the 5.1 t ha-1 yr-1 reported for an 80-year-old natural *Rhizophora apiculata* plantation in Malaysia (Putz and Chan, 1986). Other complicating variables such as forest age, species composition, management regime, and local climatic change may alter biomass allocation patterns, resulting in observed disparities between forests (Kairo et al. 2008; Tamooh et al. 2008). Plantations have been shown to accumulate more biomass than natural forests, which could be due to superior management, such as uniform spacing that reduces competition and better climatic and substrate conditions.

In this study, a high root: shoot ratio of nearly 1:1 was confirmed for the *S. alba* zone, with below-ground biomass accounting for 43 % biomass. In contrast, the *Rhizophora* zone had the lowest R:S ratio of 1:6. A BGB: AGB ratio of 1:4 was found in a 12-year-old replanted *R. mucronata* plantation in Kenya (Kairo et al., 2008). This disparity could have arisen as a result of differences in management. While the current study was conducted in a natural forest, a related prior survey by Kairo et al. (2008) was born in an *R. mucronata* plantation, where management regimes such as spacing and pruning may have influenced tree thriving. This study found a root: shoot biomass accumulation ratio ranging from 1:6 in the *Rhizophora* zone to 1:1 in the *Sonneratia* zone, with an overall forest ratio of 2:5, which was higher than the R:S ratio of 1:4 found by Ong et al. (1995) in a 20-year *Rhizophora* species plantation in Malaysia's Matang mangrove forest. It was also higher than the R:S ratio of 1:4 found in terrestrial forests (Cairns et al. 1997). These findings show that mangroves devote more of their biomass to roots to cope with the unstable, soft, anoxic, hypersaline, and nutrient-deficient sediments they grow on and secure the stability and anchorage of the tree (Komiyama et al. 2008).

Leaf phenology

The considerable variation in leaf phenology among the studied species was a notable finding. *C. tagal* showed a bimodal pattern with peak leaf emergence between April and June and November and December, whereas *A. marina* had a unimodal pattern with peak leaf production and loss between May and July. *R. mucronata* had a multimodal pattern, but *S. alba* had a continuous leaf production and losses pattern. Similar findings were made in previous research at Gazi for *R. mucronata* and *A. marina* (Wang'ondu et al., 2010). Leaf production and losses were unimodal and highly seasonal in *A. marina*, but leaf gain and loss in *R. mucronata* were constant, with peak production occurring during the wet season. Slim et al. (1996) reported comparable phenological features in *R. mucronata* while working in Gazi, with litterfall peaking during the dry season. Sherman et al. (2010) found that leaf production and losses were continuous in *Rhizophora stylosa* on Okinawa Island, Japan; however, losses were more evenly distributed.

Mangroves regulate the cellular salt concentration in one way by accumulating salts in their leaves, which are then excreted (Ball and Munns 1992). Evapotranspiration is higher during dry seasons, and species like *R. mucronata* adapt by yellowing and shedding leaves to reduce evapotranspiration. Seasonality in litter production has been seen in other mangrove species as well. Coupland et al. (2005) found that leaf production was linked to leaf fall in Australia. The vegetative and reproductive phenology timing was likely to coincide with specific climatic circumstances, including temperature and rainfall.

Regression equation on biomass accumulation

The *S. alba*, *R. mucronata*, and *C. tagal* zones showed poor connections in the equations created to represent the interaction between below ground and above ground biomass accumulation. The connection for the *Avicennia* zone, on the other hand, was stronger, and hence this equation can be utilized to predict below-ground biomass accumulation in the *A. marina* zone. The short time of data collection or a flaw in the approach employed to estimate belowground biomass accumulation could explain the low belowground biomass for the three species. In a review of the methods used to estimate the below-ground biomass, Vogt et al. (1996), working in the mangrove forest of Puerto Rico, cautioned against using this technique due to

labor difficulties, while Sánchez (2006) in South Florida mangroves pointed to the potential for using the growth core technique to estimate the below-ground biomass with significant success. Cutting the roots during the creation of the in-growth core, on the other hand, may cause root growth to be delayed, resulting in an underestimate of root output. The findings of this research could have similar consequences.

In conclusion, this study aims to investigate the accumulation of structure, biomass, and environmental factors in the four dominant mangrove areas of Mangrove Forests in Gazi Bay. The following conclusions can be drawn based on the study results: Environmental factors significantly affect the accumulation of biomass in mangrove forests. Salinity was found to play a greater role under the studied environmental variables because more biomass accumulation occurred in wet seasons with a low salinity level.

In the *Sonneratia*, *Rhizophora*, and *Ceriops* species zones, the correlation between above-ground and below-ground biomass accumulation was weak, while in the *A. marina* species zone, it was intense. Thus, only below-ground biomass accumulation of *A. marina* can be predicted from above-ground biomass accumulation using the equations used in this study.

When comparing the combined below ground: above-ground biomass of mangroves to that of terrestrial vegetation, it is clear that mangroves collect more below-ground biomass than terrestrial forests, proving their uniqueness. The current study found that different mangrove species zones have significant differences in above-ground, below-ground, and total biomass accumulation.

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Current status, utilization, succession and zonation of mangrove ecosystem along Mida Creek, Kenya

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Abstract. *Warui MW, Manohar S, Obade P. 2020. Current status, utilization, succession and zonation of mangrove ecosystem along Mida Creek, Coast Province, Kenya. Bonorowo Wetlands 10: 32-43.* Human activities have destroyed mangrove forests, posing a threat to the mangrove ecosystem and the living things that rely on it. From September 2009 through February 2010, researchers studied the current state, usage, succession, and zonation of the mangrove ecosystem along Mida Creek in Kenya's Coast Province. The goals were to assess changes in the floristic composition of the mangrove forest, identify the most preferred mangrove species, investigate whether accessibility determines utilization of mangrove species, investigate whether harvesting of mangroves affects their succession, and investigate the effectiveness of current mangrove forest management policies. The data was collected using the Point-Centered Quarter Method (PCQM) to see if access into the mangrove forest influences their use and affects mangrove succession. To evaluate the efficiency of government policies/legislation governing mangrove exploitation and the most favored mangrove species by the local community and other users, questionnaires were circulated, and interviews were conducted. Out of the 210 houses in Mida Creek, 136 respondents were interviewed, one from each household. To examine the temporal changes in the floristic composition of the mangrove forest, two sets of aerial images (1992 and 2006) were processed and interpreted. The data from the questionnaires were coded and entered into the Statistical Package for the Social Sciences (SPSS). An analysis of variance (ANOVA) was used to see if the accessibility of mangroves influences their use. To see if the utilization of mangroves affects their succession, researchers used regression analysis. To evaluate the forest structure, and importance value was derived. Descriptive statistics were used to summarize the present mangrove forest management policies and their effectiveness. Between 1992 and 2006, the floristic composition of the mangrove forest in Mida Creek changed; the area covered by mangroves decreased as follows: *Rhizophora mucronata* (Rm); 65.09-63.93ha; *Avicennia marina* (Am); 344.99-310.63ha; *Ceriops tagal* (Ct); 225.12-223.82ha; Rm and Ct; 52.87-50.22ha; Ct and Am; 143.69- 140.29ha; Rm and Am; 44.36-41.27ha; Rm and Bg; 212.75-199.75ha; Rm and *Sonneratia alba* (Sa); 47.64-46.32ha; Rm, Ct and *Bruguiera gymnorrhiza* (Bg); 129.07-128.12ha; Rm, Am, Ct, and Bg; 472.44-428.46ha. The number of cut mangroves in the adjacent settlements, center, and shoreline revealed a significant difference ($F=3.277$; $df=2$; $p=0.040$) in the ANOVA test, indicating that accessibility determines consumption. The most favored mangrove species was *Rhizophora mucronata*, according to the findings. According to regression analysis, there was a correlation between the number of mangrove seedlings and the number of cut mangroves ($F=8.529$, $df=1$, $R=0.198$, $P=0.004$). The use of mangroves has an impact on their succession. Mangrove utilization policies and legislation have been less effective. The key species in Mida Creek were *Rhizophora mucronata* and *Ceriops tagal*. The extent of mangrove forests in Mida Creek has shrunk over time.

Keywords: Mangroves, Mida Creek, status, succession, utilization

INTRODUCTION

Mangroves are woody tropical and subtropical trees or shrubs that grow in brackish seas or estuarine wetlands in the intertidal zone (Tomlinson 1986). Mangroves are found mostly in intertidal locations with groundwater discharges or seepage, not in estuaries (Ruwa and Polk 1986). In locations where there are no river discharges, this seepage is responsible for the colonization and growth of mangroves. The change in micro-environmental conditions from oceanic to brackish water caused by seepage of subterranean water to the shoreline creates acceptable micro-habitats for colonization by mangrove seedlings. It offers suitable habitats for suitable habitats mangrove development. This seepage is responsible for mangrove colonization and growth in locations with no river discharges. The change in micro-environmental conditions from oceanic to brackish water caused by seepage of subterranean water to the shoreline creates acceptable micro-habitats for mangrove seedling colonization and offers suitable habitats for mangrove development. Mangrove ecosystems are open systems that exchange matter and energy with the marine and terrestrial ecosystems that surround them (Manohar 1993).

Mangrove swamps are typical wetland habitats found along tropical and subtropical coasts in mud and silt deposits. These ecosystems are thought to cover 15.2 million hectares of the world's tropical shorelines (Spalding et al. 1997; FAO 2003, 2005). Mangrove forests provide critical functions and benefits to coastal zones and their plant, animal, and human populations as intertidal ecosystems (Cannicci et al. 2008; Kristensen et al. 2008; Nagelkerken et al. 2008; Walters et al. 2008). True mangroves (containing both major and minor components) and mangrove companions can be found in mangrove forests. True mangroves, according to Tomlinson (1986),

are defined as species that have all or most of the following characteristics: (i) Complete fidelity to the mangrove environment; (ii) Play a significant role in forest structure and can form pure stands; (iii) Morphological specialization that adapts them to their environment (e.g., aerial roots and vivipary); (iv) Physiological mechanism for salt exclusion; (v) Taxonomic isolation from terrestrial relatives at least at the generic level. Mangroves are found worldwide, with 124 nations and territories containing mangrove species out of 258 (Tomlinson 1986; Saenger et al. 1983). In 1980, as part of the FAO/UNEP Tropical Forest Resources Assessment, the first attempt was made to estimate the entire mangrove acreage globally, estimated to be 15.6 million hectares. Mangrove coverage is now estimated to be 17,075,600 hectares (Aizpuru et al. 2000). Asia accounts for 38.51 % of total mangrove coverage (FAO, 2005). Mangroves are mostly found between 30º N and 30º S latitudes. Japan (31º22' N) and Bermuda (32º20' N) are the northern limits; New Zealand (38º03' S), Australia (38º45' S), and the east coast of South Africa (32º59' S) are the southern limits (Spalding et al. 1997). Mangroves are found along the warmer eastern coasts of the Americas and Africa instead of the cooler western coasts. The presence of warm and cold oceanic currents causes this disparity in distribution. The Eastern and Western hemispheres are the two main centers of mangroves (Duke 1992). The Indo-West Pacific region, which comprises East Africa, Indo-Malesia, and Australasia, is located in the Eastern hemisphere. The Atlantic East Pacific region encompasses West America, East America, and West Africa in the Western Hemisphere. Mangroves are thought to have originated in the eastern hemisphere, so the region is known as the Old World mangroves and the Western hemisphere as the New World mangroves. There are 49 mangrove species in the Eastern hemisphere and 11 in the Western hemisphere (Duke 1992). Indonesia, Australia, Brazil, and Nigeria have the most mangrove areas, accounting for 19.5, 9.2, 6.5, and 6.4 percent of global coverage, respectively (FAO 2007).

Mangroves can be found in nearly every country along Africa's west and east coasts, from Mauritania to Angola on the west coast and Egypt to South Africa on the east, including Madagascar and numerous other islands. They aren't found in Namibia, owing to the countries. Its semi-arid, desert-like climate includes low and irregular rainfall, a lack of warming currents, and favorable topographical factors. Mangrove forests cover around 3.2 million hectares in Africa, accounting for roughly 19% of global coverage. They are found in three major coastal sections: the western Atlantic (1.5 million ha, 49%), the central Atlantic (0.4 million ha, 14%), and the western Indian Ocean (0.4 million ha, 14%). (1.2 million ha, 37 %).

In Africa, the phytogeographical distribution of mangrove species varies slightly across the continent. Avicenniaceae (*Avicennia germinans*, often known as white mangroves), Combretaceae (*Laguncularia racemosa, Conocarpus erectus*), and Rhizophoraceae (*Rhizophora harrisonii, R. mangle, R. mucronata* - usually called red mangroves) are three families comprising six species in West and Central Africa. In pure stands, especially in tidal estuaries, *R. mucronata* is very dominant in this location, characteristic of long and straight poles. *R. harrosonii* and *R. mangle*, respectively, are tiny trees and shrubs. There are ten species of mangroves in Eastern Africa, the most common being *Rhizophora mucronata*, *Ceriops tagal*, and *Avicennia marina* (Semesi 1998), which cover 1.1 million hectares (Spalding et al. 1997).

Mangrove forests can be found in Kenya's Coast Province, which runs along the Indian Ocean. Lamu, Ungwana Bay, Ngomeni, Malindi, Mida, Kilifi, Mombasa, Gazi, Funzi, and Shimoni are areas where mangrove forests may be found. Mangroves can be found as creek or fringe mangroves (Macnae 1968; Ruwa 1993). Mangrove trees that grow on low-gradient beaches in creeks and bays make up the creek mangrove community, which usually forms well-developed forests with species zonation. In front of or at the base of rocky cliffs, fringe mangroves grow solitary or in single or mixed species clusters. The most frequent mangroves along the Kenyan coast are creek mangroves, which form the pioneer population, spreading from the coastlines and colonizing mud banks in tributaries exposed at low tide.

Mangrove forests have been estimated to cover 75 % of the world's tropical coasts. Because of anthropogenic pressures, the global range of these forests has shrunk to less than half of its original extent (Saenger et al. 1983, Spalding et al. 1997). According to a recent study of worldwide mangrove forests, the Eastern African region has lost around 8% of its mangrove cover in the last 25 years, or about 3,000 ha each year on average.

Plant biomass in mangrove forests has been shown to be up to 700 t/ha, half of which is carbon. Tropical forest deforestation is thought to account for up to 17% of global carbon dioxide emissions, resulting in faster global warming (Van der Werf et al., 2009). The goals of this research were: (i) to evaluate the alterations in the floristic composition and structure of the Mida Creek mangrove forest between 1992 and 2006, (ii) to discover which mangrove species are the most common in Mida Creek, (iii) to see if the accessibility of mangrove species influences their use, (iv) to see if they use of mangroves has an impact on their succession, (v) to assess the efficiency of present mangrove forest management policies.

MATERIALS AND METHODS

Study area

Mida Creek (3º21'S, 39º59'E) lies in the Malindi district, about 25 km south of Malindi town and 88 km north of Mombasa (Figure 1). The watercourse is located in a 31.6 km2 planographic area (Brakel 1979). Watamu Marine National Reserve in Mida Creek was designated as a protected area to recognize its scenery both above and below the water level. It is one of Kenya's three protected areas containing mangroves (Spalding et al. 1997). In 1968, it was designated as a reserve, and in 1976, it was gazetted under the Wildlife Conservation and Management Act. Watamu Marine National Reserve and the surrounding

coast were declared as a Biosphere Reserve in 1979. (Kennedy 1988). Mangrove forests, sand flats, rock outcrops, seagrass beds, coral growths, and deep water make up the diverse habitats of Mida Creek. Natural elements such as mangroves, coral reefs, and mudflats may be found in reserve, serving as a haven for shorebird populations (UNEP/IUCN, 1988). Unlike most Kenya's coastal districts, Mida Creek receives no freshwater from overland sources.

Climate

The monthly temperature ranges from 23 to 27 degrees Celsius. The maximum temperature during the hottest times is 34°C, while the minimum temperature is 20°C. Strong winds, rougher water, and tidal flows during the south-eastern monsoon (April-September), known as the "Kusi," generate sea-grass deposits in some regions, particularly those bays facing that way. In the other months, the wind is calm. The average monthly wind speed is 9.6 km hr-1, with a range of 6.2 km hr^{-1} to 16.6 km hr^{-1}. The total yearly precipitation ranges from 1000 to 1600 millimeters. The long rains, influenced by southeast monsoon winds, fall from April to June, while the short rains fall between November and December. The area is generally hot and humid, with an average yearly air temperature of around 28° C and minimal seasonal change. Because of the close proximity to the sea, relative humidity is about 95%.

Geology of soils

Coral limestone rock, shallow sandy clay soils, fringing reefs, and coral gardens are all characteristics of Mida Creek. Mida Creek has a low water-retention capacity.

Socio-economic activities

People in the Mida Creek area generate income by trading coconuts and mangoes and building materials such as mangrove poles, bricks, gravel, and cement. They also create and sell tourist trinkets and souvenirs. Some members of the community work as tour guides, while others operate boats that transport tourists around scenic areas for a fee. The majority of the residents are fishers who sell seafood to supplement their income. The community owns some bandas that tourists use, which they profit from. Mida Creek has a population of about 30,000 people, with literate residents accounting for half of that.

Mangrove vegetation

Mida Creek is home to eight of the ten mangrove species discovered in Kenya (Kokwaro 1985), occupying a total area of 1746 ha (Gang and Agatsiva 1992; Kairo and Gwada 1998). *Avicennia marina, Rhizophora mucronata, Ceriops tagal, Lumnitzera racemosa, Bruguiera gymnorrhiza, Sonneratia alba, Xylocarpus granatum,* and *Pemphis acidula* are the mangrove species found near Mida Creek. *Rhizophora mucronata, Ceriops tagal,* and *Avicennia marina* are the most common species (Tomlinson 1986). Numerous researches, including some coastal research, have been undertaken in and around Mida Creek (Ouko and Manohar 1998).

Figure 1. A map showing the study area (Mida Creek), Coast Province, Kenya

Data collection

In this study, data was gathered from both primary and secondary sources.

Secondary data

Secondary sources such as textbooks, journals, economic surveys, government and nongovernmental organization reports, academic research findings, and media coverage provided the initial information and data. After that, the secondary data were combined to create information on various topics, including the socio-economic situation, the current state of mangroves, governance, policies, and management techniques.

Primary data

Interviews, questionnaires/surveys, direct observation, satellite imagery, and participant observation were all used to collect primary data. Initially, exploratory field surveys were done in the study site, followed by creating questionnaires for data collection.

Aerial photographs/ Visual image interpretation

To examine the temporal changes in the floristic composition of the mangrove forest in Mida Creek, two sets of a combination of 12 aerial pictures for each set, showing the forest's condition in 1992 and 2006, were utilized. Aerial photos from the Kenya Marine Fisheries Institute (KEMFRI) were obtained in 2006, while aerial photos from the Survey of Kenya and the Kenya Wildlife Service were taken in 1992. Aerial pictures were scaled at

1:35,000 and 1:25,000, respectively. The aerial photos were scanned and saved as jpeg files. After that, visual interpretation employed texture, shape, tone/color, and pattern. Bare patches, islands, mangroves (species level), and oceans were recognized as four different types of cover. Eleven classes of the identified mangroves were: *Rhizophora mucronata*; *Avicennia marina*; *Ceriops tagal*; *Sonneratia alba*; a mixture of *Rhizophora mucronata* and *Ceriops tagal*; a mixture of *Ceriops tagal* and *Avicennia marina*; a mixture of *Rhizophora mucronata* and *Avicennia marina;* a mixture of *Rhizophora mucronata* and *Bruguiera gymnorrhiza*; a mixture of *Rhizophora mucronata* and *Sonneratia alba*; a mixture of *Rhizophora mucronata, Ceriops tagal* and *Bruguiera gymnorrhiza* and a mixture of *Rhizophora mucronata, Avicennia marina*, *Ceriops tagal*, and *Bruguiera gymnorrhiza*. Onscreen interpretation, in which the visuals are zoomed in and out for clearer visibility, was used for a more detailed and in-depth explanation.

The photos were georeferenced using the fiducial markers that had already been marked on the aerial photographs as control points. Five sampled points were taken for each class of species identified, and the coordinates were uploaded to the GPS ready to place on the ground. To reduce overlaps during ground crossings, the sampling point was selected at an interval of 50 m. The sampling points were also representative for each class to avoid distortions, and the sampling points were distributed uniformly for each class. There are a total of 55 points taken. During ground-truthing, sampling spots were established using GPS, and observations were made in a 10m radius, with the species found are recorded. The classes were corrected when there was a discrepancy between the classes determined during photo interpretation and what was discovered on the ground.

The final classes were then digitized onscreen with ArcGIS version 9.3, which resulted in different shapefiles for each number of classes. Mangroves, bare patches, and island cover types have their respective area coverages determined. When comparing the changes between the two time periods, some modeling was done. The same cover types from the two years were intersected, and the area outside the intersection was used to determine the extent of change.

Questionnaires, interviews, and observations

Questionnaires and interviews were used to analyze the effectiveness of policies/legislation guiding mangrove exploitation and learn more about the most desired species. The total number of homes in the Mida Creek area is 210 (less than 10,000); hence the sample size was 136. This was calculated using Fisher's equation (Fisher et al. 2004).

$$N = \frac{Z^2(p)(q)}{d^2}$$

Where:

Z refers to the confidence limits of the survey results. If one would like to be 95% confident in your results, Z=1.96

prefers to the proportion of the population with the attribute you are looking for

q = (1-p)

d refers to the desired precision of the estimate

Data was gathered by interviewing 136 people in the Mida Creek area. Questionnaires were completed, and visual observations of daily life in the homes visited were made. One hundred people from the houses and thirty-six Kenya Forest Service and Kenya Wildlife Service staff members were questioned, resulting in 136 questionnaires used for numerical analysis. To avoid repeating questions from members of the same home, just one individual per household (the head of the family) was questioned. Because of the unavailability of topographic or administrative maps showing household distribution, households were approached directly in the field. Uyombo, Chafisi, Mida Msikitini, Mida Majaoni, Magangani, and Dongokundu were the locations of the interviews using stratified random sampling. The interviews were conducted in Kiswahili and/or the native Giriama, depending on the respondents' comfort level with each language. The old preferred to speak in Giriama, while the middle-aged and youth favored Kiswahili. A Kenya Wildlife Service guide, Gede, and a local resident aided in traveling around the creek, eliminating potential antagonism and allowing for a comfortable reception and fluent dialogue with the respondents in the visited families. The data was then entered into questionnaire schedules.

The questionnaire included questions to gather general information about the respondents, elicit the respondents' opinions on various issues, and reveal "the way of forest life" of the community members, i.e., how they used the mangrove forest and what kind of preferences they had. The questionnaire was semi-structured, with brief multiple-choice and some open-ended questions. The former primarily focused on narrowing down the answer categories to make data analysis easier. To ensure that the respondent and interviewer discussed the same concept and subject area, the interviews frequently began examining the respondent's comprehension of the term mangrove and how the trees look (distinguishing them from other trees). Mangrove trees were given the name mkoko, which was a Kiswahili or Giriama phrase, although only one species (Rhizophora mucronata or red mangrove) was called mkoko at the species level.

When respondents were afraid of answering specific questions because they were participating in certain behaviors, eye observation was used to obtain the necessary information (e.g., use of mangroves as poles which was easily visible). In other cases, observations were required to visually examine and complete the respondents' responses. Mangroves were used for various purposes, including furniture, cooking utensils, fishing traps, canoes, sailboats, and the construction of dwellings. The amount of the mangrove species cut was clearly evident. This method of observation supplemented the information obtained during the interviews. The few concerns that arose during the survey had to do with the sensitivity of specific topics. Given the present policy on mangrove exploitation in

Kenya, the issue has become sensitive. Questions on mangrove pole harvesting elicited a lot of skepticism, making it difficult to get information from respondents. This is because no permits for mangrove harvesting are provided in Mida Creek. It often needed a long chat to gain the respondent's trust and make them feel comfortable enough to answer questions. Community leaders were seeking to gather sensitive information. The Kenya Forest Service, Kenya Wildlife Service, and Kenya Forestry Research Institute staff were given questionnaires. Personal contact was also established with the District Forest Officer and the KWS senior warden in charge of Gede. The questions posed aided in determining the efficacy of mangrove conservation strategies and regulations.

Point-Centered Quarter Method (PCQM)

Transects and sampling locations were utilized to analyze the impacts of mangrove utilization/harvesting on mangrove succession, mangrove forest structure, and if mangrove accessibility determines their utilization Figure 2). Three 200m long line transects were created perpendicular and parallel to each other (along with the land, near the coastline, and in the middle). The distance between each transect was 100 meters. The Point-Centered Quarter Method (PCQM) was utilized, and vegetation sampling was done in 100m2 quadrats that were regularly spread out along the transects. Each transect had ten quadrats set out. In Mida Creek, seven replicates, thus 210 quadrats, were studied. This took place for over a month.

The transect was laid out with a tape measure, and the transects were marked with red tape. A compass and a GPS were used to guarantee that the transect fell in a straight line. If the mangrove roots were too large or a tree blocked the transect, a way was found to get around them, but the straight line was maintained once on the other side. To make data collecting easier and to avoid danger, a tide table was checked every day to determine the times of low and high tides. GPS coordinates and altitude were recorded in each quadrat. Mangrove species were classified, and the number of standing trees (>4cm DBH), cut stems, dead trees and stems, and seedlings/saplings were also tallied (below 4cm DBH). This was accomplished by visual observation. The diameter at breast height (D130) was measured and recorded using a tape measure. All of the trees whose DBH was measured had their canopy cover approximated. All of the trees whose DBH was measured had their canopy cover compared. Following the procedures described in Cintron and Novelli (1984) and Kairo et al., the following characteristics were determined: stem density (De), frequency (F), basal area (Ba), and importance value (IV) (2002). Photographs were taken in some of the quadrats where most of the trees had been damaged or scattered.

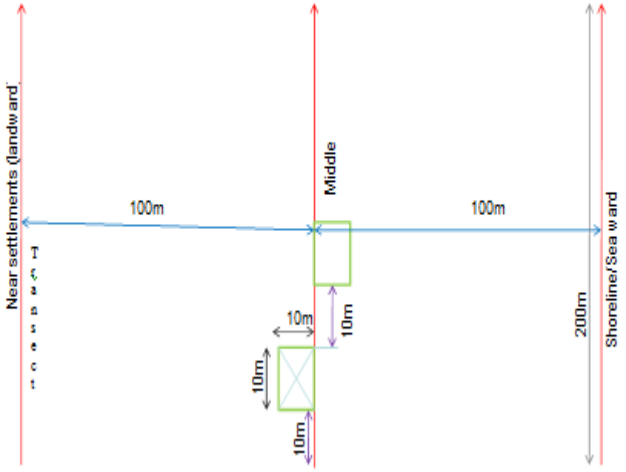

Figure 2. A transect layout for Point-Centered Quarter Method (PCQM)

The following formulas were used to compute relative density, relative dominance, relative frequency, and Importance Value: The relative density (DE_{ri}) was;

$$DE_{ri} = \frac{100DE_i}{\sum_{i=1}^{m} DE_i},$$

Where: DE_i was the density for species *I*, and *m* is the number of species. The relative dominance (DO_{ri}) was;

$$DO_{ri} = \frac{100Ba_i}{Ba},$$

Where: Ba_i was the basal area of all trees of species *i*. The relative frequency (F'_{ri}) was;

$$F'_{ri} = \frac{100F_i}{\sum_{i=1}^{m} F_i},$$

Where: *F*i was the number of sampling points in which species *i* is represented100 times. The importance value (*IV)* was calculated as;

IV=DE_{ri} + DO_{ri} + F'_{ri}

Data analysis

For data analysis, the completed questionnaires were coded and imported into SPSS. To examine if the use of mangroves affects their succession, researchers used regression analysis. The difference between the means in the number of cut mangroves in three different zones was assessed using analysis of variance (ANOVA) to see if the accessibility of mangroves affects their usage. To analyze changes in the floristic composition of the mangrove forest in Mida Creek, aerial pictures were georeferenced and visually assessed. Descriptive statistics were used to summarize data on awareness and effectiveness of policies governing mangrove exploitation. According to Dahdouh-

Guebas and Koedam, relative density, dominance, and frequency were calculated, and importance levels were assigned (2006). This was used to evaluate the structure and regeneration of mangrove forests.

RESULTS AND DISCUSSION

Mangrove knowledge levels

When interviewing the respondents, different levels of knowledge became apparent. Using different physiognomic and morphological aspects of the plants (e.g., roots, leaves, bark) and their environments, the 136 respondents were able to identify mangrove species (where mangroves are found).

Knowledge of mangroves was unaffected by education levels (none, primary, secondary, college, and university). Although the roots, leaves, and bark of mangroves could be distinguished from those of other trees, the majority of respondents described mangroves based on their habitat (those that grow in salty water or the ocean) (Figure 3).

The respondents had no trouble answering the questions during the interview and filling out the surveys because they understood precisely what mangroves were and how they looked. Some people could tell the difference between all mangrove species, while others could only say the difference between a few.

Accessibility and utilization

The ANOVA test revealed a significant difference in the mean number of cut mangroves in three areas: near settlements, middle, and shoreline (Most accessible, middle, and least accessible, respectively) ($F=3.277$; $df=2, 207$; $p=0.040$; (Table 1). Because mangrove species are more accessible (i.e., they may be entered without trouble), they are used more near communities and the seashore.

The number of cut trees nearest settlements and in the shoreline was higher than in the middle (Figure 4).

Mangrove forest structure at Mida Creek

Rhizophora mucronata and *Ceriops tagal* were the important species in Mida Creek, based on relative density, relative frequency, relative dominance, and significance values (Table 2). Mida Creek was the most structurally developed mangrove forest, followed by the *Avicennia marina*.

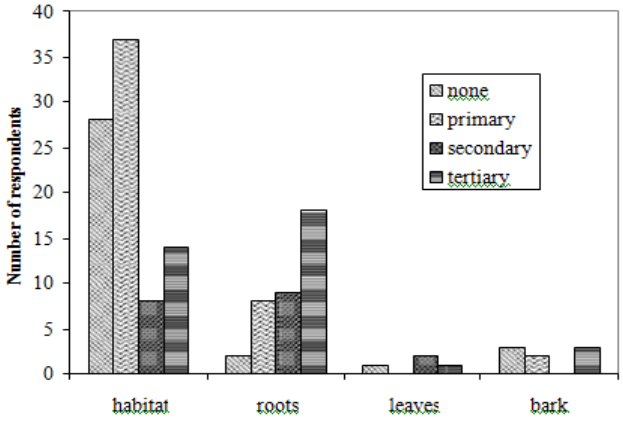

Figure 3. Mangrove knowledge levels by the local community

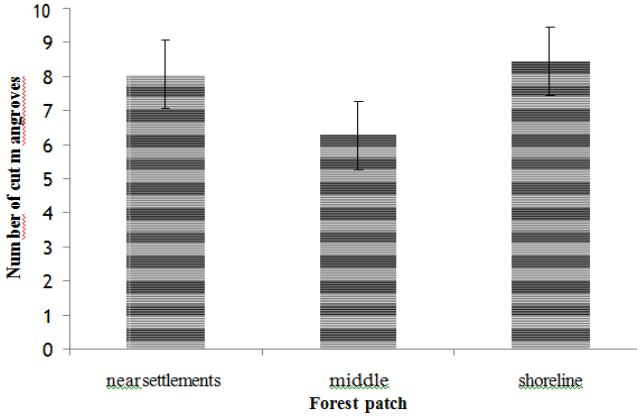

Figure 4. The abundance of cut mangroves in each forest patch

Table 1. ANOVA for accessibility and utilization for the number of cuts

	Ss	df	Ms	F	P
Forest patches	205.895	2	102.948	3.277	0.04
Error	6503.729	207	31.419	-	-
Total	6709.624	209	-	-	-

Note: Ss: Sum of squares, df: degree of freedom, Ms: Mean square, F: calculated f value, P: significant value

Table 2. Structural attributes of the mangrove species at Mida Creek, North Coast, Kenya

Species	Density	Frequency	Dominance	R/density	R/frequency	R/dominance	IV	IV/3
Avicennia marina	729.5	0.2762	0.000308	9.88	17.68	23.83	51.39	17.13
Rhizophora mucronata	3201.0	0.7048	0.000478	43.37	45.12	36.95	125.44	41.81
Ceriops tagal	3164.8	0.4619	0.000400	42.88	29.57	30.97	103.43	34.48
Bruguiera gymnorrhiza	137.6	0.0476	0.000031	1.86	3.05	2.40	7.32	2.44
Sonneratia alba	118.1	0.0571	0.000064	1.60	3.66	4.92	10.18	3.39
Xylocarpus granatum	20.0	0.0095	0.000010	0.27	0.61	0.75	1.63	0.54
Lumnitzera racemosa	9.5	0.0048	0.000002	0.13	0.30	0.18	0.61	0.20

Note: Density: Species/ha, Frequency: Proportion of subsamples which contain species, Dominance: basal area of species by total area (m^2/ha), Relative density, frequency, dominance, importance value: in percentage (%).

Mangrove forest cover at Mida Creek

According to tan o an aerial photography study, the extent of mangrove vegetation in Mida Creek has shrunk between 1992 and 2006. Mangrove forest has been lost in an area of 105.217 hectares, while bare patches and islands have risen by 32.098 ha and 107.44 ha, respectively (Table 3). Islands and bare patches acquired a total of 139.538 square kilometers. This is due to human activities, which have resulted in islands and bare patches extending into the mangrove forest. They've also reached out to the sea. Mangrove plants were present in several areas in 1992 but were not visible in 2006. (Figures 5 and 6).

Table 3. Changes in mangrove forest cover at Mida Creek (1992-2006)

	1992 (area in ha)	2006 (area in ha)	Area (ha)
Mangroves	1745.545	1640.328	105.217(-)
Bare patches	52.193	84.291	32.098(+)
Islands	165.725	273.165	107.44(+)

Note: +: gained, -: lost

According to the findings, the area occupied by different mangrove species in Mida Creek, Kenya, has altered over time (Table 4).

Clearing/felling of trees and encroachments have caused alterations in the mangrove forest in some areas (Figure 7).

Table 4. Floristic composition and changes in the area of mangrove forest at Mida Creek (1992-2006)

Mangrove Species	Coverage in Ha (1992)	Coverage in Ha (2006)	Difference (ha)
Rm	65.088	63.934	1.154
Am	344.986	310.627	34.359
Ct	225.116	223.821	1.295
Sa	7.529	7.529	-
Rm, Ct	52.874	50.219	2.655
Ct, Am	143.693	140.287	3.406
Rm, Am	44.359	41.270	3.089
Rm, Bg	212.751	199.747	13.004
Rm, Sa	47.642	46.321	1.321
Rm, Ct, Bg	129.066	128.117	0.949
Rm, Am, Ct, Bg	472.441	428.456	43.985

Note: Rm: *Rhizophora mucronata*, Am: *Avicennia marina*, Ct: *Ceriops tagal*, Sa: *Sonneratia alba*, Bg: *Bruguiera gymnorrhiza*

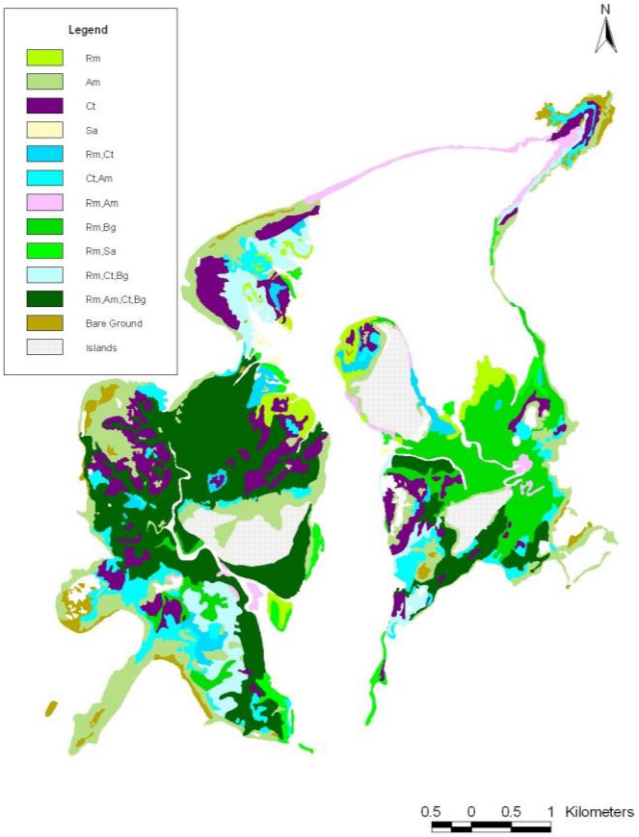

Figure 5. Aerial photograph interpretation showing the floristic composition of mangrove forest in Mida Creek in 1992

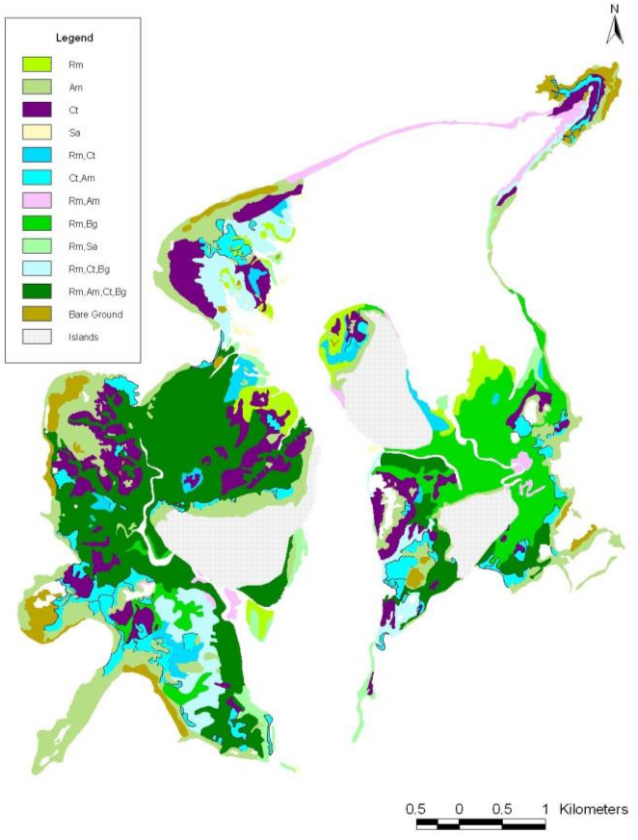

Figure 6. Aerial photograph interpretation showing the floristic composition of mangrove forest in Mida Creek in 2006

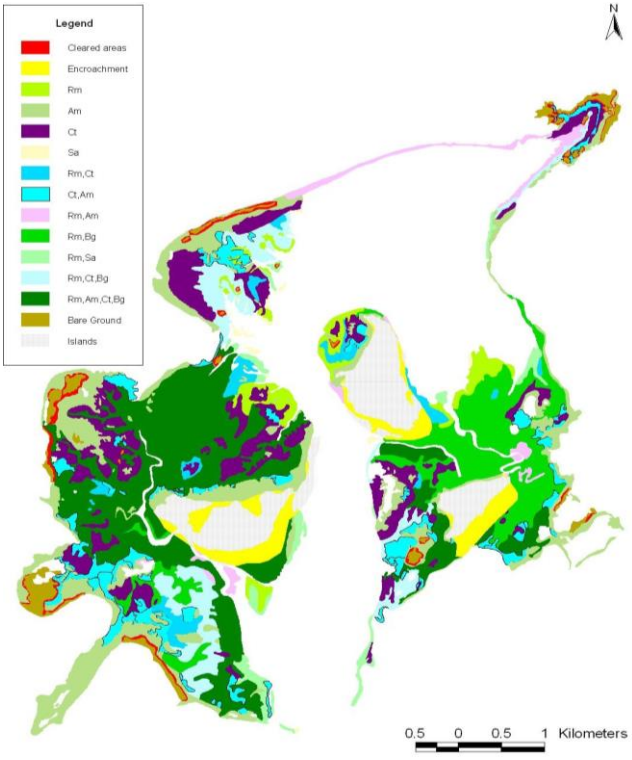

Figure 7. Aerial photograph interpretation showing changes in the floristic composition of mangrove forest in Mida Creek (1992-2006)

Alterations in the mangrove forest in Mida Creek up to the time of data collection were supported by questionnaires, with the majority of respondents stating that the mangrove forest in Mida Creek has reduced over time (Figure 8).

Mangrove preferences

Some mangrove species are more desirable than others. *Rhizophora mucronata* is the most popular species in Mida Creek due to its high-quality wood, followed by *Ceriops tagal* and *Avicennia marina* by both males and females (Figure 9).

Awareness of policies and legislation

In both sexes (Figure 10), people of all ages, occupations, education levels, and length of stay are aware of mangrove utilization policies. The policies are unknown to a few people.

Succession and utilization

Regression study revealed a significant association between mangrove succession and utilization (F=8.529, df=1, R=0.198, P=0.004) (Table 5). As a result, there existed a link between succession and utilization. The use of mangroves has an impact on their succession. In extensively used areas, natural succession was low (Figure 11). The number of chopped stumps could only explain 4% of the variation in the number of saplings, according to R2= 0.0392.

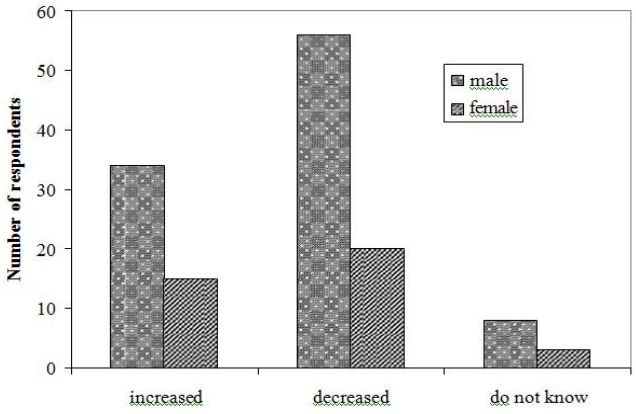

Figure 8. Awareness of changes in the mangrove forest cover along Mida Creek

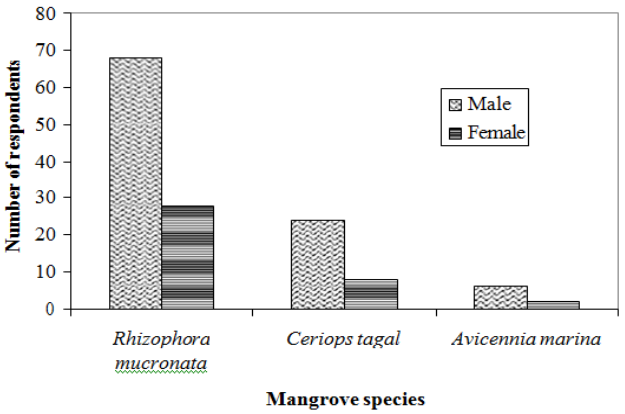

Figure 9. Preference of mangrove species by sex of respondents at Mida Creek

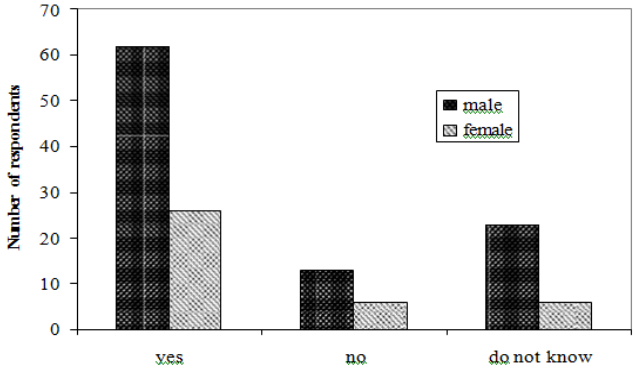

Figure 10. Effectiveness of the policies governing mangrove utilization

Table 5. A regression analysis of succession and utilization of mangroves along Mida

	Ss	df	Ms	F	P
Regression	3424.753	1	3424.753	8.529	0.004
Residual	83521.747	208	401.547	-	-
Total	86946.500	209	-		

Note: Ss: Sum of squares, df: degree of freedom, Ms: Mean square, F: calculated f value, P: significant value R: Correlation between the observed and predicted values of the dependent variable

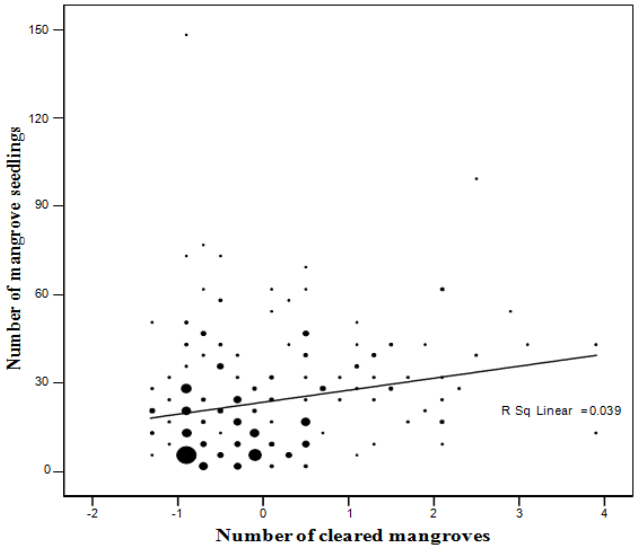

Figure 11. Scatter diagram of the relationship between the number of tree stumps and the number of seedlings at Mida Creek

Sonneratia alba was detected along the seaward forest boundary during the survey at Mida Creek, followed by mixed stands of *Rhizophora mucronata* and *Ceriops tagal*, *Lumnitzera racemosa*, *Xylocarpus granatum*, and *Bruguiera gymnorrhiza*. Then came the *Avicennia marina* (Figure 12).

According to the results of the interviews and observations, the roots, leaves, bark, and stem of several mangrove species are used in the following ways (Table 6):

The data show that mangrove stems are used more than leaves, roots, and fruits. This impacts succession since most mangrove species' embryos attach themselves to the stems of parent plants. When the stems are cut, the embryos fall and dry up, preventing natural succession. The mangrove ecosystem is home to fish, crabs, mollusks, crustaceans, and oysters, all consumed by the local populace. Fish is the primary source of protein for the people that live in that area. Thus, it is harvested daily. Some fishermen sell the fish they catch in the market to supplement their income. This money is used to purchase maize flour, other foods, and clothing. People tread on the seedlings of the mangrove species when gathering animal species, and they die. This has an impact on succession.

Discussion

Utilization and accessibility

Their accessibility determines the use of mangrove species. *Avicennia marina* is found near communities and is suitable for home use firewood; hence many mangroves have been cut for firewood in the nearby settlements, owing to the area's accessibility. Women who are terrified of going deep into the forest are the ones that do this. Most women work all day and can easily collect firewood when they get home in the evening. Because there is less water in the morning, firewood can also be easily obtained during low tides, which usually occur in the morning. The majority of mangrove cutters work at night, and because they cannot get far into the forest, they cut near communities. Mangroves gathered near communities are very easy to transport.

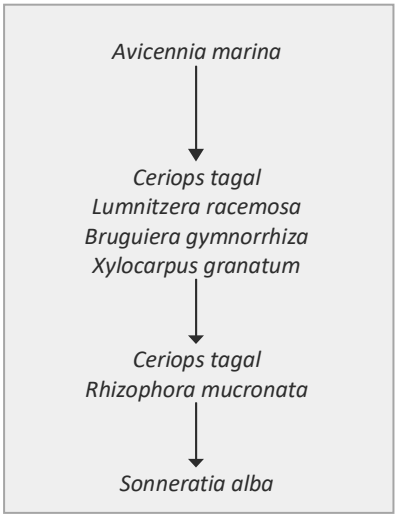

Figure 12. Successional and zonation trend of mangroves along Mida Creek, North Coast, Kenya

Table 6. Utilization of mangrove species by the local community at Mida Creek

Species name	Uses	Parts used
Avicennia marina	Charcoal, low-quality firewood, Boat making, fencing posts, hive	Stems Branches
Ceriops tagal	Poles, medium quality firewood, Paddles, oars, dyes (tannings)	Stems, bark
Rhizophora mucronate	Charcoal, poles, dyes, medicines, High-quality firewood, ointments	Stems of barks Stems and roots
Bruguiera gymnorrhiza	High-quality firewood, charcoal, Oars, beehives paddles	Stems
Xylocarpus granatum	High-quality timber, charcoal, Ointments, firewood	Stems, fruits
Sonneratia alba	Timber, medium quality firewood, Paddles, fishing nets floats Fodder, charcoal	Stems, leaves
Lumnitzera racemose	Medium quality firewood, charcoal	Stems

Because the area is difficult to access, only a few mangroves have been chopped in the middle. People find it difficult to walk in the middle due to the roots of *Rhizophora mucronata* and the thickness of the mangroves. Because of the stagnant water in the area, the earth is slippery (muddy clay soil) in the center, making it less accessible. Because the shoreline is easily accessible by boat, more trees have been felled near the water's edge. People can stroll freely in ocean arms during low tides, and they can readily reach the shorelines by cutting the mangroves they need to use. The boats can also transport harvested mangrove poles. Even though different needs of mangrove species are determined by accessibility rather than quality (Kairo 1995b), *Rhizophora mucronata*, *Ceriops tagal*, and *Avicennia marina* were preferred more than others, and this also impacts their utilization. Mangroves have zonation patterns due to individual species' environmental tolerance and physiological preferences (Rabinowitz 1975). Rhizophora mucronata can be found in the heart of the forest, and their knee roots render the area inaccessible. Each mangrove species has a variety of environmental tolerances (e.g., salinities, tidal floods, shade, land elevation, etc.) that limit it to the zones in which it thrives, e.g., the tropics. *Ceriops tagal* and *Avicennia marina* grow in the marginal dry landward side, whereas *Sonneratia alba* grows in the seaward fringe (Kairo 1995, Kairo 2001). Some mangrove species can be found on both the landward and middle side and the seaward side. People will leave the dwarf ones and go collect the huge ones in the seaward zone if the one on the landward side is dwarf and the same species is significant and is in the seaward zone, depending on the necessity. Depending on whatever mangrove species a person requires, they can travel as far as the seaward side to obtain it.

Composition of mangrove forest

Avicennia marina, Rhizophora mucronata, Ceriops tagal, Lumnitzera racemosa, Bruguiera gymnorrhiza, Sonneratia alba, Xylocarpus granatum, and Pemphis acidula are the mangrove species found at Mida Creek. Rhizophora mucronata, Ceriops tagal, and Avicennia marina are the most common species. Mangrove density, frequency, and dominance are used to make this determination. The mangrove forest in Mida Creek has been subjected to various degrees of human disturbance. Within the several species of mangroves, there were significant variances in frequency, density, and dominance. Anthropogenic factors such as indiscriminate and unregulated harvesting, pollution, and siltation have all impacted forest structure and composition.

Changes in the mangrove forest cover

Between 1992 and 2006, the mangrove vegetation in Mida Creek shrank by 6%. This was based on aerial pictures processed to show changes between 1992 and 2006. Interviews and questionnaires were also undertaken to obtain the local community's perspectives on mangrove forest changes throughout time. Most people believe that mangrove vegetation in Mida Creek has diminished over time. Further investigation revealed that this was mostly the perspective of people who had lived in the area for more than five years and had witnessed changes in the mangrove forest during that time.

Licenses/permits were once issued in Mida Creek to cut mangrove trees. Still, this practice was discontinued when overuse of mangroves was discovered, and the forest was placed under the new administration. The main reason for the observed shift is a reduction in desired tree sizes or overall tree numbers owing to tree cutting and encroachment. The exploitation of the mangrove forest for sustenance and economic reasons has come from population growth and a scarcity of food. As the population grows, additional buildings are required, such as houses, boats, canoes, and oars for use while fishing for daily bread, necessitating the utilization of mangrove trees. The islands (Sudi and Kirepwe) have grown in size, and farming has begun, reducing forest area. People have turned to traditional medicine acquired from particular types of mangrove trees due to a lack of funds to go to the hospital and traditional beliefs. Another factor contributing to mangrove decline is high water pressure caused by the overharvesting of mangroves by the local people, which has resulted in the uprooting of trees along the shoreline, diminishing the extent of the forest, as seen in Kirepwe, where trees toppled owing to strong ocean currents. Unmanaged yet exploited mangroves do not permanently vanish, although they change over time. It suggests that small human societies cannot sustainably manage mangroves (Kairo et al. 2002).

Mangroves preference

Ceriops tagal, *Rhizophora mucronata*, and *Bruguiera gymnorrhiza* are three species that are preferred over others. Because of their capacity to grow long and straight, these species are preferred. Each of these species has a specific function inside the framework of a building, such as roofing poles, side framework, and lumber (Dahdouh-Guebas et al. 2000). Because of its high quality, *Rhizophora mucronata* is the most popular species. It is a durable wood resistant to termites and soil conditions such as wetness and saline soils, which is why most people choose it to build buildings. Their roots are often regarded for their medicinal powers against constipation, fertility-related (boosts human fertility), or menstruation diseases (relieves stomach aches) (Dahdouh-Guebas et al. 2000), which is why more women than men favor it. A medication made from the bark of the roots of Rhizophora mucronata is said to exorcise demons from those who are possessed. *Rhizophora mucronata* bark is used to make dyes, including tanning chemicals, which are applied to the insides of canoes and vessels and valued for their preservation properties. Dyes are also used to seal the microscopic holes of reed and palm leaf woven trays used for storing cereal flour and embellish weaving mats, baskets, and trays. It's used in schools to make tie-dye fabric garments.

Insecticides are derived from Avicennia marina logs, which emit a large amount of smoke when burned, keeping mosquitoes and other biting night insects away. Simple one-person canoes are also created from the hefty wood of the *Avicennia marina*. Paddles and oars are constructed from *Bruguiera gymnorrhiza* or *Rhizophora mucronata* to drive boats forward. Because *Ceriops tagal* poles are thin, they are used as poles (fito) to create a network through the house's walls and roofs. *Ceriops tagal* is also used to construct shrines, cooking huts, livestock sheds, and pegs, among other buildings. Men are more focused on this work than women; thus, they prefer the species.

Policies and effectiveness

The interview/questionnaires revealed that illegal loggers destroy mangroves, but people/respondents were scared to name them for fear of being targeted. According to the respondents, the majority of the trees are harvested at night. Since most individuals do not harvest mangroves without permission, policies and legislation have been appropriately enforced. Those needing to construct poles must apply to the Kenya Forest Service for authorization to cut a certain number of poles for house construction (Pers comm., District Forest Officer, Gede). Some people take advantage of this to increase their crops. A management policy that consists of regulations and prohibitions in the absence of the instruments to apply them has been the greatest impediment to rational use and conservation of mangroves in Kenya (Ferguson 1993; Kairo 1995). It was evident that patrols in mangrove forests do not occur daily, therefore monitoring occurs only a few times per week. Because some areas within mangrove forests where unauthorized mangrove cutting occurs are inaccessible, patrol groups cannot visit them. Bans on all harvesting operations, for example, cannot be implemented effectively without first considering the effects for local subsistence populations. Some activities have emerged for the long-term utilization of mangroves, such as beekeeping by the Vimoyoni women's organization. The importance of mangroves has been taught. This type of environmental education instills in locals a sense of responsibility for their actions in the forest, but community-based natural resource management must be effectively institutionalized (Agrawal and Gibson 1999). Mangrove management in Kenya currently restricts the use of mangrove wood products for industrial fuelwood or charcoal. However, it is unclear how strictly the rule is enforced. Dahdouh et al. (2000) demonstrated how dangerous the situation might become if the government banned the cutting of mangrove timber. Several alternatives to mangrove cutting have been proposed (Kairo et al. 2002). However, it appears that the government provides few other options in return.

Succession and utilization

The forest conditions (cut or not cut), tides, and soil stability influence whether mangroves propagate by self-planting or self-regeneration mechanisms. Harvesting too many trees from the forest reduces soil stability, causing propagules and saplings to be carried away by the tides and preventing natural succession. Because no propagules fall to become seedlings when the mother plant is cut, the number of seedlings that emerge is reduced, and some mangrove species may become extinct as a result. People trample on the tiny mangrove plants that occur when mangroves and other mangrove resources are being harvested, preventing them from germinating and affecting succession and trends. They also drag the mangrove poles across the ground when hauling them out of the forest, destroying the seedlings.

In conclusion, (i) The mangroves and their resources are extremely important to the residents of Mida Creek. (ii) The floral composition of the mangrove forest in Mida Creek has changed through time, reducing the size of the mangrove forest. This is due to the encroachment of the mangrove forest to perform various activities to support the local people in the area. (iii) *Rhizophora mucronata* is favored over other mangrove species due to its superior wood quality. (iv) In Mida, accessibility impacts how the mangroves are used. Mida Creek residents travel deep into the mangrove forest to gather the mangrove species. (v) Mangroves are impacted by human activity, altering succession patterns. (vi) Mangrove harvesting policies are ineffective since there are illegal mangrove cutters/harvesters because no licenses to exploit mangrove in Mida Creek are provided.

As a result of the findings of this study, the mangrove forest in Mida Creek has declined, and the subsequent efforts should be undertaken: (i) Mangrove replanting should be expanded to regions where it has not before been done. To provide young trees for replanting, more mangrove nurseries should be constructed. (ii) Environmental regulations are enforced to safeguard mangrove forests. (iii) More research on the succession/zonation of mangroves at the species level in Mida Creek is needed. This research should be carried out to compare artificial and natural mangrove regeneration. (iv) To prevent overexploitation of mangroves, sustainable utilization, such as beekeeping and crab farming, should be encouraged. The government should support local communities in obtaining resources to make this possible. (v) Educating and raising awareness about the value of the mangrove environment among the local people. This should be done at the school level so that the young ones can grow up with this knowledge. (vi) Monitoring unauthorized mangrove degradation as part of community participation in mangrove protection. To survive, the inhabitants need to plant different tree species, such as casuarinas.

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Isolation and identification of lipolytic bacteria from the digestive tract of eel (*Anguilla bicolor bicolor*)

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Abstract. *Hapsari RT, Susilowati A, Pangastuti A. 2020. Isolation and identification of lipolytic bacteria from the digestive tract of eel (*Anguilla bicolor bicolor*). Bonorowo Wetlands 10: 44-50. Anguilla bicolor bicolor* is commonly found in the waters of western Indonesia. Currently, the demand for eel is increasing, causing an increase in the economic value of eel and being used as an export commodity. This study aims to isolate and identify lipolytic bacteria found in the digestive tract of eel (*A. bicolor bicolor*). Lipolytic bacteria isolated from the digestive tract of eel (*A. bicolor bicolor*). Bacterial isolation uses minimal media enriched with olive oil. When lipolytic bacteria were screened using Rhodamine B agar media, the presence of an orange glow in the colony upon exposure to 350 nm UV light indicated lipolytic activity. Identification of lipolytic bacteria was based on observing the morphological characters of bacterial colonies (edges, elevation, colonies, and colors) and the gene sequences encoding 16S rRNA. DNA sequence characteristics of lipolytic bacteria were analyzed by BLAST Nucleotide on the NCBI website (www.blast.ncbi.mlm.nih.gov/blast.cgi). Based on the screening results, 7 isolates of lipolytic bacteria were obtained from 30 successfully isolated isolates. The DNA sequence characteristics of the bacterial isolates were analyzed by BLASTN and found 5 species, namely *Pseudomonas azotoformans, Providencia vermicola, Providencia* sp., *Aeromonas veronii,* and *Aeromonas hydrophila.*

Keywords: *Anguilla bicolor bicolor,* identification, isolation, lipase enzyme, lipolytic bacteria

INTRODUCTION

Indonesia is a country that is rich in eel species. According to Sasongko et al. (2007) and Fitri et al. (2019), there are 6 species of eel in Indonesia, namely *Anguilla marmorata*, *A. borneoensis*, *A. bicolor,* and *A. ancertralis*, *A. mauritinia,* and *A. celebesensis.* In the species *A. bicolor,* there are 2 subspecies, namely *A. bicolor bicolor* and *A. bicolor pacifica*. *Anguilla bicolor bicolor* is mostly found in the waters of western Indonesia, while *A. bicolor pacifica* is mostly found in the waters of eastern Indonesia (Sugeha and Suharti 2008). Today eel is increasingly popular for consumption, thus increasing its economic value and potential for export.

The increasing popularity of eels for consumption has encouraged researchers to research eels, one of which is the study of normal microbiota found in the digestive tract of eels. Naturally, every fish has a microbiota in its body. The microbiota can be in the form of bacteria that can be found on the skin, gills, digestive tract, and light-emitting organs (Austin, 2006; Ridwan et al., 2019). These bacteria have various functions, such as self-protection, digestive processes, and preventing diseases in the fish's body. In the digestive tract, eels have various bacteria used in the digestion process of food and protection from pathogenic bacteria that enter the body.

According to Aslamyah (2006), a fish's digestive system is simpler than that of land animals, so the digestive mechanism of fish is very limited. It affects the availability of digestive enzymes, such as amylase and lipase enzymes, in lower amounts because they have little secretion. In contrast, the protease enzyme is secreted more because the eel is a carnivorous fish. One of the enzymes that play an important role in the digestive process, namely lipase, plays a role in breaking down triacylglycerol into fatty acids and glycerol.

The lipase enzyme in the digestive tract of the eel produced by bacteria is usually extracellular (Pramiadi et al., 2014). Lipases, also known as triacylglycerol hydrolases (triacylglycerol acyl hydrolases, EC 3.1.1.3), are naturally occurring enzymes that catalyze the hydrolysis of triacylglycerol (fats/oils) into fatty acids, monoacylglycerol, diacylglycerol, and glycerol (Poedjiadi and Supriyanti 2009). According to Kurniasih et al. (2013), high enzymatic activity in the digestive tract will increase the digestibility of food by releasing enzymes that help the digestive process. The enzyme-producing bacteria will also regulate the condition of microorganisms in the intestine and suppress the growth of pathogenic bacteria in the digestive tract of fish. Increasing the enzymatic activity of the digestive tract of the eel through microbiological development can improve the quality of the eel. However, to develop and increase eel production, it is necessary to know the types of bacteria that have lipolytic activity in the digestive tract of eels.

Knowledge of lipolytic bacteria in eel can be applied as a candidate for probiotic bacteria to increase feed digestibility. It is expected to increase the quality and weight of eel. According to Fuller (1992), probiotics are food additives in the form of live microbes that benefit the

host by improving the balance of microbes in the digestive tract. The use of lipolytic bacteria as probiotic candidates can also suppress the growth of pathogenic bacteria because probiotic candidate bacteria can produce antibacterial compounds. With the addition of probiotics, the mortality of eels during cultivation can be reduced be suppressed. According to Telussa (2013), the development of microorganisms is currently very intensively carried out to be applied in all fields because it is considered more environmentally friendly and more economical.

Based on this description, it is necessary to study the microbiota of eel (*A. bicolor bicolor*) through the isolation and identification of lipolytic bacteria present in the digestive tract of eel. This study will be known what lipolytic bacterial microbiota is contained in the digestive tract of eels that can be used as probiotic candidates and help digest food.

The objectives of this study were (i) to isolate lipolytic bacteria from the digestive system of an eel (*A. bicolor bicolor*) and (ii) to identify lipolytic bacteria isolated from the digestive tract of an eel (*A. bicolor bicolor*).

MATERIALS AND RESEARCH

Materials

The lipolytic bacteria isolated and identified in this study were sourced from the digestive tract of *A. bicolor bicolor*, which was taken from the eel farm at Universitas Sebelas Maret, Surakarta, Central Java, Indonesia in August-November 2018 with a length of 27-32 cm.

Ways of working

Isolation of lipolytic bacteria

The eels used as samples are fasted for 24 hours to clean the digestive organs from food debris. *Anguilla bicolor bicolor* was dissected by cutting the lower part of the abdomen from the anterior of the body to the ventral fin, then cutting towards the dorsal of the eel to the lateral line, and then cutting towards the anal part of the fish. The stomach and intestines are taken, and then the intestinal contents are removed to reduce impurities from the fish's stomach contents. Then the organs were washed with physiological saline (0.85% NaCl). Then the fish digestive tract samples were homogenized in 0.85% sterile NaCl and then made serial dilutions of 10^{-1}-10^{-4}. A total of 0.1 mL of the 10^{-3}-10^{-4} dilution series was spread on minimal media with olive oil and incubated at 27ºC for 48-72 hours using the spread plate method with 2 repetitions. Colonies growing on the surface of the media and showing different morphologies can be used as candidates for lipolytic bacteria that can be stored in slanted agar at 4ºC (Gayathri et al., 2013).

Screening for lipase producing bacteria

The bacterial screening was carried out on bacterial isolates purified by taking 1 ose of isolate and then streaking on rhodamine B agar media, then incubation at 27ºC for 48 hours. Bacteria with lipolytic activity are characterized by bacterial colonies that glow pink to orange when observed under UV light with a wavelength of 350 nm (Carissimi et al., 2007).

Identification of lipolytic bacteria

Observation of the colony morphology of lipolytic bacteria. Observation of the morphology of lipolytic bacterial colonies was carried out by observing bacterial colonies growing on NA media. Aspects observed included color, shape, elevation, and the edge of the bacterial colony.

Gram stain. Bacterial isolates were taken and scratched on a glass object, sterilized, and then fixed for fixation. Furthermore, 1 drop of crystal violet was added and allowed to stand for 1 minute, and then rinsed with distilled water until the dye faded. After drying, 1 drop of iodine solution was dripped on the bacterial preparation for 1 minute and then washed with distilled water. Furthermore, the preparations were dripped with 96% alcohol for 20 seconds and then flowed again with distilled water. Then after drying, the preparation was dripped with 1 drop of safranin for 45 seconds, then drained with distilled water and dried. The preparations were observed under a microscope with 1000x magnification (Pratita and Putra 2012).

Identification of lipolytic bacteria using the 16S rRNA coding gene sequence. The lipolytic bacterial genomic DNA obtained from the screening process was extracted with the presto^{TM} mini gDNA bacteria kit. The lipolytic bacterial 16S rRNA gene was amplified using My TaqTM HS Red Mix, utilizing a primer consisting of 63 forward primers (63f: 5'-CAGGCCTAACACAT GCAAGTC-3') and 1387 reverse primers (1387r: 5'-GGGCGGAWGTGTACAAGGC-3'). The PCR reaction was started by mixing 12.5 µl Kapa2G fast ready mix, 1.25 µl 63 forward primers with a concentration of 10 pmol, 1.25 µl 1387 reverse primer with a concentration of 10 pmol, 1 µl DNA template, and 9 µl ddh2o. The pre-denaturation process was carried out at 95ºC for 3 minutes. One PCR cycle of 30 cycles consisted of denaturation at 95ºC for 15 seconds, annealing at 56ºC for 15 seconds, and elongation at 72ºC for 30 minutes. Finalizing was carried out at 72ºC for 2 minutes, and then the PCR was stopped and stored at 4ºC. The PCR amplification products were then separated by gel electrophoresis (Marchesi et al. 1998). The PCR product for the gene encoding the 16S rRNA of lipolytic bacteria was then sequenced by 1st base Singapore.

Data analysis

The lipolytic bacteria isolates were analyzed descriptively by observing cell colony morphology in shape, color, elevation, and bacterial margins. The characteristics of lipolytic bacterial DNA sequences resulting from the extraction process were analyzed using bioinformatics techniques with the BLAST Nucleotide device on the NCBI website (www.blast.ncbi.mlm.nih.gov/blast.cgi).

RESULTS AND DISCUSSION

Isolation and screening of lipolytic bacteria from the digestive tract of eels

Isolation of bacteria from the digestive tract of the eel was carried out by taking all parts of the stomach and intestines of the fish 3 times so that more varied and more bacterial isolates were obtained. This study resulted in 30 bacterial isolates coded sd01 to sd30 (Table 1).

As many as 30 bacterial isolates could grow and thrive on minimal media enriched with olive oil. Pure culture isolates were distinguished based on the morphological characters of the colonies. In several studies, such as that conducted by Lestari (2016), it was stated that the isolation of bacteria from the digestive tract of eel obtained as many as 8 bacterial isolates, which colony morphological characters could distinguish. Another study conducted by Lestari et al. (2016) stated that in the digestive tract of eel, 11 isolates of bacteria could be isolated. In his research, Floris (2010) stated that the microbiota community in the digestive tract of fish is known to have an essential role in the digestive tract because it can help the process of micronutrient metabolism, synthesis of enzymes, and vitamins such as B12, which the digestive tract can directly absorb.

In the digestive tract, microbiota exists in a mixture of various microorganisms that play a role according to their function in the digestive process. Separating bacteria to form a pure culture consisting of single cells must be carried out to study the morphological characters, growth properties, physiological properties, and their role in the digestive tract (Fardiaz 1992). The obtained bacterial isolates were then screened for lipolytic ability (Figure 4).

Based on the screening results, six bacteria had lipolytic activity in the digestive tract of eels. The isolates were SD01, SD09, SD12, SD13, SD22, SD29. The presence of bacterial isolates with lipolytic activity indicates the presence of microbiota in the digestive tract of fish that functions in the digestive process of food in the breakdown of lipids into fatty acids and glycerol. Rani et al. (2005) explained that the orange glow occurs when the lipids in the medium are hydrolyzed with a lipase catalyst from bacteria into fatty acids and glycerol. Then the fatty acids enter the bacterial cell through an assisted diffusion process facilitated by a helper protein, and the indicator rhodamine B enters the bacterial cell through simple diffusion. Fatty acids in cells will bind to rhodamine b to form complex bonds, which occur due to the reaction between cationic rhodamine b with uranyl ions from fatty acids (Carissimi et al. 2007).

The presence of lipolytic activity in the digestive tract of eel has previously been known in a study conducted by Lestari (2016) through a lipid hydrolysis test on bacteria isolated from the digestive tract of eel (*A. bicolor*). Bacteria with lipolytic activity are microbiota that can come from fish's eating habits and the living environment of eels. In their research, Taufik et al. (2017) stated that eating habits would affect enzyme activity and microbiota communities in the digestive tract. Lipolytic bacteria are producers of extracellular lipase enzymes and are classified into three types: non-specific lipase, 1,3-lipase, and specific fatty acid lipase. Lipase synthesis by lipolytic bacteria is affected by temperature, nitrogen and carbon ratio, inorganic salts, oxygen, and lipid sources such as olive oil, lard, and fatty acids. Microbiota in the gastrointestinal tract can cause lipolysis in two ways: by contributing to TAG breakdown through bacterial performance and then by altering pancreatic lipase secretion or inactivating pancreatic lipase by protease enzymes (Ray et al. 2012).

Table 1. Bacterial isolates obtained from the digestive tract of eels obtained from each fish sample

Fish sample	Number of bacterial isolates	Isolation code
Fish 1	2	SD01, SD02
	1	SD03
Fish 2	6	SD04, SD05, SD06, SD07, SD17, SD18
	9	SD08, SD09, SD10, SD11, SD12, SD13, SD14, SD15, SD16
Fish 3	6	SD19, SD20, SD21, SD22, SD23, SD24
	6	SD25, SD26, SD27, SD28, SD29, SD30
Total isolate	30	

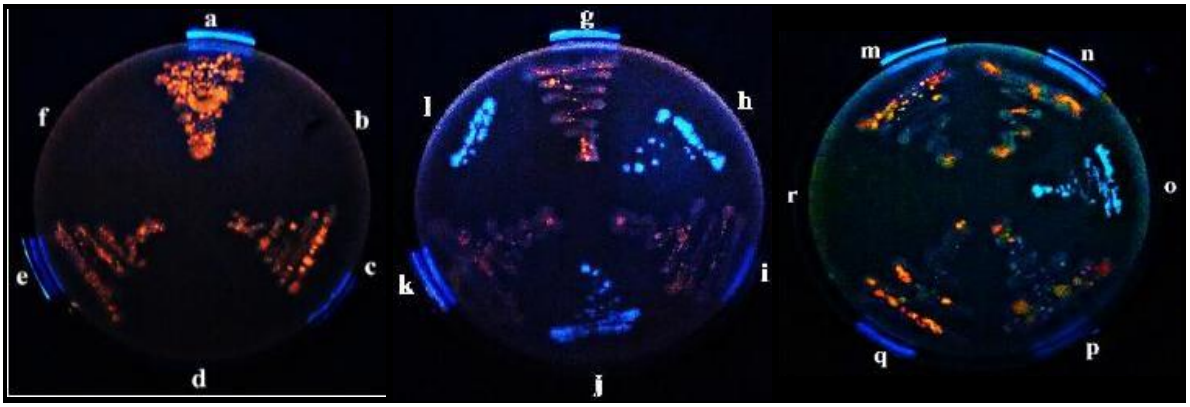

Figure 4. Colonies of lipolytic bacteria from the digestive tract of eels on Rhodamine B Agar media under UV lamp exposure of 350 nm. Bacterial isolates that had lipolytic activity were SD01 (a), SD09 (c), SD12 (e), SD13 (g), SD22 (n), SD29 (q)

Morphological characteristics of lipolytic bacteria in the digestive tract of eels

The colony and cell morphology characterized six bacterial isolates with lipolytic activity. The characteristics of bacterial colonies include the shape, color, margins, and elevation of the colonies. Bacterial cell characteristics include gram staining and cell shape (Figure 4x). The characterization can be seen in Table 2.

The physical characteristics of the lipolytic bacteria found in the digestive tract of eel revealed that the bacteria were rod-shaped and red, indicating that they were gram-negative bacteria. In the morphological characterization of the colony, the whole colony had an irregular shape, while the character of color, margin, and elevation varied in some isolates. The isolates SD01 and SD12 were yellowish-white. SD22 is beige, while the rest is white. The edges of the colonies were lobate except for isolates SD01 and SD12, which were undulate. The elevations of the colonies were flat for SD01, SD09, SD12, and SD13, whereas SD22 and SD29 had convex elevations.

Identification of lipolytic bacteria by gene sequences encoding 16S rRNA

Bacterial isolates that were detected to have lipase activity were identified molecularly based on the gene sequences encoding 16S rRNA. Lipolytic bacteria genomic DNA samples were amplified using PCR with 63F and 1387R. Marchesi et al. (1998) stated that the two primers could amplify the 16S rRNA coding gene sequence with a size of about 1300 base pairs. Based on the visualization of the PCR product (Figure 5), it can be seen that the six isolates had sizes between 1000 and 1500 bp.

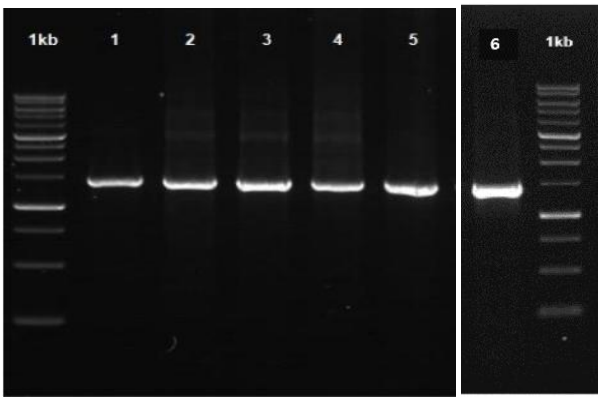

Figure 5. Electrophorogram of Lipolytic Bacteria PCR Products. Description: 1kb= Marker, 1= Isolate SD01, 2= Isolate SD09, 3= Isolate SD12, 4= Isolate SD13, 6= Isolate SD29

Table 2. Cell morphology and colony characteristics of lipolytic bacteria in the digestive tract of eels

Isolate code	Characteristics					
	Cell morphology			Colony morphology		
	Gram	Cell shape	Shape	Color	Edge	Elevation
SD01	-	Rod-shaped	Irregular	White	Undulate	Flat
SD09	-	Rod-shaped	Irregular	Yellowish white	Lobate	Flat
SD12	-	Rod-shaped	Irregular	White	Undulate	Flat
SD13	-	Rod-shaped	Irregular	Yellowish white	Lobate	Flat
SD22	-	Rod-shaped	Irregular	beige	Lobate	Convex
SD29	-	Rod-shaped	Irregular	White	Lobate	Convex

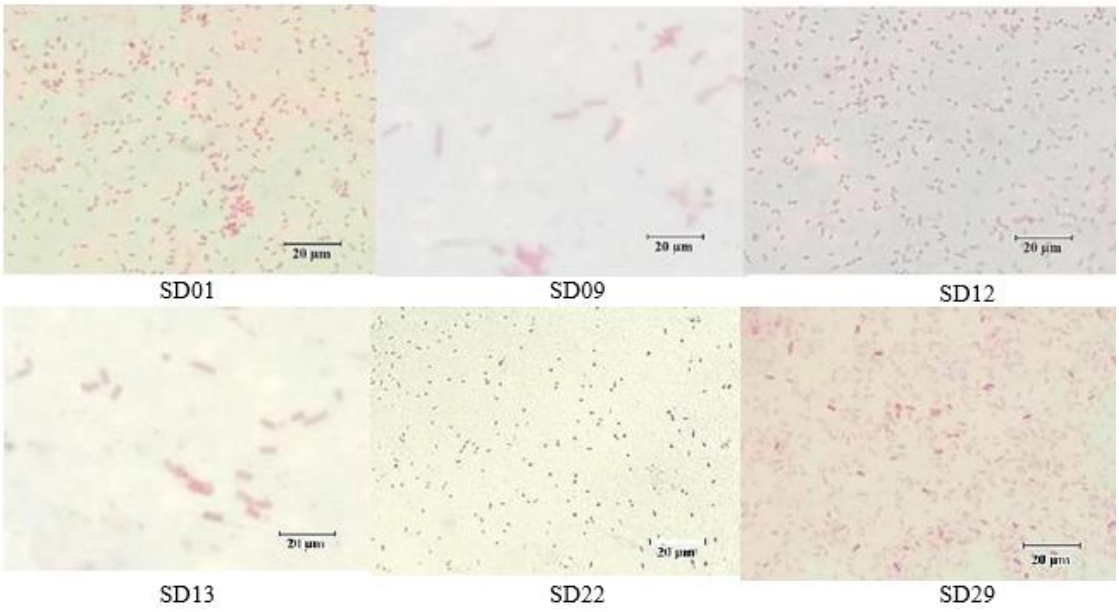

Figure 4x. Gram stain of lipolytic bacteria from the digestive tract of eel (*A. bicolor bicolor*). 1000x magnification

Table 3. BLASTN analysis of gene sequences encoding 16S rRNA for lipolytic bacteria in the digestive tract of eel

Isolate code	Identity	Query cover	Closest relatives
SD01	99%	96%	*Pseudomonas azotoformans* KGGI28
SD09	93%	92%	*Providencia vermicola* 79C1
SD12	99%	98%	*Pseudomonas azotoformans* KGGI28
SD13	94%	73%	*Providencia*
SD22	98%	92%	*Aeromonas hydrophila* M_81
SD29	99%	93%	*Aeromonas veronii* FC951

The amplicon of the 16S rRNA gene is then sequenced and will produce a sequence of nucleotide bases. The characteristics of DNA sequences were analyzed using the BLASTN program (Table 3). Janda and Abbott (2007) stated that a bacterial species is the same as the reference in GenBank if it has a minimum similarity of 97%; if the base sequence similarity is <97%, it can indicate a new species. In contrast, Hagstrom et al. (2000) stated that if the sequence of bases had a similarity of 93%-97%, it was said that the isolates had similarities at the genus level.

The BLASTN analysis that has been presented shows that of the 6 identified isolates, 4 of them had 98-99% identities, while two other isolates, namely SD09 and SD13 isolates, had 93% and 94% identities, respectively. The presence of microbiota in the digestive tract of eels in this study (Table 3) is supported by research conducted by Esteve and Garay (1991) which found much microbiota associated with *Anguilla anguilla* in freshwater, including *Aeromonas hydrophila*, *A. sobria*, *Pseudomonas azotoformans*, and *Plesiomonas shigelloides*. In his research, Huang et al. (2018) stated that the most commonly found bacteria associated with the digestive tract of *A. anguilla* eels were from the genus *Aeromonas* (52.18±23.81%). Huang et al. (2018) also stated that bacteria from the genus *Aeromonas* were more commonly found in adult eels (81.03±2.99%) than in eels in the previous phase.

The genus *Pseudomonas* has been widely known as a microorganism isolated from various natural sources, such as soil, plants, water, and aquatic animals. Fendri et al. (2010) stated that bacteria in this genus have capabilities in food technology, medicine, environmental microbiology, and natural degradation agents and produce extracellular lipase enzymes. *Pseudomonas azotoformans* is a gram-negative bacterium with rod-shaped cells measuring 0.6-0.8 x 1.4-2.0 µm, does not produce spores, is obligate aerobes, and is motile with a polar flagellum. These bacteria produce fluorescent pigments with an optimum temperature for growth in the range of 25-30º C and an optimum pH of 7 (Feliatra et al. 2004).

Research conducted by Fendri et al. (2010), *P. azotoformans* is said to be able to be used as a lipid degrading agent. This bacterium is grown in media containing 2% triacylglycerol, and it produces hydrolysis products in diacylglycerol, monoacylglycerol, and fatty acids with an optimum temperature of 30ºC and pH 6. Another study conducted by Gram et al. (2001) stated that *P. azotoformans* bacteria could play a role in suppressing the death of aquatic animals due to vibriosis in rainbow trout, and in vitro is antagonistic to *Aeromonas salmonicida*. According to research by Haba et al. (2000), the genus *Pseudomonas* is a producer of lipase enzymes with the highest lipase activity among the genera *Bacillus*, *Rhodococcus*, and *Staphylococcus*, which is 1.703 U/L, *P. azotoformans*, a lipase producer with olive oil as a substrate, has lipase activity of 4.4 units/L. L.

Providencia vermicola, belonging to the Enterobacteriaceae family, is a gram-negative bacterium. Its rod-shaped cells measure 2.14-5.0 x 0.57-0.71 µm. Colonies are round, shiny, and have a convex elevation. Bacteria species *P. vermicola* can be found as a microbiota in the digestive tract of freshwater fish (Ramkumar et al., 2014). Cultivation on NA and TSA media will produce a distinctive aroma. These bacteria can grow up to a temperature of 41ºC. *P. vermicola* can also produce acids from L-arabinose and 2-ketogluconic and L-erythritol, D-glucosamine, and D-glucuronic acid (Somvanshi et al. 2006). Tanu et al. (2012) reported that these bacteria could be isolated from the digestive tract of seahorses, and in some freshwater fish, these bacteria are pathogenic. *P. vermicola*, isolated from the digestive tract of seahorses, has a role in synthesizing extracellular lipase enzymes because seahorse feed contains a lot of unsaturated fatty acids. In his research, Bala et al. (2018) found that he could isolate *P. vermicola* bacteria from water disposal in palm oil refineries. Bala et al. (2018) also stated that the mycobacteria could be used as oil degradation agents that pollute oil refinery areas because they have lipase activity and live in areas with high lipid levels.

Aeromonas is a genus of bacteria found in aquatic areas, and several species can be found as pathogenic bacteria in aquatic animals (Ruzauskas et al., 2017). In Thenmozhi and Ahilan's (2014) research, *Aeromonas* species were isolated from *Cyprinus carpio*, namely *A. salmonicida* and *A. hydrophila*, and showed lipolytic activity. *Aeromonas salmonicida* showed lipolytic activity of 69.23%, and *A. hydrophila* showed lipolytic activity of 75%. Cahill (1990), in his research, stated that the genus *Aeromonas* is one of three bacteria other than *Pseudomonas* and *Vibrio*, which are most commonly found as microbiota in the digestive tract of freshwater fish.

Aeromonas veronii is a gram-negative, rod-shaped bacterium, ornithine decarboxylase positive, motile with polar flagella, growing at an optimum temperature of 35-37°C (Skwor et al. 2014). This bacterium is associated with leeches, found in aquatic environments and human fecal specimens, identified as a cause of disease in fish and humans by gastrointestinal tract attack (Pemberton et al. 1997). *Aeromonas veronii* is reported as a lipolytic bacterium and can produce toxins known as extracellular

products (Pramudita et al., 2013). Nawaz et al. (2010) stated that *A. veronii* has optimum lipolytic activity at 35° C and is found in the digestive tract of catfish. This bacterium can produce four different lipase enzymes that can play a role in the host infection process and damage the host cell plasma membrane.

Aeromonas hydrophila is a gram-negative, rod-shaped, facultative anaerobe, motile, resistant to tetracycline. *Aeromonas hydrophila* was isolated from fresh and marine waters, causing disease in several fish (Skwor et al. 2014). *Aeromonas hydrophila* has various sizes ranging from 1.0 to 3.5 microns in length and 0.8 to 1.0 microns in width, with the optimum temperature for growth of 28-37°C (Arwin et al. 2016). This type of bacteria is found as the main microbiota found in the digestive tract of freshwater fish. In fish cultured in ponds, this bacterium is ubiquitous due to the presence of fish feces that contaminate pond waters, which often causes disease in fish, so it can be indicated that the source of *A. hydrophila* in pond waters comes from fish feces.

Aeromonas hydrophila species were identified as decomposers of chitin in the digestive tracts of freshwater fish. Additionally, *A. hydrophila* was capable of producing extracellular enzymes capable of metabolizing cellobiose. Under anaerobic conditions with many colonies, *A. hydrophila* produced lipases with high activity (Cahill 1990). Cascon et al. (1996) stated that *A. hydrophila* could produce glycerophospholipid-cholesterol acyltransferase, which is analogous to the mammalian plasma lecithin-cholesterol acyltransferase, which is part of the lipase enzyme. Sholikhah's research (2009) stated that *A. hydrophila* produces enzymes and extracellular toxins that contain hemolytic and protease activities that cause disease in fish.

In conclusion, this study isolated 30 bacterial isolates from the digestive tract of eel (*A. bicolor bicolor*) and as many as six isolates with lipolytic activity. The identity of the lipolytic bacteria obtained from the digestive tract of eel were isolates SD01 and SD12, identified as *P. azotoformans*; isolate SD09 was identified as *P. vermicola*; SD13 was identified as *Providencia* sp.; SD22 was identified as *A. hydrophila*, and isolate SD29 was identified as *A. veronii*.

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Review: Phytochemical composition, medicinal uses and other utilization of *Nypa fruticans*

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Abstract. *Nugroho GD, Wiraatmaja MF, Pramadaningtyas PS, Febriyanti S, Liza N, Naim DM, Ulumuddin YI, Setyawan AD. 2020. Review: Phytochemical composition, medicinal uses and other utilization of* Nypa fruticans. *Bonorowo Wetlands 10: 51-65. Nypa fruticans* Wurmb or "*nipa/nipah*" is a palm plant in mangrove ecosystems along tropical and subtropical river estuaries affected by tides. Nipa has a high abundance in the mangrove forest ecosystem. Therefore, knowledge and utilization of this nipa plant need to be sought and examined more deeply to optimize its utilization. This review aims to find out the phytochemical composition, medicinal uses, and other utilization of nipa plants. The main phytochemical composition of nipa is polyphenols, phenolics, alkaloids, tannins, flavonoids, and saponins. However, other chemical compounds such as ethyl acetate, chloroform, hexane, triterpenoids, phenol hydroquinone, diterpenes, and steroids are still available. The many chemical compounds in nipa can be used as raw materials in a product, especially in modern medicines. Based on people's beliefs in several tropical countries that nipa has the potential of herbal medicine to treat fever, gout, kidney stones, energy booster, aid digestion, as a cure for certain chronic diseases and metabolic syndromes such as diabetes and hypertension, treat asthma, leprosy, tuberculosis, sick throat, liver disease, snakebite, as a pain reliever, and also be used as a sedative and able to expel excess wind in the body. Then in the pharmacological aspect, nipa has antioxidant, antidiabetic, antimicrobial (antifungal and antibacterial), anticancer, anti-inflammatory, antinociceptive, antihyperglycemic and analgesic activities. In addition to other benefits outside of drugs, nipa is used as a roof for houses, cattle pens, huts in the garden, broomsticks, handicrafts, fishing gear, food and drink sources, and sources of renewable fuel.

Keywords: Mangrove plant, medicine, *Nypa fruticans*, phytochemical, utilization

INTRODUCTION

Mangroves consist of trees, shrubs, palms, or ferns found above sea level in the intertidal zone (Duke et al. 1998; Romañach et al. 2018). Mangroves dominate intertidal sedimentary habitats in the tropics and subtropics. Most mangroves are associated with soft silty sediments on sheltered tropical beaches such as bays, estuaries, and lagoons. Then, unlike other terrestrial or aquatic ecosystems, mangrove ecosystems are unique because they exist in terrestrial, freshwater, and marine environments. Also, mangrove ecosystems are regularly inundated by tides, watered by freshwater, and most of the time inundated by water (Giri et al., 2015; Xaverius et al., 2019; 2020).

Mangroves are one of the most productive ecosystems on earth. Mangroves are one of the natural resources that have various functions and benefits in terms of ecological and economic aspects. The ecological function of mangroves can be seen from the physical, chemical, and biological aspects (Nyangon et al., 2019). Mangroves are a renewable resource that provides food, shelter, and habitat for various terrestrial and marine fauna (Okugbo et al., 2012; Irwansyah et al., 2021). In addition, various types of products derived from various flora and fauna as well as environmental services, such as effective sediment trapping, shoreline protection from erosion, seawater intrusion control, nutrient recycling, reduction of sea wave height and speed, cleaning of water pollutants, and other things that are very important to support human life (Estoque et al. 2018; Kusmana 2018). The primary productivity of mangroves can exceed tropical terrestrial forests (Alongi, 2014), and their carbon storage is greater than terrestrial forests on a per unit area basis (Donato et al., 2011; Mahli et al., 2011). Although mangrove forests account for only 0.5% of global coastal areas, they can

absorb 14% of the carbon in coastal oceans (Alongi 2014). In addition, mangrove forests also offer many necessities and goods such as food, firewood, and wood (Wulan et al. 2021) and have aesthetic, historical, and cultural values (Grasso 2000).

Mangroves are commonly found along the coasts of tropical and sub-tropical developing countries where coastal populations rely heavily on these forests for their livelihoods. According to Kusmana (2018), mangrove forests currently grow in 124 tropical and subtropical countries with around 15.2 million ha areas, of which about 36% (5.4 million ha) grow in Southeast Asian countries. Most of the mangrove forest in Southeast Asia grows in Indonesia; the area is currently around 3.2 million ha (59% of the mangrove forest in Southeast Asia, or 21% of the mangrove forest in the world). The Southeast Asia region, especially Indonesia, has extensive mangrove forests and many mangrove species.

Centuries ago, coastal communities traditionally used various species of mangrove plants for food, clothing, shelter, and traditional medicines. Several plant species in mangrove ecosystems have the potential to be utilized by humans in daily life, such as *Rhizophora mucronata*, *R. apiculata*, *R. stylosa*, *R. racemosa*, *R. harrisonii*, *R. mangle*, *Avicennia marina*, *A. alba*, *Bruguiera cylindrica*, and *Sonneratia* spp. These species also contain phytochemicals or secondary metabolites that can be used in ethnomedicine to treat various diseases and play an important role in modern medicine. Some of the phytochemicals present in mangroves are steroids, triterpenes, saponins, flavonoids, polyphenols, alkaloids, tannins, fatty acids, resins, and phenols (Mani et al. 2012; Soonthornchareonnon et al. 2012; Rengasamy et al. 2013; Subhashini et al. 2013; Yadav et al. 2014; Genilar et al. 2021). This species is very potential because the mangrove ecosystem is always under pressure, leading to the production of certain compounds for its survival (Sasidhar 2020). Then, mangrove plant species can also contain toxic substances that exhibit biological activities such as antifungal, antibacterial, and pesticide properties (Azman et al., 2015). Extracts from mangrove plants are reported to have various medicinal properties such as antibacterial, anthelmintic, antidiabetic, antioxidant, antifungal, antimicrobial, anti-inflammatory, anticancer, analgesics, and others (Neamsuvan et al. 2012; Prabhu and Guruvayoorappan 2012; Yang et al. 2015; Das et al. 2016; Reddy and Grace 2016; Sachithanandam et al. 2019).

Various diseases can be cured using plants that live in the mangrove ecosystem, such as angina, diarrhea, dysentery, hematuria, flatulence, epilepsy, smallpox, diabetes, asthma, rheumatism, and stomach pain, malaria, cholera, hepatitis, cancer, ulcers, nausea, vomiting, stop bleeding from wounds, hemostatics, AIDS, ulcers, fever, leprosy, minor injuries, plaster fractures, tuberculosis, elephantiasis, hematoma, cough, diabetes, eye infections, gastrointestinal diseases, headaches, insect stings, animal bites, liver disease, etc. (Prabhakaran et al. 2012; Shilpi et al. 2012; Arumugam et al. 2014; Revathi et al. 2014; Manilal et al. 2015; Vinoth et al. 2019; Rajani et al. 2020).

In addition to the plants mentioned above, one of the plants that also lives in the mangrove ecosystem and has the potential to contain chemical compounds to treat various diseases and other benefits is the *nipa/nipah* plant (*Nypa fruticans* Wurmb). Then, parts of nipa commonly used by the public include roots, rhizomes, leaves, leaf bones, sap, fruit, and seeds. In addition, nipa provides livelihoods for the community, such as providing food sources and building materials. Nipa plants are the only palms and the oldest globally that grow in coastal areas and along estuary flows affected by tides (Gee 1989; Badve and Sakurkar 2003; Robertson et al. 2020). Nipa plants are the dominant plant species in mangroves in many tropical and subtropical areas, covering large coastal areas with low salinity and deposition of sediments (Hossain and Islam 2015). This plant was also introduced to river mouths in West Africa and Panama (Numbere 2018).

Materials derived from nipa are used to build and produce fuels, sugar, beverages, and pharmaceuticals in Southeast Asia and Oceania (Tsuji et al., 2011; Yahaya et al., 2021). Nipa forests can serve as an effective barrier against damage from tsunamis (Hossain and Islam, 2015). Nipa plants can also contribute significantly to the carbon budget in coastal ecosystems (Ghani et al., 2017). Furthermore, significant literature on livestock and the economy supports the sustainable use of nipa resources throughout the Indo Pacific Ocean region (Carandang et al., 2009).

Although, nipa forest plantations are often used for human use, such as for commercial and industrial use (Akpakpan et al. 2012) or land clearing to support aquaculture and subsistence agriculture (Veettil et al. 2018; Arifanti et al. 2019). However, nipa continues to grow rapidly and predominantly in estuarine areas, covering tens of thousands of hectares in the lower salinity areas of major river deltas (Robertson et al. 1991). Therefore, knowledge and utilization of this nipa plant need to be sought and studied more deeply. Therefore, this review aims to find out the phytochemical composition, medicinal uses, and other utilization of nipa plants.

MANGROVE FOREST PLANT: *Nypa fruticans*

Local name

Nipa is a type of palm that has been known as a plant with various uses and benefits by people in various regions of the world, especially coastal areas of the tropics and subtropics. Therefore, this plant also has various local names that differ in each region, such as *poothada* (Andaman Islands); *rola*, *ki-bano*, *tacannapoon* (Austalia); *golpata*, *nipa palm* (Bangladesh); *dani* (Myanmar); *shui ye* (China); *gabna gulag*, *nipumu* (India); *nipa* (Malaysia); *chickenatangh*, *ayangmbakara* (Nigeria); *biri -biri* (Papua New Guinea); *anipa*, *pinok*, *tata*, *pinóg*, *sasa*, *saga*, *nipa*, *lasá*, *pauid*, *pawid* (Philippines); *attap palm* (Singapore); *gim-pol* (Sri Lanka); *chaak*, *lukchaak*, atta (Thai); *dừa nước*, *dùa lá* (Vietnam) or commonly called nipa palm (or simply nipa) or mangrove palm (Baja-Lapis et al. 2004; Lim 2011; GRIN 2017). Meanwhile, this plant is also

known by many names in various regions, communities, tribes, or islands in Indonesia, among others, as in Sundanese it is *daon, daonan*; Javanese or Balinese people call it *buyuk*; *bhunyok* by the Madurese; *bobo* or *boho* by the people of Manado, Ternate and Tidore; The people of Halmahera call it *boboro*; then in the area of Seram Island, Ambon and its surroundings it is commonly called *palean, palenei, pelene, pulene, puleanu, pulenu, puleno, pureno, parinan, parenga, parena* (Heyne 1987; Crawfurd 2017).

Description

Nipa consists of roots, stems/rhizomes, leaves, flowers, fruits, and seeds. Unlike most palms, nipa stems grow underground and are submerged in water. Only the leaves and flower stalks grow upward above the surface so that the nipa looks trunkless. The bark of this nipa plant has a very hard green texture and will turn brown when the nipa condition is old. However, the inside remains softer like a cork. According to Tomlinson (1986), the nipa is a monoecious and pleonanthic palm. It also exhibits viviparous germination, as in many other mangrove plant species. The stature and some organs of the nipa can be seen in Figure 1.

Roots. Its fibrous roots can reach a length of 13 m. Because the nipa roots are only located in unstable soil/sand/mud, the nipa clumps can be washed away by water to the sea.

Trunks. Like the *Metroxylon* spp. tree, the nipa trunk spreads along the ground, forming a rhizome about 60 cm thick, which is submerged by a layer of mud and water and can reach a length of about half a meter.

Leaves. From the rhizome emerges compound pinnate leaves typical of palms. The leaves are erect or nearly erect, rising 7 to 10 m above the ground. The leaf blade has a stocky petiole which is round at the base, 1-1.5 m long, green, and will turn brown when the condition of the nipa plant is old. The leaflets are ribbon-shaped elongated, and tapered at the end, have a leaf bone called a stick (as in coconut leaves). Leaflets can reach 60-130 cm long and 5-8 cm wide. The old nipa leaves are green, while the young leaves are yellow (shiny on the top surface), resembling coconut leaves. The number of leaflets in each stalk reaches 25-100 strands.

Inflorescences. Compound wreaths appear in the axils of sturdy leaves, 1-2 m long, single, male and female flowers are separate with female flowers collected at the end to form a ball (round head) with a diameter of about 25-30 cm, and male flowers are arranged in panicles. Strand-like, red, orange, or yellow on the underlying branches. Each strand has 4-5 male flowers that reach 5 cm in length.

Flowers. Male nipa flowers are yellowish red, protected by a flower sheath, but the pollen-filled part is still sticking out. Then, the female nipa flowers are round. The length of the flower stalk reaches 100-170 cm. These flower bunches can be tapped to take the juice. Four to five months after the release of nipa flowers, the flower bunches can be tapped because the amount of sap produced is maximum.

Fruits. The fruit structure is similar to a coconut, with a smooth exocarp, a fibrous mesocarp, and a hard endocarp called a shell. Type of stone fruit, oval, upside down and flattened with 2-3 ribs, reddish-brown, 11 x 13 cm, collected in tight groups resembling a ball, with a diameter of about 30-45 cm. In one bunch, the fruit can reach between 30 and 50 grains. The ripe fruit falls into the water and floats with the ebb and flow of water until it gets stuck in a new growing place. Often the fruit has germinated while still being carried by the current to a new place.

Seeds. Seeds are protected by a shell, white, egg-shaped, measuring about 5 x 4 cm.

Habitat and distribution

Mangrove is an ecosystem term that refers to the diverse collection of trees and shrubs that form the dominant plant community in tidal wetlands near the seafront along sheltered tropical and subtropical coasts. Nipa is one of the mangrove plants that grow in that place with a temperature of 20-35^{0}C and a rainfall of 1,000 mm/month, evenly distributed throughout the year. This is the only palm (Arecaceae) that is able to live and adapt in the mangrove ecosystem. The genus *Nypa* and the subfamily Nypoideae are monotypic taxa because nipa is the only members (Dowe 2010). They are common on beaches and rivers flowing into the Indian and Pacific Oceans, from India to the Pacific Islands. This plant is a native species from China (Hainan); South Asia, such as Sri Lanka and Bangladesh; Southeast Asia, such as Indonesia, Malaysia, Thailand, Myanmar, Cambodia, Singapore, Vietnam, and the Philippines; Australia (Queensland and Northern Territory); and Pacific Islands such as Solomon, Mariana, Bismarck Islands, New Guinea, and the Caroline Islands. Later, it was also reported that this species was introduced to several regions and countries, such as Cameroon, Guyana, Marianas, Nigeria, Panamá, Society Islands, and Trinidad-Tobago (POWO 2022) (Figure 2).

Nipa plants can grow in soft mud and slow-moving tides, and river water that carries the nutrients these plants need. Usually, nipa can grow by forming its stand, but it grows mixed with other mangrove tree species in some areas. Nipa is one species that is best adapted to growing in coastal mangrove areas with moderate and less extreme salt content. According to Setyawan (2005) and Theerawitaya et al. (2014), this plant will suffer if exposed to pure seawater and prefer brackish waters at river mouths. This species can live in bays, tidal plains, and creeks as long as high tides and freshwater flow. They can be found inland, as far as the tide can store the seeds of this plant. Nipa can withstand short-term drying in its environment. The rhizome that creeps horizontally along the riverbank can stabilize the soil and prevent soil erosion. Then, new leaves can emerge quickly after damage and protect from storm winds, and can be used to produce useful products for local residents.

Figure 1. Some parts of *Nypa fruticans*. A. Flower (a. Female, b. Male); B. Fruit (insert: individual fruits); C. Tree stature with roots and rhizomes submerged in water and leaves standing upright above the water (Photo by YIU)

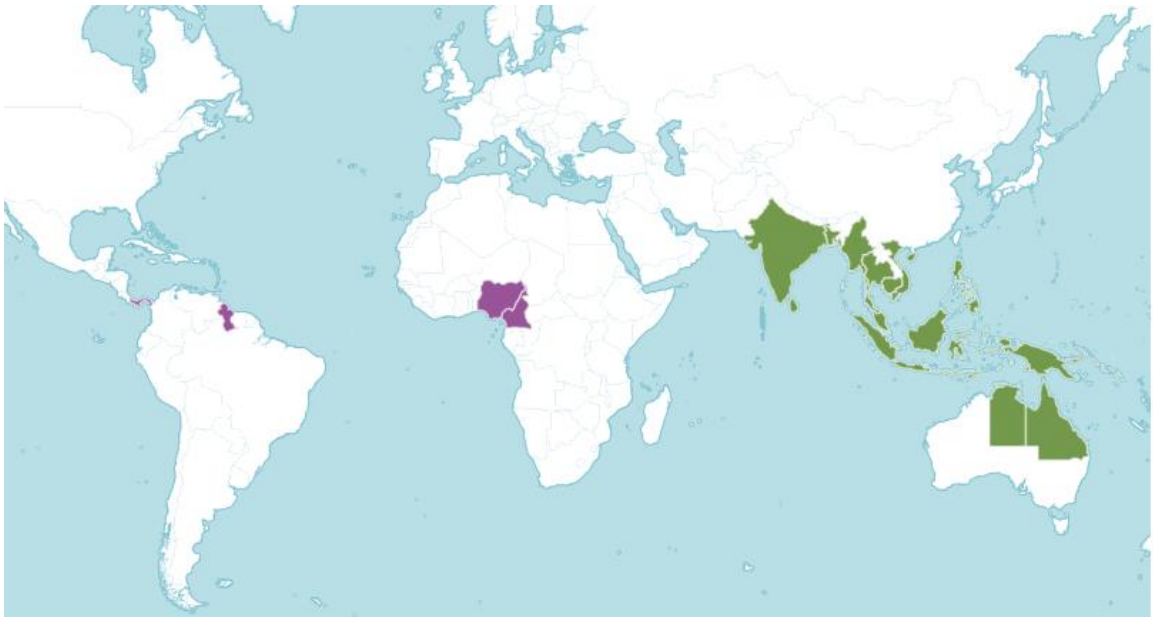

Figure 2. Distribution of *Nypa fruticans* in the world. Green borders: Natively found in China (Hainan); South Asia such as Sri Lanka, India, and Bangladesh; Southeast Asia such as Indonesia, Malaysia, Thailand, Myanmar, Cambodia, Singapore, Vietnam, and the Philippines; Australia (Queensland and Northern Territory); and Pacific Islands such as Solomon Islands, Mariana, Bismarck Islands, New Guinea, and the Carolinas. Purple borders: Introduced to Cameroon, Guyana, Marianas, Nigeria, Panamá, Society Islands, Trinidad-Tobago (Source: Plants of the World Online 2021)

PHYTOCHEMICAL COMPOSITION

The phytochemical analysis is one way to determine the presence of chemical compounds (primary and secondary metabolites) in plants (Hayne 1987). Primary metabolite products are compounds that can be used to fulfill plant life itself, either helping the plant grow, develop, or survive climate change. Meanwhile, secondary metabolite products are compounds that can protect plants from disturbances and pathogens in the vicinity to continue to grow properly. These secondary metabolite products are also often referred to as bioactive.

All plants can be found in the phytochemical analysis, including nipa. Nipa plant is a palm-shaped mangrove plant that can produce secondary metabolites with various biological properties and activities. This can be caused by the extreme conditions in which it grows. As also stated by Boopathy and Kathiresan (2010) that plants that live near the sea and around the coast contain more and more unique bioactive compounds than plants that live on land. The reason is that the environmental conditions in which they live are extremely extreme. Many external disturbances need to be faced, such as wind, waves, water currents, salinity, etc.

At least 25 chemical compounds were detected by Azuma et al. (2002) on nipa. These chemical compounds consist of fatty acid derivatives, terpenoids, carotenoid derivatives, benzenoids, and several unknown compounds. In addition, Choi et al. (2020) have shown in their research that nipa has the potential to reduce UVB-induced photoaging. Furthermore, the phytochemical analysis also found protocatechuic acid, catechin, chlorogenic acid, epicatechin, kaempferol, and pengxianencins in nipa. In vivo studies by Zhao et al. (2012) and Al-Numair et al. (2015) suggest that phytonutrients such as kaempferol may be important in protecting biological systems from oxidative stress. Meanwhile, in general, the active compounds produced by nipa include phenolics, saponins, flavonoids, and tannins (Sahoo et al., 2012; Astuti et al., 2020). The many chemical compounds in nipa can be used as raw materials in a product, especially in modern medicines. Therefore, by knowing the content of chemical compounds through phytochemical analysis, the utilization of nipa can be optimized and can be a reference and innovation in the utilization of mangrove ecosystems.

Phytochemicals in roots

Nipa roots contain alkaloids, steroids, triterpenoids, phenolics, flavonoids, and tannins (Radam and Purnamasari 2017). Still, research by Radam and Purnamasari (2017), through testing nipa root solutions with various treatments, the results obtained that the presence of alkaloids in nipa roots was characterized by the formation of a white precipitate in the test solution after being reacted or added with reagents (Wagner's reagent, Meyer's reagent, and Dragendof's reagent). Then, the presence of steroid content was marked by a color change to green in the solution when tested. Then, the presence of triterpenoids was indicated by a change in color to bluish in the test solution. Meanwhile, the presence of flavonoid compounds in this test was marked by a red-orange to black color change in the test solution. This content indicates antioxidant activity as a defensive metabolite capable of fighting reactive oxygen (Lovly and Marlee 2018). Similar results were also shown in Yusoff et al.'s (2015a) study, which showed that nipa extract contained phenolic and flavonoid compounds. Furthermore, the presence of hydroxyl indicates phenolic compounds (-OH) functional groups (Park et al. 2013) and carboxylic acids (COOH) (Ha et al. 2012), which are structurally similar and are very well used as antioxidants (Zhao et al. 2014).

Phytochemicals in leaves

According to several studies, nipa leaves contain phytochemicals. To test the phytochemicals of nipa leaves, the leaves must first be extracted. Then the extract was experimented with to determine what content was contained in the nipa leaves. Based on research that has been done, nipa leaves contain ethyl acetate, chloroform, and hexane extracts (Lovly and Marlee 2018). The ethyl acetate extract of nipa leaves has a very strong antioxidant activity. Bakshi and Chaudhuri (2014) research found that methanol, ethyl acetate, and acetone in nipa leaves showed antibacterial activity against *Escherichia coli*, *Agrobacterium tumefaciens*, *Streptococcus mutans*, and *Staphylococcus aureus.* Osbor et al. (2008) said that nipa leaves contain polyphenols and alkaloids. Similar results were also shown by research conducted by Lestari et al. (2017), which showed that positive nipa extract and leaf fraction contained natural phytochemical compounds. The crude extract contains polyphenols, flavonoids, triterpenoids/steroids, saponins, and alkaloids. The methanol fraction contains polyphenols, flavonoids, saponins, and alkaloids, while the ethyl acetate and n-hexane fractions contain triterpenoids/steroids.

In addition, the results of research by Ebana et al. (2015) reported that nipa leaf samples in the Niger Delta Region of Nigeria revealed the presence of alkaloids and polyphenols. Then, the proximate analysis carried out also showed that the plant was very rich in ash, lignin, cellulose, hemicellulose, moisture, and nitrogen (Ekpunobi and Onuegbu 2012). Then, from research by Gazali and Nufus (2019), it was shown that nipa leaves contain almost all bioactive compounds; antioxidants, namely flavonoids, phenolics, tannins, saponins, steroids, and triterpenoids. Then, the intensity of the phenolic test precipitate was higher than the other components. The phenolic and flavonoid compounds in the sample indicated that the sample's activity had potential as an antioxidant. This is also reinforced by Imra et al. (2016) report that nipa leaf extract contains active chemical compounds, including flavonoids, tannins, phenol hydroquinone, diterpenes, steroids, and saponins. From all the research results that have been mentioned, the main ingredients in nipa leaves are polyphenols, phenolics, alkaloids, tannins, flavonoids, and saponins. However, the nipa leaf extract contains other chemical compounds such as ethyl acetate, chloroform, hexane, triterpenoids, phenol hydroquinone, diterpenes, and steroids. Nipa leaves also have methanol extract and have been shown to have antidiabetic and analgesic effects by research by Reza et al. (2011).

Phytochemicals in fruit

Nipa contains fruit that can be tapped to produce abundant sap called nipa palm sap (NPS) (Hafizi et al., 2018). NPS is also known as a source of traditional medicine used to treat various diseases. Research by Yahaya et al. (2021) identified good antiradical activity in NPS with an IC_{50} value of 33.36 g/mL using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test. DPPH radical scavenging assay, FRAP assay, and CUPRAC assay were performed to determine the anti-free radical activity in nipa (Islam et al. 2020a). The NPS tested consisted of water content (72.44%), ash (1.04%), protein (7.04%), carbohydrates (19.48%), fat (0%), and energy levels (106 kcal). The large protein content in NPS shows that it can be used as a protein supplement for muscle development and maintaining the health of the human body (Wakili et al. 2015) and with a fat content of 0%, indicating that NPS is a food source with low cholesterol levels and is healthy (Olalude et al. al. 2015). In addition, maleic acid, cinnamic acid, chlorogenic acid, and kaempferol are the main compounds revealed by ultra-high-performance liquid chromatography. Overall, NPS is a potential source of

antioxidants with significant health benefits. Then according to Gordon et al. (2012), Prasad et al. (2013), Sukairi et al. (2019), and Phetrit et al. (2020), NPS showed high phenolic and flavonoid content, as well as antioxidant activity. In addition, the fruit is also rich in carbohydrates, fiber, minerals, sucrose, glucose, fructose, and vitamin A (Osabor et al., 2008; Saithong et al., 2019).

Other parts of the fruit, especially the fruit's skin, also found other biochemical compounds. The skin of nipa fruit is like coconut but has a smaller size and has fewer skin fibers than coconut. The skin of the fruit is then investigated to determine the phytochemical content. The phytochemical content in the nipa fruit peel was investigated by Herfayati et al. (2020), who obtained the results that the skin of nipa fruit contains anthocyanins. This is indicated by a change in the color of the solution from red to blue during the test. The red color is lost due to the high pH of the solution (Khoo et al., 2017), so the anthocyanins lose their red color due to the formation of quinoidal anones (Rajesh et al., 2014; Utami et al., 2016; Saptarini and Herawati 2018). In addition, nipa also contains polyphenols (Osabor et al. 2008). Polyphenols are one of the chemical compounds that are classified as natural antioxidants found in plants (Putri et al. 2015).

Besides conducting phytochemical analysis on nipa organs, nipa plants can also be tested for their phytochemical content through their product, namely flour from the fruit. The results obtained the average nutritional value of nipa flour, namely the average water content of 5.57%, ash content of 2.35%, protein 4.23, carbohydrates 52.14%, crude fiber 24.14%, fat 1.06%, the calorific value is 226.29 cal/100 g, zinc 17.13 mg/kg and iron 405.32 mg/kg (Radam et al. 2019). So, it can be said that the phytochemical content in nipa flour is very diverse. The content of compounds in nipa plants is influenced by the content of chemical compounds, heavy metals in media water, and the environment as a place to live (Nafieet al. 2019).

From the results of phytochemical studies on various nipa organs, various contents and benefits of nipa are produced to be utilized optimally; many more studies have been carried out and processed with the most appropriate method for human needs in the future.

MEDICINAL USE

Traditional medicinal use

Human awareness of health is also increasing; medicinal products with natural ingredients are preferred because they are believed to be cheaper, easier to obtain, and will not cause prolonged effects on the body in the future. Some medicines derived from nature or herbs have been passed down and passed down from generation to generation and have become the main choice of the community (Az-Zhara et al., 2021), one of which is drugs derived from nipa.

Coastal communities have realized the abundance of nipa plants. People have long used nipa to be processed into various traditional medicines. This is also supported by Gazali and Nufus's (2019) statement, which states that mangroves, including nipa, are plants that have millions of benefits, which can be processed into various kinds of support for the life needs of coastal communities. Almost all parts of the nipa plant can be processed and used further in herbal medicines.

Young shoots, wood, roots, or nipa leaves are sometimes used for medicinal purposes (Tsuji et al., 2011). Then according to Bandaranayake (1998) and Rahmatullah et al. (2010), traditionally, the leaves, stems, and roots of nipa are used to treat asthma, leprosy, tuberculosis, sore throat, liver disease, snake bites, as a pain reliever, and can also be used as a sedative and able to expel excess wind in the body.

Vinegar from nipa, locally known as nipa palm vinegar (NPV), is a traditional preparation produced by the fermentation of sap. It is commonly consumed throughout East Asia (Päiväke et al. 1984). Added to drinking water, NPV is taken before meals and at bedtime. It has been shown that consumption of nipa vinegar reduces postprandial hyperglycemia in type 2 diabetic patients who receive a diet with a moderate glycemic index (Liatis et al. 2010). Likewise, the local community in Malaysia considers that nipa vinegar can treat diabetes, and this is scientifically justified by the research of Yusoff et al. (2015b) that vinegar exerts an anti-diabetic effect by delaying the absorption of carbohydrates from the small intestine through selective inhibition of intestinal glucose transporters, thereby suppressing postprandial hyperglycemia. Then, in the research of Yusoff et al. (2015a), chronic administration of NPV aqueous extract at a dose of 1,000 mg/kg caused a significant effect of lowering blood glucose and increasing insulin in diabetic rats.

In some countries, based on public belief from Malaysia, Philippines, Indonesia, Thailand, Sri Lanka, and India that NPS has medicinal potential to treat fever, gout, kidney stones, energy booster, helps the digestive process, and a cure for certain chronic diseases and metabolic syndromes such as diabetes and hypertension (Tamunaidu et al. 2013; Yusoff et al. 2015a; Hafizi et al. 2018; Sukairi et al. 2019; Phetit et al. 2020).

Then, there are some uses of nipa in other traditional medicine. According to Burkill (1935), Päiväke (1996), and Lim (2011), one of the traditional recipes in Malaysia to treat herpes is to drink the juice of young shoots with coconut milk. In other countries, such as Patuakhali and Barguna District of Southern Bangladesh, ash from burned nipa plants is used to treat toothaches and headaches (Islam et al. 2020b). In the Philippines, a decoction of fresh leaves of nipa is used as a lotion for boils. Fresh leaves of nipa are widely used to treat ulcers in the form of cataplasm or lotion. Nipa alcohol is used as an eyewash for inflammation of the eyelids.

Meanwhile, in Indonesia, the coastal community of Banyuasin, South Sumatra, has processed nipa as a medicine for stomach pain, diabetes, and fever-reducing medicine. The same thing can also be found in Aceh, where people use nipa leaf extract as medicine for canker sores and toothaches. Meanwhile, the people of Kalimantan use

leaf ash and nipa roots as medicine for toothaches and headaches. Other parts of nipa are also used to treat abdominal pain, diabetes mellitus, fever, and canker sores (Imra et al., 2016). The bones of nipa leaves have also been traditionally used for a long time by the coastal communities of Kakap River, West Kalimantan, as a toothache medicine. The young leaves are used as a thrush medicine (Lestari et al. 2016). Then the ashes from the leaves and roots are burned to produce salt ash called salt nipa, which is used for headaches and toothaches in Kalimantan (Lim 2011). Although the target disease is not described, nipa wood can potentially be used as medicine in Borneo (Kalimantan) (Burkill 1935).

Mineral content as medicine

Research conducted by Tamunaidu and Saka (2011) revealed that the nipa fruit contains eleven minerals that are good for use by the body. Three of the most abundantly contained minerals in nipa fruit are potassium, sodium, and magnesium. These results are also strengthened by Herman et al.'s (2011) research that the mineral content of magnesium, sodium, and potassium in nipa fruit reached 7.97 ppm, 9.35 ppm, and 3.79 ppm, respectively. Furthermore, potassium reduces the risk of cardiovascular disease and stroke and protects the body from bone loss (Weaver et al., 2018; Sun and Weaver, 2020). Meanwhile, according to Strazzullo and Leclercq (2014), sodium plays an important role in regulating cell homeostasis, body fluid, electrolyte balance, and blood pressure. Lastly, magnesium is also known to have an important role in the body. Whereas magnesium can stimulate ATPase reuptake of calcium from the sarcoplasmic reticulum and modulate insulin signal transduction and cell proliferation, it is important for cell adhesion and transmembrane transport (Jahnen-Dechent and Ketteler 2012). However, the consumption of nipa must also be in accordance with the body's daily needs. Minerals contained in nipa, if consumed too much, will produce effects that are not good for the body.

Fiber content as a cholesterol drug

Furthermore, nipa is also known to have a fairly high fiber content. Fiber is a type of carbohydrate that is resistant to digestion and absorption in the human small intestine. Instead, fiber generally undergoes partial or complete fermentation in the human large intestine (Santoso 2011). The benefit of fiber for the human body is that it can prevent hypercholesterolemia in humans who are overweight or obese (Fairudz and Nisa 2015). In addition, Santoso (2011) also revealed that fiber could be used as an agent for controlling diabetes, cancer, and cardiovascular disease. Research conducted by Dalming et al. (2018) revealed that nipa fruit contains 46.18% crude fiber, which can bind cholesterol in vitro. Then, cholesterol is adsorbed on the nipa fruit flour to allow cholesterol to be lowered or suppressed. Another possibility is that the fiber component of nipa fruit can stimulate the excretion of cholesterol in the body by binding to bile acids in relation to the satiety effect produced by nipa fruit (Dalming et al. (2018).

Pharmacological properties

There is still a lot of knowledge of plants or animals that can be trusted to cure diseases by indigenous peoples. The search for bioactive compounds from mangrove plants in pharmacology is still neglected, although many studies have proven that mangrove plants can be a good source of natural medicine (Das et al., 2015). Climatic conditions and rampant pollution in the world cause an increase in the risk of disease suffered by humans. Various types of diseases continue to infect humans, both old and diseases that have only emerged recently. Such conditions encourage researchers to look for mechanical and medicinal treatments from existing natural resources to grow up the world of pharmacology.

One of the natural resources that can be used as medicine for many years is the mangrove plant. This aligns with Mahmud et al.'s (2014) statement that mangrove plants have been shown to contain phytochemicals that have significant antioxidant, antinociceptive, antihyperglycemic, antimicrobial, and anticancer activities. Further research on one of the plants in the mangrove ecosystem, the phytochemical content of nipa, has also been carried out.

From studies that have been carried out in various countries, it is found that nipa is full of benefits for the human body. Some of these benefits include being an antioxidant (Sabri et al. 2018; Shin et al. 2018), antibacterial (Nopiyanti et al. 2016), anti-inflammatory (Bae and Jung 2016), a stimulator of insulin secretion (Yusoff et al. 2017), and as cytoprotective (Sari et al. 2018). Then it was also supported by the positive results of the antioxidant activity test on nipa parts, such as leaf, stem, bark, and root extracts (Abdel-Aziz et al. 2016).

The results of other studies have also been carried out, such as the results of research by Reza et al. (2011), which revealed that there were antidiabetic and analgesic activities in methanol extracts of twigs and leaves of nipa plants. Reza et al. (2011) also stated that nipa leaf extract could stimulate the residual function of pancreatic cells, produce an antihyperglycemic effect through the extrapancreatic mechanism, and increase peripheral glucose utilization. Likewise, the research of Yusoff et al. (2015b) was conducted to evaluate the effect of aqueous extract (AE) on postprandial hyperglycemia to understand the mechanism of antidiabetic action. In vitro intestinal glucose absorption, in vivo carbohydrate tolerance test, AE was tested, and spectrophotometric enzyme inhibition test. One mg/mL AE showed comparable results to phloridzin (1 mM) in vitro. It delayed glucose absorption through the isolated rat jejunum and was more effective than acarbose (1 mg/mL). Later, in vivo confirmatory tests showed AE (500 mg/kg) caused significant suppression of 30 min postprandial hyperglycemia in mice. In contrast, AE showed somewhat weak inhibitory activity against -glucosidase and -amylase compared to acarbose in the spectrophotometric enzymatic assay. These findings suggest that NPV exerts an anti-diabetic effect by delaying carbohydrate absorption from the small intestine through selective inhibition of intestinal glucose transporters, thereby suppressing postprandial hyperglycemia.

Furthermore, Bae and Jung (2016) stated that nipa fruit extract could inhibit the production of nitrite and pro-inflammatory cytokines at a dose of 200 g/mL. Then Kang and Hyun (2020) revealed research results showing that nipa leaf extract with a 500 mg/kg bodyweight concentration could reduce the expression of TRPV1, COX 2, inflammatory and pro-inflammatory expression in experimental rats. These studies are also supported by Khairi et al. (2021) research, who revealed that nipa's fruit and leaf extract showed enormous potential to be further developed into drugs or inflammatory inhibitory agents. Other pharmacological activities of nipa, such as antinociceptive and anticancer, have been revealed in various scientific studies (Yusoff et al. 2015a; Kang and Hyun 2020). Other compounds found contained in nipa in fruit, leaves, and stems were polyisoprenoid compounds. According to Sari et al. (2018) and Istiqomah et al. (2020), polyisoprenoid compounds from nipa showed anticancer activity in vitro. They could reduce cell proliferation and induce apoptosis in colon cancer cells.

Nipa exhibits a variety of pharmacological activities, including antioxidant, anti-inflammatory, antibacterial, antidiabetic, and analgesic activities. This plant's traditional herbal medicines are associated with no side effects, toxicity, and increased efficacy and safety. The following potencies are presented in more detail regarding the pharmacological activity of nipa as an antioxidant, antibacterial, and anti-inflammatory.

Antioxidant

Natural antioxidants from plants have gained considerable scientific interest due to their natural origin and lower adverse side effects (Lourenço et al., 2019). There is also increasing public consumption of exogenous antioxidants, such as ascorbic acid (i.e., Vitamin C), network tocopherols (i.e., Vitamin E), carotenoids, and polyphenols found in fruits, vegetables, cereals, beverages, and other natural food products. This is due to the belief that these products can support the antioxidant defense system (Lourenço et al., 2019). In addition, mangrove plants tend to have more compounds that can act as antioxidants than the activity of other compounds (Rahman 2018).

A study by Blanch et al. (2020) reported that the salicylic acid present in nipa could reduce the high salt stress experienced by nipa in the environment by increasing the activity of the antioxidant system. Then according to Choi et al. (2022), chemical compositions including phenolic acids and flavonoids can be found in nipa. Moreover, PCA shows the high relevance of each antioxidant potential. This finding can be used for wide commercial applications in the food and pharmaceutical industry for nipa.

Furthermore, the phenolic compounds, alkaloids, and flavonoids found in nipa have also been shown to have antioxidant properties (Gordon et al., 2012; Manojlovic et al., 2012; Soonthornchareonnon et al., 2012; Maqsood and Benjakul 2013; Sharief et al. 2014). According to Margaretta and Handayani (2011), these compounds are indeed proven to be compounds that can act as antioxidants. Antioxidants can counteract free radicals and autoxidation reactions in lipid oxidation. In biological systems, phenolic compounds and flavonoids act as free radical scavenging agents with antioxidant activity (Lourenço et al., 2019). Therefore, the beneficial effect of the bioactive compounds present in NPS may have antioxidant activity that provides a protective element from chronic diseases.

According to Yahaya et al. (2021), ascorbic acid in nipa also showed the highest antioxidant activity with an IC_{50} value of 21.29±0.74 g/mL. According to Sowndhararajan and Kang (2013), the lower the IC_{50} value, the higher the antioxidant activity. While the antioxidant properties of the sample were determined by the radical scavenging activity of DPPH and showed positive results, namely EC_{50} 112.90 mg/mL (Sukairi et al. 2018). The antioxidant compounds contained in nipa can combat free radicals in our body system and have great potential to be commercialized as healthy foods and beverages with scientific evidence and validation (Thyagarajan and Sahu 2018).

To assess the antioxidant activity of NPV, three in vitro antioxidant assays were used: 2,2-diphenyl-1-picrylhydrazyl and 2,2'-azinobis-3-ethyl benzothiazoline-6-sulphonic acid-free radical scavengers, and a reducing power assay (Yusoff et al. 2015a). Analysis of the chemical profile of the NPV aqueous extract revealed the presence of acetic acid (35.25%). Then, the ethyl acetate extract in nipa triggers a significant antioxidant effect caused by its high phenolic content. There are several reports on the antioxidant activity of plants affected by the content of their ethyl acetate extract (Nishidai et al., 2000; Kawano et al., 2010). Thus, it can also be concluded that the antioxidant component is present in the ethyl acetate extract.

Anti-inflammatory

Mangrove plant extracts have shown positive results and are important in indigenous medicinal practices. Research shows that one of the activities found can cause a very potent anti-inflammatory agent. An important aspect needed by mangrove extract to be useful as an anti-inflammatory drug is its flavonoid composition. Nipa has been used as a drug to treat inflammatory diseases because it has flavonoid compounds (Sahoo et al., 2012; Astuti et al., 2020). Flavonoids are a group of secondary metabolites widely found in plants and generally consist of 15 carbon atoms. Many flavonoids directly affect the enzymes responsible for the inflammatory process. In addition, some flavonoids can inhibit the induction of adhesion molecules such as blood neutrophils, which are required for the inflammatory process (Middleton et al. 2000).

Then, the results of the research of Bae and Jung (2016) showed that the aqueous extract of nipa alone had no cytotoxic effect at a concentration of 200/mL in 264.7 RAW cells. Nipa treatment inhibits nitrite production, and pro-inflammatory cytokines including IL-1β, IL-6, and TNF-α are dose-dependent. In addition, nipa treatment inhibited LPS-induced activation and nuclear factor (NF)-κB translocation. These results suggest that nipa treatment can reduce LPS-induced inflammatory response through

the inactivation of NF-κB. This study may indicate that nipa can be a useful drug or agent to prevent inflammation (Bae and Jung 2016).

Nipa, which is used in traditional medicine, is a plant that has received attention because of its various effects. The study of Kang and Hyun (2020) investigated the anti-inflammatory effect of nipa extract by controlling the neurological function of TRPV1 in experimental mice. The validation of the TRPV1 channel as a therapeutic target for controlling pain and inflammatory conditions in various disease and injury states has prompted the development of several TRPV1 agonists and antagonists that have entered clinical trials (De Petrocellis and Moriello, 2013). Then, quantification of the sciatic nerve and spinal cord protein L4-L6 showed a decrease in the expression of TRPV1, inflammatory expression factors, COX2, and pro-inflammatory factors in the group treated with nipa extract, thus indicating that nipa extract affects inflammation by controlling TRPV1 in neuropathic sciatic pain, so that produce an anti-inflammatory effect.

Antibacterial

Antibacterial is a drug that kills bacteria, especially pathogenic bacteria that can harm humans. Drugs that can be used to eradicate microbes must have the highest possible selectivity, meaning that the drug must be highly toxic to microbes. Antibacterial can also be used as a prevention against various pathogenic bacterial infections.

Most of the natural antioxidants reported in the literature also have antibacterial activity (Lourenço et al., 2019). The antibacterial properties of nipa have been investigated by Osabor et al. (2008), Chaudhuri and Guha (2010); Prabhakaran and Kavitha (2012); Shamsuddin et al. (2013), and Bakshi and Chaudhuri (2014). Then, Ebana et al. (2015) decided to analyze the leaves of nipa plants through phytochemical tests by testing the antibacterial properties of various extracts against *E. coli*, *Klebsiella pneumonia*, *S. aureus*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa*. Nipa is a rich source of various biochemical compounds such as alkaloids, cardiac glycosides, polyphenols, phlorotannins, saponins, and anthranoids. The presence of polyphenols indicates excellent antibacterial activity. Aqueous and ethanolic extracts of the tested nipa sections showed good antimicrobial resistance against all test organisms. Variations in the leaf ethanol extract concentration showed that a concentration of 5% and above gave absolute inhibition of *E. coli*. Another study showed that this plant also has excellent antibacterial and antifungal activity against *Vibrio* species (Shamsuddin et al. 2013; Imra et al. 2016), *Fusarium oxysporum* (Chaudhuri and Guha 2010), *B. cereus* (Lestari et al. 2016), and *B. subtilis* (Nopiyanti et al. 2016).

Then, another study showed that the highest concentration of crude extract of nipa leaves to inhibit Aeromonas hydrophila and Streptococcus agalactiae was 60%, with inhibition zones of 15.90 mm and 16.85 mm, respectively. The MIC results of crude extract of nipa leaves against *A. hydrophila* and *S. agalactiae* were found at concentrations of 100% and 75%, respectively. The MBC value with the lowest growth of *A. hydrophila* bacteria was 1.33a±0.52, and the highest was 4.33b±0.82. MBC with the lowest growth of *S. agalactiae* bacteria was 2.17a ± 0.75, and the highest was 4.67b ± 0.52. (Sari 2017).

OTHER UTILIZATION

Other utilization in Indonesia

nipa is a palm plant often used by coastal communities because of its many benefits. The part of nipa that can be utilized by the people of West Kalimantan, Indonesia, consists of leaves, shoots, bone leaves, fruit, and *Mayang* (flowers that have not yet bloomed) (Suparto et al., 2019). Then, nipa leaves are also used as raw materials in making roofs and walls of buildings. Nipa leaves as a roof are usually applied to houses, cattle pens, or huts in the garden (Febriadi and Saeni 2018). The roofing of nipa leaves is carried out by drying the old leaves to remove the moisture content, then the leaf's midrib is removed, and the leaf skeleton is folded to two-thirds of its length (Hamilton and Murphy 1988). Then, the prepared leaves are stitched together with vines overlappingly until they reach a length of 1-2 m. The advantages of this roof are that it can last 3-5 years before being replaced with a new one and can withstand the heat, especially in coastal areas.

In the Bawean Islands, Indonesia, woven from nipa leaves, produces a product in mats sold as souvenirs for visiting tourists (Trimanto et al. 2016). Nipa leaf stalks are quite strong and flexible and can also be used as a broomstick. In Makassar, broomsticks from nipa leaf stalks are traded for economic value. The economic benefit value from the production of the broomstick is IDR 2,055,333 or 13.57% of the total economic benefit value of nipa in Tallo District, Makassar City (Muthmainnah and Sribianti 2016). In Sumatra, the young nipa leaves (named shoots) were used as cigarette leaves in the past, namely wrapping sheets for rolling tobacco. After the thin epidermis is removed, the leaves are dried in the sun, then bleached to whiten and cut into cigarette sizes (Heyne 1987).

Besides being processed into various unique items, parts of the nipa plant also produce promising food resources. As many as 2.55 tons of nipa fruit can be produced from one hectare of vegetation (Dalming et al., 2018). People usually consume nipa fruit directly or further processed for preservation purposes. Nipa fruit tastes like coconut meat and can be processed as drinks and sweets that have economic value for people living in mangrove ecosystems. The manufacture of candied fruit nipa can be done because the method of manufacture is easy and uses simple technology (Khotimah et al. 2020). Old nipa fruit is used as flour in Kalimantan, precisely in Sangkimah Lama, Sangatta Village, East Kutai District, East Kalimantan Province. Flour made from old nipa fruit is produced by separating the fruit from the shell, cleaning the epidermis, grinding it by pounding it or blending it, drying it, and finally sieving (Subiandono et al. 2011). Further management of this nipa fruit can be a new source of economy for the community. Furthermore, food production

from processed nipa fruit can be further packaged and marketed to visiting tourists.

Furthermore, the community has widely used nipa resin and syrup for processing and have economic value in their lives. Actually, not only in Indonesia, according to research conducted by Tamunaidu et al. (2013), the sap from the nipa plant derived from its *mayang* can be used as a potential raw material for ethanol production. One nipa plant can produce 0.4-3 L of sap per day, and each stalk can be harvested continuously for 20 days. Therefore, the amount of ethanol produced per 1,000 ha of nipa is estimated at 4,550-9,100 L per hectare per year. Ethanol comes from the fermentation of raw materials containing glucose using the bacteria S. cerevisiae (Hadi et al., 2013).

The advantage of using nipa as the main raw material for bioethanol is that nipa is not the main food source, so it will not compete with other food needs. The part used as raw material for bioethanol is the juice so that it does not damage its ecology (Hamilton and Murphy 1988). Knowing the influencing environmental factors is necessary to produce maximum bioethanol from nipa plants. One of the environmental parameters where nipa grows to produce the best juice as raw material for producing bioethanol is salinity. The results of fermentation can be an environmentally friendly alternative fuel. This utilization has the potential to reduce dependence on fewer fossil fuels. However, the utilization of nipa sap for fermentation into bioethanol has not been widely carried out. This is motivated by the lack of knowledge and public capital in processing nipa sap into bioethanol.

Then, the syrup tapped from nipa is mostly used by the public to be processed into consumption and flavoring ingredients. The Indonesian people know the syrup produced from this tapping as sap which can be used as a sweetener for food and beverages after further processing. Nira has a clear color with a sweet taste and a distinctive fragrant aroma (Heriyanto et al. 2011). Tapping was carried out on young nipa fruit stalks. Further processing, the sap that has been collected from the tapping process is boiled for five hours to make brown sugar. Brown sugar producers in East Kalimantan, in one day, can process 50 L of sap and produce approximately 200 grams of brown sugar (Heriyanto et al. 2011). In South Kalimantan, the sap from the nipa plant is processed by crystallization to make brown sugar in the form of granules. Other Indonesians also use the sap to ferment it into a sweet alcoholic beverage. The drink is known as *tuak* in North Sumatra Province, Indonesia, and has the local name *saguer* in North Sulawesi Province (Kurniawan et al. 2018). Fermentation of nipa to be used as an alcoholic beverage is generally found in areas with a non-Muslim majority population.

Other utilization in other countries

The Southeast Asia peoples use some of the results from tapping sap from nipa to make traditional vinegar (Cheablam and Chanklap 2020). Flower bunches (inflorescences) can be tapped to produce a sweet, edible sap collected to produce a local alcoholic drink called *tuba*, *bahal*, or *tuak*. A bunch of fruit is ready to be tapped when the unripe fruit is at its peak of sweetness. The bunches are cut from the stalks about six inches down, and mud is rubbed on the stalks to induce sap flow. The sap begins to flow immediately if the ripeness of the fruit is measured correctly. A bamboo tube or bottle is placed over the cut stems, and the sap is collected twice daily, cutting half a centimeter from the end of the stem after each harvest to prevent it from clumping. The sap flow will continue for 30 days per stalk, and the nipa will flower continuously throughout the year, providing a continuous supply of sap. In Thailand alone, vinegar production from nipa plant sap reaches 12% of the total use of sap to manufacture other products. Khanap Nak is one of the places where there is a vinegar producer from nipa. Every day, farmers can produce as much as 7.5-200 liters of vinegar, depending on the number of workers. The valuation of vinegar production in Thailand's coastal communities reaches 40-66.7 USD per day. The vinegar produced can also be used for household purposes as a condiment for various local dishes.

Before World War II, Malaysia was dealing with the manufacture of alcohol from nipa, which was used as a vehicle fuel (Baja-Lapis et al. 2004). Two factories produced alcohol from nipa in Sarawak, Malaysia (Chai and Lai 1984) until the 1980s. Simultaneously, similar studies were conducted in the Philippines (Halos 1981) and Papua New Guinea (Newcombe et al. 1980). The manufacture of industrial alcohol from nipa was also an important industry in the Philippines in the early decades of the 20th century (Whitmore 1973). However, the industry was short-lived due to political problems, and competitive gasoline prices prevailed during that period (Fong 1984; Whitmore 1973). Nevertheless, the use and study of alcohol in nipa continued into the middle of the 20th century.

In the Philippines and Malaysia, *tuba*/alcohol can be stored in a tapayan (earthly balloon vase) for several weeks to make a type of vinegar known as *sukang paombong* in the Philippines and nipa vinegar in Malaysia. *Tuba* can also be distilled to make *arak*, locally known as *lambanog* in Filipino. Young shoots are also edible; The flower petals can be infused to make an aromatic tea. In Cambodia, the leaves are used to wrap cakes (such as *num katâm*), and the flowers are sometimes used to make sugar, vinegar, and alcohol (Phon et al., 2000).

Then, nipa leaves are flexible, making them easy to shape and use for handicrafts. For example, in Nigeria, the leaves are woven to form the hats known locally as "*ikpoto*" (Udofia and Udo 2005). The hats are sold as souvenirs for beach visitors and used by local people to accompany them in activities such as selling fish. The people of Nigeria also use nipa as bio-ethanol (Okugbo et al., 2012).

According to Hossain and Islam (2015), in Bangladesh, nipa leaves are part of people's lives that can be used as cigarette wrappers as we know that some people habits are smoking. Smoking is a medium to relieve stress or socialize with neighbors and is mostly done in rural and urban communities. Therefore, nipa leaves also have social value in their utilization. The leaf stalk can be used as a

float for fishing nets that have been spread, the main stalk is used as a net framework, and the middle bone is used as a rope to pull the net. Nipa, locally called *'Golpata,'* is used for multipurpose such as thatched roofs, partitions, food, and as a source of firewood. The newly developed shoots will be used as a vermicide. Dried leaves, petioles, woody stems, fruit residues, and other parts are used as fuel. In fishing, nipa rhizomes are widely used to make it easier for fishing nets to float on the water surface. The sweet sap from the inflorescence stems is used as a source of syrup (cane molasses), amorphous sugar, vinegar, and alcohol. Nipa plant sap is an important factor in producing sap/sugar. The growth properties of nipa fruit stems and their water content have affected sap production (Matsui et al., 2014).

In conclusion, the active compounds produced by nipa include phenolics, saponins, flavonoids, and tannins. The many chemical compounds in nipa can be used as raw materials in a product, especially in modern medicines. In some tropical countries, based on people's beliefs that nipa has the potential of herbal medicine to treat fever, gout, kidney stones, energy booster, aiding the digestive process, as a cure for certain chronic diseases and metabolic syndromes such as diabetes and hypertension, treat asthma, leprosy, tuberculosis, sick throat, liver disease, snakebite, as a pain reliever, and can also be used as a sedative and able to expel excess wind in the body. Then, it was also proven that nipa could act as an antioxidant, antidiabetic, antimicrobial (antifungal and antibacterial), anticancer, anti-inflammatory, antinociceptive, antihyperglycemic and analgesic. Nipa plants other than medicine are used as a roof for houses, cattle pens, or huts in the garden, broomsticks, handicrafts, fishing tools, as a source of food and drink, to a source of renewable energy fuel. Further research is needed to find out more about the actual potential of the drug and educate the public about the importance of the benefits of nipa as a renewable resource for the future of human life.

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