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Journal of Fisheries Science

Volume 4•Issue 1•March 2022 ISSN 2661-3387(Online)



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Volume 4 Issue 1 • March 2022 • ISSN 2661-3387 (Online)

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ARTICLE

Microbiological and Proximate Evaluation of *Tagelus adansonii*, Bosc, 1801 (Mollusca: Bivalvia, Solecurtidae) in Mangrove Swamps of Iko Estuary, Southeast, Nigeria

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ARTICLE INFO

Article history

Received: 27 December 2021

Accepted: 21 January 2022

Published Online: 11 February 2022

Keywords:

Nutrition

Health

Energy

Seafood

Growth

Clam

ABSTRACT

Tagelus adansonii has served as man's food around the world from time immemorial. However, the aquatic ecosystem in which they live is constantly polluted. Microbial and proximate compositions and energy value of *T. adansonii* were evaluated as indices for food safety and biomarker of pollution. Standard microbiological techniques and standard methods of AOAC were employed. Results showed that *Bacillus subtilis*, *Micrococcus species*, *Proteus species*, *Klebsiella species*, *Staphylococcus aureus*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Enterobacter sp*, *Escherichia coli*, *Bacillus cereus*, and *Chromatium species* were the probable bacteria while *Rhizopus stolonifer*, *Aspergillus niger*, *Penicillium species*, *Candida tropicalis*, *Fusarium species* and *Aspergillus flavus* were the probable fungi isolated from the sample. Total Heterotrophic Bacterial Count (THBC), TVC, TCC, TSC and TFC in fresh sample were $2.01 \pm 0.14 \times 10^5$, $2.77 \pm 0.27 \times 10^5$, $2.79 \pm 0.81 \times 10^5$, $6.08 \pm 0.21 \times 10^5$, and $2.08 \pm 0.21 \times 10^4$ cfu g⁻¹ respectively and concentrated mostly in the gut. The mean crude protein, moisture, carbohydrate, ash, lipid and crude fibre contents of the soft tissues were 60.92 ± 2.38 , 40.75 ± 1.85 , 26.58 ± 2.91 , 5.99 ± 0.43 , 5.56 ± 0.51 and $4.13 \pm 0.10\%$ respectively, while the energy or caloric value was 397.65 ± 11.97 . Proper monitoring and surveillance should be adopted by Government to check pollution of the aquatic environments and proper processing should be adopted before consumption for good public health.

1. Introduction

Shellfish and finfish are the most important sources of animal protein in the diet because of their good quality

and quantity of protein^[1]. Anthony *et al.*^[2], reported that shellfishes contain crude protein and fat higher than 20 and less than 5 percent respectively. They are relatively

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DOI: <https://doi.org/10.30564/jfs.v4i1.4277>

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good sources of calcium, phosphorus, magnesium and potassium; and are also a major source of income for coastal towns and villages^[3]. Tagelus is a genus of marine bivalve molluscs belonging to the family Solecurtidae, in the order Cardiida, an order of bivalves belonging to the class Bivalvia. In previous centuries, Bivalvia referred to as the Lamellibranchiata and Pelecypoda, is a class of marine and freshwater molluscs that have laterally compressed bodies enclosed by a shell consisting of two hinged parts. Adanson's tagelus is a short razor clam commonly known as knife clam^[4]. It has wide distribution across eastern Atlantic^[5].

Due to geometric rise in human population, the aquatic ecosystems have been grossly polluted. The primary sources of nutrient pollution include fertilizer from agriculture farmlands, animal manure, sewage treatment plant discharge, detergents, storm water runoff, cars and power plants, failing septic tanks and pet waste, with considerable regional variation in the relative importance of each^[6]. Nutrient pollution is of great environmental concern globally. It is a matter of concern due to excessive amount of nitrogen and phosphorus in water and air. Marine pollution has become one of the biggest threats due to industrialization and agricultural activities. It is hazardous not only for the water kingdom but for human beings directly or indirectly. In many forms, marine pollution alters the physical, chemical, and biological characteristics of the ocean and coastal areas, negatively impacting the health of biodiversity and ecosystems^[7], and may increase susceptibility to other stressors, including disease.

For the past decades, marine mollusks have received special attention and become a natural resource of economic importance^[8]. Apart from being valued as food and their ornamental importance, marine mollusks from polluted waters have been widely implicated in the outbreak of foodborne illnesses such as typhoid fever, hepatitis and other similar intestinal disorders in various parts of the world^[9,10].

Microbiological and proximate analyses of seafood have great importance in food safety and quality assurance since, they are major constituents of living matter^[11,12]. It is very useful in nutrient content compartmentalization and thus often affords a great deal of attention to consumers due to its potential benefits. It can also serve as biomarkers of aquatic pollution. This research therefore seeks to establish baseline data which will be useful in food safety, quality assurance and future monitoring of aquatic pollution.

2. Materials and Methods

2.1 Study Area

Live specimens of *T. adansonii* were obtained from the

intertidal zone of the Iko Estuary, a brackish water habitat characterized by tides, mangroves, several species of fin and shellfish (Figure 1).

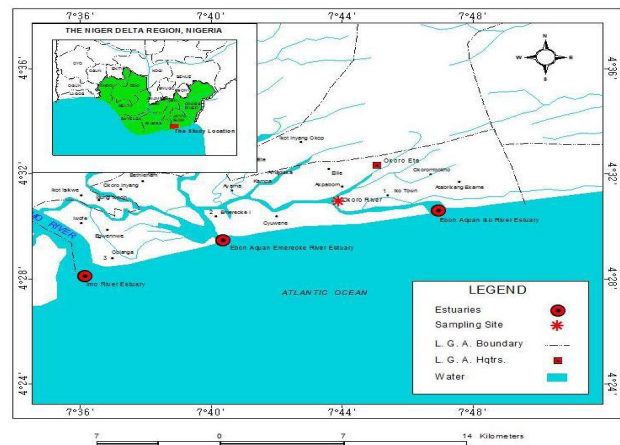


Figure 1. Location of the study area on the map of Niger Delta Region, Nigeria

2.2 Sampling Procedure and Processing

Samples were collected monthly from February 2019 to January 2020 in Iko Lagoon in Nigeria. This receives its catches from the Qua Iboe and Imo river estuaries. A total of five hundred and thirty (530) live specimens of *T. adansonii* were handpicked from the sediments in randomly sampled areas and washed with water from the creek and then transported in a sterile ice-packed coolers containing mud collected from the tidal mudflat to the Microbiology Laboratory University of Uyo, Uyo, Nigeria for analysis. Prior to analysis, sample was washed with a brush and water to remove all debris on the shells, spread on laboratory table and allowed to air-dry at room temperature. Selection of the healthy-looking clams was done through visual examination. A total of one hundred and fifty (150) samples each were selected for gut, shell and soft body analyses. Thereafter, extraction of the samples' soft body was done using a sterile scalpel. After each sampling date, samples' flesh weighing 10 g were homogenized in a blender with 90 mL of sterile distilled water, corresponding to a 10^{-1} dilution^[13]. A sterile surgical blade was used to obtain samples' guts through dissection then pulverized using a sterile mortar. The clam shell was also pulverized separately and then all the specimens were used for analysis. Part of the samples were weighed prior to laboratory analysis of its proximate composition^[14].

2.3 Microbiological Analysis

2.3.1 Serial Dilution of the Homogenate

Prior to culture, serial dilution of the homogenate was

conducted according to the method of Fawole and Oso^[15]. The samples were first blended using Laboratory Blender (Lab Blender 400 series, UK). Next was the weighing of ten (10) grams of samples and homogenization in 90 mL of sterile distilled water. This was shaken vigorously to dislodge bacteria and other microbes attached to it. Sterile pipette was then used to prepare tenfold dilution of the homogenates after which the aliquot (1 mL) was serially transferred to other test tubes containing 9 mL of distilled water up to 10^{-6} . Molten agar was prepared, and one (1) mL of the diluents of 10^{-4} was aseptically dispensed into the Petri dishes containing 15 mL of the agar. Samples culture was done in triplicates. All plates were incubated at 37 °C for 24 hours. In order to obtain pure colonies, subculture of the samples was carried out. Biochemical test of the isolates was carried out confirmed by microscopic examination prior to characterization and identification. Nutrient Agar (Oxoid, USA), Thiosulphate citrate bile-salt agar (Oxoid, USA), Eosin Methylene Blue (Oxoid, USA), Mannitol salt agar ((Difco Laboratories, Detroit, Mich), and Sabourad Dextrose Agar (Difco Laboratories, Detroit, Mich) were used for the enumeration of Total Heterotrophic Bacteria Count (THBC), Total Vibrio Count (TVC), Total Coliform Count (TCC), Total Staphylococcal Count (TSC) and Total Fungal Count (TFC) respectively. Nutrient agar slants in McCartney bottles was used to maintain the isolates and preserved in a refrigerator at 4 °C for further analysis. A sterile inoculation needle was used to pick up morphologically different colonies and aseptically transferred to sterile nutrient slant for further characterization. Purity check and characterization was done following the standard characterization key^[16] partitioned into Gram reaction, catalase, coagulase, motility, starch hydrolase, citrate, urease, MR, VP, Kovac's oxidase, oxidation/fermentation (O/F) test, indole, mannitol, glucose, sucrose and lactose tests.

2.4 Experimental Procedure

Completely Randomized Design (CRD) with four (4) treatments and three (3) replicates was used. Total Heterotrophic Bacteria Count, TVC, TCC, TSC and TFC stood for treatments 1, 2, 3, 4 and 5 respectively. Each quarter year sampling represented a replicate; the one year sampling represented three replicates.

2.5 Analysis of Proximate Composition

2.5.1 Moisture Content Determination

Weighing bottle was washed, oven-dried at 80% for five (5) minutes, cooled and weighed and recorded as beaker weight (a). Two (2) grammes of the sample was weighed into the weighing bottle, now bottle weight plus sample recorded (b). The weighing bottle with the sample was

then oven-dried at 105°C for 24 hours. The bottle allowed to cool in a desiccator to room temperature, weighed with a minimum exposure to atmosphere. This was repeated till constant weigh is obtained (c). Percentage moisture was therefore calculated thus:

$$\text{Percentage Moisture (\% wet weight)} = \frac{b-c}{b-a} \times 100$$

The dried sample was ground with grinding machine into powder form often necessary to pass through sieve of particular mesh size and then stored at low temperature in dry air-tight container.

2.5.2 Preparation of Sample for Subsequent Analysis

After taking part of the fresh sample for moisture content determination, the remaining sample was to be dried to a constant weight before subsequent analysis. Low temperature (50-60 °C) was employed to reduce any possible effect of high temperature on the protein (and probably other nutrient) in the food sample, such effects include Protein denaturation, Loss of vitamins, decomposition of anions. However, the oven dried material was ground in a mortar into a powdered form, often necessary to pass through a sieve of a particular mesh size and then stored at a low temperature in dry air-tight container specifically having a plastic cover.

The following parameters were determined from the ground samples; Ash, crude fibre, lipids, crude protein, fat, etc.

2.5.3 Ash and Organic Matter Determination

This was done by ignition of crucibles with lid in a muffle furnace at 105 °C for an hour, and transferred to a desiccator to cool and the weight was recorded as weight of empty crucible (a). Next, 1 – 5 grammes of dry sample, finely pulverized, was transferred into the pre-weighed crucible and the weight of the crucible plus sample (b) was recorded. Then, crucible plus sample were charred on a heater or Bunsen flame in a fume cupboard, to drive off most of the smoke (until smoking ceased), then transferred to a muffle furnace heated at (500-600 °C) to burn off all the organic matter; then left for 2 hours. The crucible was taken out, when cool, covered and placed in a desiccator and weighed (c). Percentage ash was determined as follows:

$$\text{Percentage Ash} = \frac{c - a}{b - a} \times 100$$

The portion of sample which burnt off is organic matters. Therefore the percentage organic matters were determined thus:

$$\text{Percentage organic matters} = 100 - \% \text{Ash.}$$

2.5.4 Crude Fiber Estimation

To estimate crude fibre, 2 grammes of sample was

defatted with petroleum ether for 2 hours, boiled under reflux for 30 minutes with 200 mL of a solution containing 1.25% of H_2SO_4 for 100 mL solution. Residue was obtained by filtering the solution through linen fixed on a fluted funnel washed with boiling water until the washing was no longer acidic. This was transferred to a beaker and boiled for another 30 minutes with 200 mL of a solution containing 1.20 g of NaOH for 100 mL. The final residue was filtered and washed with boiling water several times until it is base (NaOH) free. The residue was finally washed twice with ethanol, and qualitatively transferred into a pre-weight crucible, oven dried at 105°C (W_1). This was incinerated in a furnace at 550°C for 2 hours (W_2). It was then cooled in a desiccator and weighed. Loss in weight after incineration was also taken.

$$\text{Percentage crude fibre} = \frac{W_2 - W_1}{\text{Weight of original sample}} \times 100$$

2.5.5 Ether Extract Determination

Two grammes of the sample was weighed into a thoroughly-washed and oven-dried extractor thimble and plugged lightly with cotton wool. Next, 150 mL of petroleum ether (boiling point $60 - 80^\circ\text{C}$) was formed into a 250 mL round bottom flask. The Soxhlet extractor was fitted into the round bottom flask on a heated mantle. The Soxhlet apparatus was assembled and allowed to reflux for about 4 hours and the extract was poured into a dried pre-weighed beaker (W_1) and the thimble was rinsed with a little quantity of the ether back to the beaker. The beaker was oven-heated to drive off the excess solvent. The beaker was cooled in a desiccator and weighed (W_2). Crude fat was estimated thus:

$$\text{Percentage Ether Extract} = \frac{W_2 - W_1}{\text{weight of sample}} \times 100$$

2.5.6 Crude Protein Determination

The crude protein content was determined by Kjeldahl method. One gramme of the sample was put in a standard 250 mL Kjeldahl flask containing 1.5 g of CuSO_4 and 1.5 g of Na_2SO_4 as catalyst and 5 mL concentrated H_2SO_4 . The Kjeldahl flask (digestion) was heated gently to prevent frothing for some hours until clear bluish solution was obtained, allowed to cool and quantitatively transferred to 100 mL standard flask and made up to the mark with distilled water. Twenty (20) milligrams of the digest were pipetted into a semi micro Kjeldahl distillation apparatus and treated with equal volume of 40% NaOH solution. The ammonia evolved was steamed, distilled into a 100 mL conical flask containing 100 mL solution of saturated boric to which 2 drops of Tashirus indicators (double indicator) have been added. The tip of the condenser was immersed into the boric

acid double inductor and then the distillation continued until about 2/3 of the original volume obtained. The tip of the condenser was rinsed with a few millimeters of distilled water in the distillate which was then titrated with 0.1 M. HCL until a purple-pink end point was observed (Sample titre, T_1). The blank determination was also carried out in similar manner except for the omission of the sample (Blank titer, T_1). The crude protein was obtained by multiplying the % nitrogen content by a factor (6.25). Percentage crude protein is estimated thus:

$$\text{Percentage crude protein} = \frac{T_1 - T_2 \times 0.1 \times 0.04 \times 20 \times 100}{\text{weight of sample} \times 6.25/10}$$

Note: Most protein contain about 16% Nitrogen; that is 16 mg. Nitrogen equals 100 mg protein, therefore one milligram of nitrogen equals $100/16$ equals to 6.25 mg of protein. The Nitrogen value is therefore multiplied by 6.25 to get the weight of protein.

2.5.7 Estimation of Nitrogen Free

Nitrogen free Extract (NFE) was determined as the difference obtained after subtracting total organic Nitrogen (protein) lipid, ash and fibre from the total dry matter thus:

$$\text{Percentage Nitrogen Free Extract} = 100\% - (\% \text{ EE} + \% \text{ CP} + \% \text{ Ash} + \% \text{ CF}).$$

2.5.8 Estimation of Calorific Value (Energy)

The Atwater system^[17] was used in the determination of the total calorific value of the sample by employing the 4-9-4 method. This system applies energy conversion factors to the macronutrients carbohydrate, fat, protein and fiber.

2.6 Statistical Analysis

Data on microbial and fungal loads on the body, gut and shell were log transformed (\log_{10}) for the purpose of statistical analysis while those on proximate composition were used directly. One – way analysis of variance (ANOVA) was used to test the variation in mean values and significance accepted at $P \leq 0.05$ level. Where significant difference existed, *post hoc* test was performed using Duncan Multiple Range Test (DMRT). The IBM SPSS statistics version 20 computer program for Windows was used.

3. Results

3.1 Characteristics of Bacterial Isolates

The cultural, morphological, structural and biochemical characteristics of bacterial isolates from *T. adansonii* are presented in Table 1.

The isolates were *Bacillus subtilis*, *Micrococcus sp*,

Table 1. Cultural, Morphological, Structural and Biochemical Characteristic of Bacterial isolate from *Tagelus adansonii* caught in Mangrove Swamps of Iko Estuary, Southeast, Nigeria

Bacteria shape	Gram reaction	Catalase	Coagulase	Motility	Starch hydrolase	Citrate	Urease	MR	VP	Form spore	H ₂ S	Hae	Glucose	Maltose	Lactose	Fructose	Sucrose	Galactose	Mannitol	Probable organism
Rod	+	+	-	+	+	+	-	-	+	+	+	-	AG	A	-	A	-	A	-	B. subtilis
Cocci in pair	+	+	-	-	+	+	+	+	-	-	-	-	-	A	+-	A	-	A	-	Micrococcus sp
Rod	+	+	-	+	+	+	+	+	-	-	-	-	AG	A	AG	AG	AG	AG	-	Proteus sp.
Rod	-	+	-	+	-	+	+	+	-	+	+	-	AG	AG	AG	-	AG	AG	-	Klebsiella sp.
Cocci in cluster	+	+	+	-	-	-	-	-	+	-	-	B	A	A	-	AG	A	A	AG	Staphylococcus aureus
Cocci	-	+	-	+	+	-	-	-	+	-	-	-	A	A	-	A	AG	A	-	Vibrio chelera
Rod	-	+	-	-	+	+	-	-	+	+	-	-	A	A	AG	A	-	A	-	Vibrio parahaemolyticus
Rod	-	+	-	-	+	-	-	-	-	-	+	-	AG	AG	AG	AG	AG	AG	-	Enterobacter sp.
Rod	-	+	-	+	-	-	-	+	-	-	-	-	A	AG	-	-	-	AG	-	Escherichia coli
Rod	+	+	-	+	+	-	-	-	+	-	+	-	AG	A	AG	A	-	-	AG	Bacillus cereus
Rod	+	-	-	+	+	+	-	-	+	+	+	-	AG	AG	+	-	A	AG	-	Chromatium sp.

Proteus sp, *Klebsella sp*, *Staphylococcus aureus*, *Vibrio cholera*, *Vibrio parahaemolyticus*, *Enterobacter sp*, *Escherichia coli*, *Bacillus cereus* and *Chromatium sp*.

3.2 Characteristics of Fungal Isolates

The type of fungal pigmentation, soma, special vegetative structure, asexual spore, special reproductive structure, conidial head, vesicles shape and nature of hyphae identify were used to isolate *Rhizopus stolonifer*, *Aspergillus niger*, *Penicillium sp*, *Candida tropicalis*, *Fusarium sp*, and *Aspergillus flavus* as probable fungi found in *T. adansonii*. This is shown in Table 2.

Table 3 shows the microbial loads of the sample. Mean THBC of 2.01×10^5 cfu g⁻¹ with the highest (2.83×10^5 cfu g⁻¹) in the gut and the least (1.60×10^5 cfu g⁻¹) seen in flesh. Mean THBC was significantly higher ($P < 0.05$) in the gut than in other body organs. Mean TVC was 2.77×10 cfu g⁻¹ with the highest (2.90×10 cfu g⁻¹) in flesh and least

(2.68×10 cfu g⁻¹) in gut. However, there was no significant difference ($P < 0.05$) in the mean TVC among different organs. Mean TCC was 2.79×10 cfu g⁻¹ with the highest (2.88×10 cfu g⁻¹) in gut and least (2.70×10 cfu g⁻¹) in clam flesh. No significant difference ($P < 0.05$) in the mean TCC among different organs.

Mean TSC was 6.08×10 cfu g⁻¹ with the highest (6.63×10 cfu g⁻¹) in fresh and no staphylococcal count on the shell. Total staphylococcal count (TSC) was significantly higher ($p < 0.05$) in the flesh. Mean TFC was 2.08×10^4 cfu g⁻¹ with highest (2.30×10^4 cfu g⁻¹) in clam gut and least (1.72×10^4 cfu g⁻¹) in shell. Total fungal count (TFC) was significantly higher ($p < 0.05$) in the gut and flesh than the shell.

Proximate Composition of knife clam is presented in Table 4. The moisture content of the mollusc ranged from 38.27% to 43.14% with $40.75 \pm 1.85\%$ being the mean. Ash ranged 5.12 – 6.23% and the mean was $5.99 \pm 0.42\%$. The

Table 2. Cultural, Morphological, Structural and Biochemical Characteristic of Fungal isolates from *Tagelus adansonii* from caught in Mangrove Swamps of Iko Estuary, Southeast, Nigeria.

Pigmentation	Type of Soma	Nature of hyphae	Special vegetable structure	Asexual spore	Special reproductive structure	Conidial head	Vesicles shape	Probable organism
White becoming greyish	Filamentous	Coenocytic	Stolon rhizoids	Ovoid sporangiospores	Tall sporangiophores in group, black brown sporangia	-	-	Rhizopus stolonifer
Black colony	Filamentous	Septate	Foot cell	Globose conidia	Smooth wall erects conidiophores	Globose	Globose	Aspergillus niger
Dark green colony	Filamentous	Septate	Broom like appearance	Globose conidia produce in long columnar	Erect conidiophores terminating in whorls of phialides	-	-	Penicillium sp
Creamy white colony	Pseudo-hyphae	Septate	Apothelium	Blastoconidia	Conidia	Radiate	Globose	Candida tropicalis
pink	Filamentous	Septate	-	Micro conidia	Short branch conidiophores	-	-	Fusarium sp
Yellow	Filamentous	Septate	Foot cell	Globose conidia	Phialides born directly on the vesicle sclerotia	Radiate	Sub-globose	Aspergillus flavus

Table 3. Microbial load (cfu g⁻¹) of *Tagelus adansonii* samples caught in Mangrove Swamps of Iko Estuary, Southeast, Nigeria

Anatomical sites	THBC (10 ⁵ cfu g ⁻¹)	TVC (10 ¹ cfu g ⁻¹)	TCC (10 ¹ cfu g ⁻¹)	TSC (10 ¹ cfu g ⁻¹)	TFC (10 ⁴ cfu g ⁻¹)
Gut	2.82±3.1 ^c	2.68±2.3 ^a	2.88±3.5 ^a	5.53±2.6 ^a	2.30±2.5 ^{bc}
Flesh	1.60±1.7 ^a	2.90±2.3 ^a	2.70±3.6 ^a	6.63±3.1 ^c	2.21±5.4 ^c
Shell	1.61±1.3 ^a	2.75±2.8 ^a	2.78±4.0 ^a	-	1.72±2.2 ^a
MEAN	2.01±0.14 ^b	2.77±0.27 ^a	2.79±0.18 ^a	6.08±0.21 ^b	2.08±2.10 ^b

Values are mean ± SD. Mean±sd in the same column with different alphabets are significantly different ($p < 0.05$). THBC = Total Heterotrophic Bacterial count, TVC= Total Vibrio Count TCC = Total Coliform Count, TSC = Total Staphylococcal Count, TFC = Total fungal count.

fibre content ranged from 3.96 – 4.21% while the mean was $4.13 \pm 0.10\%$. The protein content ranged from 56.45 – 63.31% and mean was $60.92 \pm 2.38\%$. Fat content ranged 4.99 – 6.03% while mean was $5.56 \pm 0.51\%$. Carbohydrate content was within the range of 21.26 – 29.46% and had $26.58 \pm 2.91\%$ as the mean, while the caloric value was ranged from 384.97 – 410.39 kcal and had 397.65 ± 11.97 kcal as the mean.

Table 4. Mean proximate composition of flesh of *Tagelus adansonii* caught in Mangrove Swamps of Iko Estuary, Southeast, Nigeria

Parameters	Composition (%)
Moisture	40.75 ± 1.85
Ash	5.99 ± 0.42
Crude fibre	4.13 ± 0.10
Crude protein	60.92 ± 2.38
Ether extract	5.56 ± 0.51
Nitrogen free extract	26.58 ± 2.91
Caloric value (Kcal)	397.65 ± 11.97

Values are means \pm standard deviation of triplicate samples.

3.3 Monthly Variation in the Proximate Composition

The monthly variation in the proximate composition of *T. adansonii* is presented in Figure 2. The highest crude protein content was recorded in September while the least was seen in February. However, there was no significant difference ($P > 0.05$) in the monthly composition of crude protein in this clam. The highest moisture content was recorded on September while the least was seen in July. However, there was no significant difference ($P > 0.05$) in the monthly composition of moisture in this clam. The highest carbohydrate content was recorded in January while the least was seen in April but was not significantly different ($P > 0.05$). Ash content showed the highest content in September and the least in February and were not significantly different ($P > 0.05$). Lipid content showed the highest value in July while the least was seen in October. However, there was no significant difference ($P > 0.05$) in the monthly composition of lipid in this clam. Crude fibre content of *T. adansonii* showed the highest value in October while the least was seen in November and was not significantly different ($P > 0.05$).

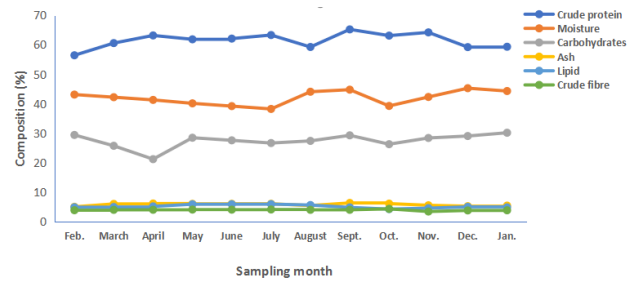


Figure 2. Monthly variations in the proximate compositions of *Tagelus adansonii* caught in Iko Estuary Southeast Nigeria

3.4 Monthly Variation in Caloric Value

The monthly variation in the caloric value of *T. adansonii* is presented in Figure 3. The highest caloric value was recorded in July while the least was seen in April. However, caloric values were significantly higher ($P < 0.05$) in June and July.

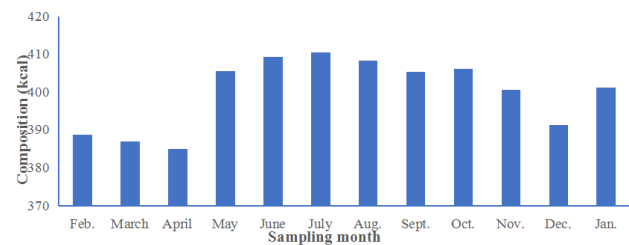


Figure 3. Monthly variation in caloric value of *Tagelus adansonii* caught from Iko Estuary Southeast, Nigeria

4. Discussion

Tagelus adansonii caught in Iko region of the Niger delta estuaries accumulate and concentrate microbial pathogens and those that are GRAS (Generally Regarded as Safe) present in the water. In all, eleven (11) bacterial species and six (6) fungal species were isolated. *Bacillus subtilis* isolated from the sample was an indication that not only pathogenic microbes but useful ones were also isolated. For instance the isolation of *B. subtilis* was an indication that commercially useful bacteria (GRAS) was presented in the samples. According to Martinez ^[17], *B. subtilis* is important in biotechnology for enzyme production. In food industry, it is used in the production of animal feed additives, sweeteners, fermented products and flavour enhancers. The production of household detergent, vitamins and antibiotics is also accomplished using *B. subtilis*. Above all, it is used in the development of vaccines and in the development of sporicides. *Micrococcus species* are very useful in cheese

production. It helps in the ripening cheeses made from farm animals' milk; halotolerant *M. Species* is a major component of micro flora of some cheeses and help in desired body, texture and flavour of soft cheese. Although not considered a pathogen, *M. Species* has generally been regarded as opportunistic pathogen since some strains such as *M. luteus* has been implicated as the causative agent of septic arthritis, recurrent bacteraemia etc. and pneumonia in patient with acute leukaemia^[18].

Some of the isolates may have been derived from external sources during handling and as such, the clams become transient carriers of such microbes. For instance, isolation of *Proteus Spp.* may mean that the sample was not prepared hygienically so that it entered from the environment and when consumed by humans it can cause osteomyelitis^[19]. However, in this study most isolates from the clam gut and homogenized flesh may be accounted for mainly by the filter feeding effect of the clam^[20]. *Klebsiella Spp* can cause diseases and spoilage therefore its isolation from *T. adansonii* mean that if eaten raw or not well processed it can cause UTIs, wound and respiratory infection as well as serious pneumonia in humans^[21]. *Staphylococcus aureus* is pathogenic and has been isolated in the sample. This means that consumption of this sample by human can result in the type of food poisoning known as SFP (staphylococcal food poisoning) and late on-set infection in neonates^[22]. *Vibrio cholera* and *Vibrio parahaemolyticus* were also isolated from *T. adansonii* and according to Ababouch *et al.*,^[23] they both cause gastrointestinal disease. It can hereby be deduced that eating of raw or unprocessed *T. adansonii* from this study area can result in outbreak of cholera *V. parahaemolyticus* infection. According to Powel *et al.*^[24] *V. parahaemolyticus* has been responsible for several recent seafood-associated outbreaks and therefore considered an emerging bacterial pathogen in Europe.

The isolation of *Enterobacter Spp.* from the sample is an indication that consumption of unprocessed clam from this study area can result in enterobacter infections. Such infection include but not restricted to UTIs, bacteraemia, endocarditis, osteomyelitis etc. *Escherichia coli* isolation is an indicator for pathogenic organisms. The isolation of *E. coli* from *T. adansonii* in this study area is an indication of fecal contamination by man and his livestock. This is of public health significance as these organisms have generally been agent of gastroenteritis in humans^[25,27]. For instance *E. coli* have been reported to be responsible

for foodborne illnesses and deaths^[28] through poor processing and handling of foods or farm animals. *Bacillus cereus* is known to cause cutaneous disease. Its isolation from this sample is an indication that eating of unprocessed clam from this water body may result in skin infection as well as pneumonia, meningitis and necrotizing fasciitis^[29]. *Chromatium Spp* was also isolated from the sample. It is not pathogenic, rather it is used in photosynthetic sulfur oxidation which is an indication that it entered the clam body via the water body.

The six (6) species of pathogenic fungi isolated from *T. adansonii* in this study area include: *Rhizopus stolonifer*, *Aspergillus niger*, *Penicillium Spp*, *Candida tropicalis*, *Fusarium Spp* and *Aspergillus flavus*. This shows that consumption of unprocessed clam from this study area can result in mucormycosis, rot, food spoilage, candidiasis, opportunistic mycosis and production of aflatoxins which can be of serious health implication to human and his livestock. According to Zhai, *et al.*^[30] *C. tropicalis* can cause fatal digestive system disease and septicemia. *Aspergillus Spp* has been associated with outbreaks of seafood diseases known as nosocomial aspergillosis which is a serious threat for severely immune-compromised patients^[31]. The level of mean THBC reported in this study was higher than the range reported by Udoh *et al.*^[32] for *G. paradoxa* in Cross River Estuary and Volta Lake while TCC fell within the range^[32]. Total heterotrophic bacteria and coliform may be directly influenced by many anthropogenic activities and rainfall. Run-off from rain might carry raw sewage from the surrounding villages and leachate from waste sites in the catchment area into the freshwater; the clams being filter feeders are able to accumulate the isolates in their tissues to level twice that in the surrounding waters^[33]. This is in agreement with Antai^[34] who reported that the high microbial load in the sample is a clear indication that clam serves as a medium through which microbes multiplied rapidly. Udoh, *et al.*^[32] added that these species of microbes are demonstrating strong resistance to novel. Generally, microbial load was higher in the gut than other parts confirming the fact that clam should be degutted before processing for consumption. *Tagelus adansonii* are protein rich food and therefore serves as a suitable substrate in supporting growth of different types of bacteria and fungi; the microbial growth in these fresh seafood will encourage food spoilage and

seafood poisoning. On the other hand estuaries or rivers are constantly polluted with faecal matter from riverine dwellers. This finding agrees with the work of [35] that shellfish and coastal waters contaminated by human pathogens could be sources of shellfish-borne or water-borne outbreaks. In fact, shellfish can accumulate and concentrate microbial pathogens present in waters by their filter-feeding activities. The risk of disease from these agents varies by pathogen, dose, host and characteristics of the seafood matrix. The extent to which a microbial hazard is likely to be present in seafood and give rise to a public health and safety risk depends on numerous factors, including the biology of the particular seafood species, its growth environment and the specific activities along its production and processing supply chain [36]. This gives relevant information regarding the food safety and sanitary conditions of *T. adansonii* and the coastal water [37]. The results obtained from this study also showed that *T. adansonii* appears in the river between the months of February – January.

The moisture content of 40.75 ± 1.85 obtained from the mollusk was lower than the range of 66.17 – 99.20% reported on snails, *Pila globosa* and Whelk found in Europe, Asia and Africa [38]. Comparison with other molluscs of economic importance shows that the moisture content was lower than that of oyster, clam, rough and smooth periwinkle and whelk which recorded 73.37, 73.72, 84.80, 80.22 and 60.97 percent respectively [1]. Moisture content of 79.60% – 81.20% had earlier been reported for periwinkle [38]. These variations could be due to the effect of environment as reported by Osibona *et al.* [39]. The ash content of 5.99 ± 0.42 is similar to 5.84% observed by Obande *et al.* [40] for fresh water snail (*Pila ampullacea*) from River Benue, Nigeria and smaller compared to 6.85% for rough periwinkle to 14.02% for whelk [1]. This value can be attributed to the fact that aquatic mollusk absorbs more minerals from the water as rivers serve as effluent to some industries whose chemical waste discharge into the water body may increase the absolute minerals in the water. The crude fibre content of $4.13 \pm 0.10\%$ was higher than the range observed by Eneji *et al.* [41] of 0.50-1.50% for land and water snails. It is also high as compared to other shellfishes [42]. The protein content of 60.92 ± 2.38 of *T. adansonii* is higher than that of *Pila ampullacea* (10.40%), fresh water fishes and the giant land snail *Archchatina maginata* [40]. It is also far higher than 9.97% to 13.96% recorded for other aquatic

molluscs [1]. On the other hand, the value is similar to that ($61.9 \pm 4.3\%$) reported by Rodríguez *et al.* [43] for mussels *T. peruvianus* from the Gulf of Nicoya, Puntarenas, Costa Rica. However, it is still comparable to values obtained in other livestock [44]. Bender [45] has responded that the amino acids in the protein of aquatic molluscs could be used to compliment the cereal sources of protein making good their relative deficiency of lysine. The result had shown that molluscs constitute a rich source of protein which according to Egonmwan [46], are of high biological value. Protein is the major structural component of cells and is responsible for the building and repair of body tissues. Thus, with increased consumption, the serious problem of protein deficiency can be mitigated. The Ether extract of $5.56 \pm 0.51\%$ is higher than 0.09% in *P. ampullacea* [40] and other species of aquatic molluscs [1]. It is high as compared to other species of animals except mullet and octopus [41]. This could be related to location and origin of the mollusc [47]. Judith and Jenny [42] indicated that consumption of molluscs in large proportion reduced the risk of hypercholesterolemia which is capable of causing cardiovascular disease, due to its high omega-3 fatty acid content. The NFE value of 26.58 ± 2.91 is higher than 0.92%, 0.72%, 0.55%, 0.26%, and 0.93% for oyster, clam, rough periwinkle, smooth periwinkle and whelk respectively reported by Kiin-kabari *et al.* [1] and 7.66% reported earlier by Obande *et al.*, [40] for the Fresh water snail (*Pila ampullacea*) from River Benue. High carbohydrate content indicates that high consumption of molluscs can be supplemented with low energy-rich foods to balance the energy-protein intake requirement. This is further supported with high caloric values (397.65 ± 11.97 kcal) recorded for this species of molluscs.

5. Conclusions

These findings showed that Knife clam contains considerable number of pathogenic microorganisms which are major sources of water borne diseases and death. It also contains considerable amount of protein and other nutrients, and high caloric value. From the foregoing, it has been established that the aquatic ecosystem is under threats of pollution and so clams as well as other aquatic food organisms must be processed properly before consumption. Considering the importance of shellfishes as primary protein source, proper monitoring and surveillance should be adopted by Government to check pollution of the aquatic environments.

Authors' Contributions

Imefon Udo conceived the work, designed the experiment, supervised the work and prepared the manuscript. Dora Udoh carried out all microbiological analyses and also read and approved the manuscript. Otobong Isang did the sampling, carried out the proximate analysis and also approved the manuscript.

Conflict of Interest

The authors have declared no conflict of interest for this work.

Acknowledgments

The authors acknowledge the support of laboratory Technologists who assisted in all the analyses carried out during this project.

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ARTICLE

Length-weight Relationship and Condition Factor of *Sarotherodon Melanotheron* (Perciformes: cichlidae) from Forcados River Estuary, Niger Delta, Nigeria

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ARTICLE INFO

Article history

Received: 22 December 2021

Accepted: 7 February 2022

Published Online: 14 February 2022

Keywords:

Sarotherodon melanotheron

Length-weight relationship

Condition factor

Forcados River estuary

Niger Delta

ABSTRACT

Length-weight relationship (LWR), condition factor (k) of the black chin tilapia, *Sarotherodon melanotheron* (Rüppel, 1852) from Forcados River estuary Nigeria was investigated. The fish were collected monthly from fishermen for a period of 24 months (between April 2012 and March 2014). 699 specimens of the fish species were collected. The Length-weight relationship (LWR) of the fish was evaluated using the equation: $W = a L^b$ while the condition factor of the fish was determined using the equation; $K = 100W L^{-b}$. The standard length of sampled *S. melanotheron* ranged from 4.15 to 18.92 cm, total length 6.01 and 22.5 cm while the weight ranged from 7.85 - 286.71 g. The b value 2.1299 was less than 3 indicating that the growth pattern of the fish was allometric. The correlation co-efficient (r) value for *S. melanotheron* was 0.7528. The condition factor for the combined sexes fluctuated monthly. The length-weight relationships and condition factor of *S. melanotheron* in Forcados river estuary indicated that the fish were above average condition.

1. Introduction

Cichlids are important ecological and commercial inexpensive food fishes of tropical fresh and brackish water bodies (King and Etim 2004; Abdul 2009) ^[1,2]. With *Sarotherodon melanotheron* commonly called “Black chin tilapia” reported as one of the most important brackish water species in the Niger Delta region of Nigeria (Ayoade

and Ikulala, 2007) ^[3]. *S. melanotheron* has also been found in Central Africa lagoons and estuaries (Amoussou et al., 2018) ^[4].

This fish, is highly valued for its edible flesh, low cost and availability for local populations (Guissé and Niass, 2021) ^[5]. However, information on its growth characteristics in Forcados River estuary seems to be scarce, hence this study. According to Egborge (1994) ^[6], *S.*

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DOI: <https://doi.org/10.30564/jfs.v4i1.4255>

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melanotheron is recognised by the possession of truncate caudal fin, moderate pectoral fin, and an anal fin which has long rays which project over half the caudal fin.

Length weight relationship (LWR) and condition factor ("K") are used for assessing the general health of a fish population and are important tools for fish biology study (Le Cren 1951)^[7] Lizama et al., (2002)^[8]. This is done by drawing a mathematical relation between the length and weight of the aquatic species (Beyer 1987)^[9]. Thereby changing growth-in-length calculations to growth-in-weight used in stock evaluation models (Pauly 1993)^[10].

Morey et al.^[11] stated that the mathematical relationships between length and weight are a very potent tool which can be used in predicting weight of fish from length measurements taken in the course of stock assessment and monitoring.

Growth is a basic characteristic of living things and the growth rates and pattern are species-specific (Olopade et al.^[12]) and vary from one water body to another even within the same species.

Anene (2005)^[13] reported that, in fish biology, condition factor gives an overview of the "condition" of fish founded on hypothesis that fish that have more weight of a certain length are in a healthier physiological state (Begenal 1978)^[14].

Condition factor can give details on the physiological situation of the fish as well as its variations in relation to its welfare (Ighwela et al., 2011)^[15] It is also used in comparing different populations of fish living in certain grazing, climate, and certain other conditions; during the determination of the period of coming into full development and when measuring up the rate of grazing activity of a species to determine if it is making meaningful use of its feeding source (Anyanwu et al. (2007), Wootton (1990)^[16,17].

According to Lizama et al., 2002 [8], condition factor determination enables the understanding of the life cycle of fish species easier and also contributes to the adequate management of these species. Therefore, balancing the ecosystem^[8].

Previous studies have been documented on the LWR and condition factors of fishes in Nigeria, West Africa. Ayoade and Ikulala (2007)^[3] worked on the biology of *Hemichromis bimaculatus*, *S. melanotheron* and *Chromidotilapia guenthsi* in Eleiyale Lake, Nigeria. Oribhabor et al. (2009)^[18] studied the length-weight relationship of *S. melantheron* and *Tilapia guineensis* in a Niger Delta Mangrove Creek, Nigeria. Fafioye and Oluajo (2005)^[19] worked on five different fish species in Epe Lagoon.

Reports from other places in the world on *S. melanotheron* includes works by Guissé and Niass, (2021)^[5] on the length relationship and condition factor of the fish in the special wildlife reserve of Gueumbeul in Senegal. Mireku et al.,^[20] reported on aspects of the biology of the cichlid in Brimsu Reservoir, Ghana while Chikou, 2019^[21] worked on the growth characteristics of *S. melanotheron* in Benin Republic.

The study was carried out to assess the length-weight relationship and condition factor of *S. melanotheron* in Forcados River estuary. The result will be useful in managing and conserving this tilapia in the estuary.

2. Materials and Methods

The study area lies between latitude 5° 35' - 5°354'N and longitude 5° 501' - 5°370' E and enjoys a tropical climate, with two defined seasons; rainy and dry seasons. The dry season commences from November to April while the rainy season is mainly from May to October (Opute 2000)^[22]. The vegetation covers include *Eichhornia crassippes*, *Fern*, *Pistia*, *Cenchrus ciliaris*, *Nymphaea spp*, *Trapa spp*, *Lemna spp*, *Ceratophyllum spp*. Human activities here include fishing, bathing, swimming and transportation.

S. melanotheron samples were obtained between April 2012 and March 2014 on a monthly bases from artisanal fishermen and immediately transported for analysis and identification in the laboratory. Keys used for identification includes that of Fischer et al. (1981)^[23], Schneider (1990)^[24], Paugy et al. (2003)^[25], Tesch, 1971^[26]; Thomas et al. 2003^[27]. The weights of the fish was measured with the aid of a sensitive Sartorius top loading balance and the lengths (standard and total) were measured using a measuring board.

The length-weight relationship was estimated using the least square regression on log transformation given the equation, cited as Equation 1:

$$\log W = \log a + b \log L \quad (1)$$

Where,

W = weight (g),
 TL = total length (cm),
 a = constant (intercept),
 b = exponent (slope).

The condition factor, was evaluated using this formula, cited as Equation 2:

$$K=100W/Lb \quad (2)$$

Where,

K = condition factor,
 W = total weight (g),
 L = total length (cm) and
 b = the regression coefficient.

3. Results and Discussion

A total of 699 *S. melanotheron* (combined sexes) were captured. The standard length of sampled *S. melanotheron* in this study ranged from 4.15 to 18.92 cm, total length varied from 6.01 to 22.5 cm while the weight ranged from 7.85 to 286.71 g. The b value was 2.1299 which was less than 3 indicating that the growth pattern of the fish was allometric. That is, as the fish increased in length so it increased slightly in weight. The correlation co-efficient (r) value for *S. melanotheron* was 0.7528 which is quite high depicting that there was a strong positive relationship between weight and total length of *S. melanotheron* in the estuary.

This result (negative allometry) is in line with results reported by Guissé and Niass, ^[5] who recorded 2.77 and 2.69 values for the regression coefficient for females and males respectively, and 2.76 and 2.72 for the wet and dry seasons in Gueumbeul (RSFG) in Senegal). Chikou ^[28] in multiple water reservoirs in Benin (b values ranged from 2.55 to 2.76), and those recorded by Ecoutin and Albaret ^[29] in the Ebrié lagoons in Côte d'Ivoire (b = 2.78) and Sine Saloum in Senegal (b = 2.81). These outcomes are unlike those established by Mireku et al. ^[30] in Brimsu reservoir, Ghana with b values that ranged from 4.08 to 4.99 for females and 3.95 to 4.94 for males by Lalèyé ^[31] in the Ouémé River in Benin where the b values (3.07) were not significantly different from 3 and those obtained by Ndimele et al. ^[32] for similar species (*S. melanotheron*) in Ologe lagoon, Nigeria with b values spanning from 3.09 to 3.12.

Le Cren (1951) ^[7] stated that change in growth rate of the same species of fish throughout different months are subjected to many factors such as food supply, environmental factors as well as ecological state of the habitats.

The growth pattern of fish can be greatly influenced by season, population, food availability, sex or physiology (Silva et al. 2015; Cella-Ribeiro et al. 2015; Dieb-Magalhães et al. 2015; Giarrizzo et al. 2015; Freitas et al. 2017) ^[33-37].

In such cases exponential value must be exactly '3' but owing to fluctuating environmental conditions of the fish, the real relationship between the variables usually does not follow cube law (Le Cren, 1951) ^[7]. According to Wootton (1990) ^[17], if fish growth is isometric the exponential value recorded will be exactly 3.0. On the

contrary, a value significantly larger or smaller than the given standard indicates an allometric growth pattern.

In a related statement, Froese (2006) ^[38] reaffirmed that for isometric growth to be recorded, the exponential value must read between 2.5 and 3.5. A lesser value shows a negative allometric growth indicating that a fish becomes lighter with increase in its size whereas a positive allometric is seen when values are higher which indicates that the fish obtained a heavier weight for a particular length. That is, as the fish increased in length so it increased slightly in weight. The correlation co-efficient value for *S. melanotheron* was 0.7528 which is quite high portraying that there was a great positive relationship existing between weight and total length of *S. melanotheron* in the estuary.

The monthly changes in condition factor of *S. melanotheron* showed that there was an increase in value from April 2012 to May 2012. A peak was recorded in June 2012 and a decrease in value recorded to August 2012. Other sharp decreases recorded were in the months of December 2012 and November 2013. This shows that the fish were in better condition during the raining season months.

Furthermore, the result showed that the condition factor was not size dependent, a rise in K value was recorded from size 4.1-5.0 cm to size 6.1-7.0 cm, while gradual drop was recorded from size 6.1-7.0 cm to 9.1-10.0 cm (Figure 2). A sharp rise was then recorded in value in size 10.1-11.0 cm and a gradual drop in value recorded from 11.1-12.0 cm to 13.1-14.0 cm. There was an increase thereafter to size class 18.1-19.0 cm.

The total length recorded in this study was within the range recorded by Ayoade and Ikulala 2007 ^[3] and Oribhabor et al. 2009 ^[10]. The value reported on the growth equations for the fish species was within the bounds of 2 and 4 described by Tesch (1971) ^[26] for most fish. Most aquatic organisms change shape as they increase in size, thus deviating from isometric growth (Thomas et al., 2003) ^[27]. This changes is usually influenced to a larger extent by the productivity of environment of such organism (Abdul et al. 2010) ^[39]. This is similar to the report of Laléyé (2006) ^[31], Ayoade and Ikulala, 2007 ^[3] for *S. melanotheron*. The correlation coefficient (R^2) of 0.7528 for *S. melanotheron* indicates increase in length with increase in weight. This findings is in agreement with earlier studies which has to do with fish species from unrelated water bodies (Laléyé 2006; Ayoade and Ikuala 2007) ^[31,3].

The correlation coefficient (R^2) values of 0.7528 reflects that there is a robust correlation linking the length and weight of *S. melanotheron* species in Forcados

River estuary. This result was in line with reports from Gueumbeul basin for this cichlid species.

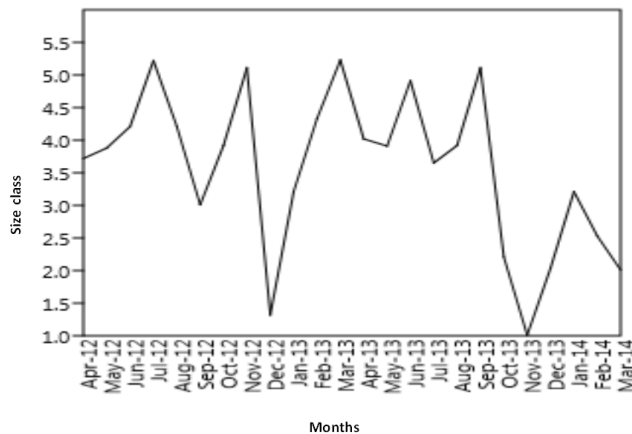


Figure 1. Monthly variation in condition factor (K) of *S. melanotheron* (A)

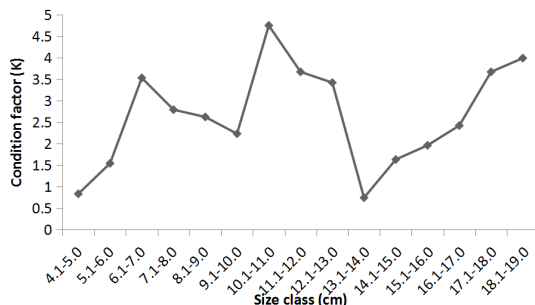


Figure 2. Variation in condition factor (K) of *S. melanotheron* according to size

The condition factor for the combined sexes fluctuated monthly in the estuary. According to Abdul (2009) ^[2], condition factor of fish is a function of phosphate availability in water. Possible sources capable of influencing high phosphate concentration in the estuary include leaching of dead macrophytes, precipitation, surface run off and domestic sewage containing human faeces (Ekhatior *et al.* 2015) ^[40]. The condition factor of fish species in this study was not size specific. This agreed with previous observation by Arawomo (1982) ^[41] and Fagade (1983) ^[42] in their studies. Pauly (1983) ^[43] reported that the differences in weight for the sampled size could be attributed to individual condition factor in relation to the degree of fatness and well-being.

According to Le Cren (1951) ^[7] fluctuations in the condition factor of a fish is associated to the maturity cycle of the fishes. It is also worthy of note that full development of digestive system can be linked to the 'K' factor. Kund *et al.* (2011) ^[44] reported that condition factor of smaller fishes are higher when compared to the large ones due to their edacious feeding nature. Bakare (1970)

and Fagade (1979) ^[42] postulated that the condition factor relatively reduces due to the gradual increase in length.

4. Conclusions

The length-weight relationships and condition factor of *S. melanotheron* in Forcados river estuary indicated that the fish were above average condition. The condition factor for the combined sexes fluctuated monthly in the estuary. The condition factor of fish species in this study was not size specific. Information obtained from the study can be used for better management of *S. melanotheron* in the water body.

Authors' Contribution

Efe Ogidiaka conceptualize the research work, conducted part of the laboratory analysis and drafted the manuscript. John Atadiose did part of the laboratory analysis while Betty O. Bekederemo sourced for literature material used and also reviewed the write up.

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ARTICLE

Planktonic Scenario of the River Ganga & Yamuna at Prayagraj in COVID-19 Lockdown: A Case Study

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ARTICLE INFO

Article history

Received: 22 December 2021

Accepted: 7 February 2022

Published Online: 15 February 2022

Keywords:

River Ganga and Yamuna

Plankton

Diversity

COVID-19

Lockdown & Prayagraj

ABSTRACT

Ganga is the most prestigious river of India. The COVID-19 lockdown may have forced us to stay indoors, but it has been boon for pollution-ridden Ganga and Yamuna. Plankton is tiny organisms drifting with water current, influenced by river physical and chemical factors. During lockdown anthropogenic factors were reduced which affected water and plankton quality. Plankton samples were collected from the upstream of the river Ganga (Shankerghat, latitude 25°30'28" N and longitude, 81°52'10"E) and Yamuna (near boat club, latitude 25°24'29"N and longitude 81°54'50"E) at Prayagraj, during national lockdown. In the before lockdown period (2019), total 28 planktonic taxa were recorded from the river Ganga, among them 10 taxa from Bacillariophyceae, 15 from Chlorophyceae and 3 from Myxophyceae. While during LD period total 54 genera with 86 species was recorded (Bacillariophyceae 10 taxa, Chlorophyceae 23 taxa, Myxophyceae 9 taxa, Euglenophyceae 2 taxa, Dianophyceae, 1, Rotifera 7 taxa, Protozoa 2 taxa). Various species of green algae were observed in this small period of lockdown, some species were not observed since a long, like *Pediastrum tetras*, *Scenedesmus abundans*, *Ankistrodesmus fusiformis*, and *Brachionus angularis*. Various species of phytoplankton and zooplankton were in reproductive phase because river was flowing silently, without any internal and external disturbance. Ganga was more affected by anthropogenic activity and factory discharge than Yamuna So lack of chemicals in the water and minimum human interference favoured auto rejuvenation of Ganga in terms of plankton quality, diversity and reproduction behaviour. Such type of environmental changes may stimulate for origin of new species and disappear or reappear of various aquatic species.

1. Introduction

Phytoplankton are the base of aquatic food web and

of global importance for ecosystem functioning and

services as they account for 1% of the photosynthetic

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DOI: <https://doi.org/10.30564/jfs.v4i1.4275>

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biomass on earth. The dynamics of these cells are linked to annual fluctuations of temperature, water column mixing resource (nutrients) availability and consumption. Climate can modify these environmental factors and alter phytoplankton structure, seasonal dynamics and taxonomic composition. These modifications affect various phytoplankton processes. Climate warming also affects phytoplankton species composition and favours species traits best adapted to changing conditions associated with climate change.

Due to the COVID-19 outbreak, India has declared country-wide lockdown in two phases from 25 March - 14 April 2020, and 15 April - 03 May 2020 [1]. Because of this, all the industrial activities other than essential were closed, and people asked to confine themselves in their houses. The lockdown resulted in minimum disturbance to the nature, especially, the Ganga River [2]. There was less industrial waste effluent in the water, minimum anthropogenic activities along its banks due to restricted pilgrim visits, and other activities along its course [3]. It was reported that the water quality, in terms of clarity or turbidity, of the river has improved at many places along its course during this short time period [2,3]. The corona virus lockdown may have forced us to stay indoors, but it has been boon for the environment and pollution- ridden Ganga and Yamuna. It is stated and proved in several studies that anthropogenic activities are considered as one of the key drivers of pollution in all spheres of the environment [4]. Domestic waste as well as industrial effluents from towns situated near these rivers are the main source of water pollution in the rivers near Prayagraj. With factories discharging toxic industrial waste into the river were closed, improvement in water quality of the river Ganga was observed [5]. This period can be considered as anthropause.

The Ganga river sustains a variety of aquatic biodiversity [6]. As aquatic organisms are very sensitive and important component of the ecosystem which response environmental disturbances [7]. Plankton, the tiny micro-organisms floating in water are important fish food and is in the base of food chain of aquatic ecosystem. Therefore any change in the water leads to the alteration in their groups in relations to tolerance, abundance, diversity and dominance in the environment [8].

Plankton the tiny floating micro-organisms, being in the base of food chain of aquatic ecosystem are important fish food. They response to the surrounding water quality, climate, habitat, disturbance other ecological and anthropogenic factors. Therefore, in this backdrop present study were carried out to perceive the response of phytoplankton and zooplankton to COVID-19 lockdown

in the river Ganga and Yamuna at Prayagraj during April 2020 and May 2020 and results were compared with those of before lockdown period. As algae were the primitive cell of evolution so these are the most important living organisms, and sensitive to the environment. Their quantitative structure and qualitative behaviour are some important tools for bio monitoring of the river Ganga.

2. Materials and Methods

National Lockdown (LD) in India was started from 22 March and samples were collected during the month of April, May and June 2020, from the river Ganga and Yamuna at Prayagraj. Plankton samples were collected from the upstream of the river Ganga (Shankerghat, latitude 25°30'28" N and longitude, 81°52'10"E) and Yamuna (near boat club, latitude 25°24'29"N and longitude 81°54'50"E), by filtering 50 litres of river water and fixed in 4% formalin solution for qualitative and quantitative analysis in the laboratory under high power microscope [9,10].

3. Results and Discussion

3.1 Planktonic Scenario in River Ganga

In the river Ganga plankton abundance recorded were 710 u/l (1st LD), 1130 u/l (2nd LD) and 1340 u/l (3rd LD). Plankton composition revealed all the major groups (Figure 1) as Bacillariophyceae ranged from 32.7-47.8%, Chlorophyceae from 33.8-43.2%, Myxophyceae from 5.3 to 18.3%, Dianophyceae 2.6%, Rotifers from 7.5 to 23%, and Protozoa 2.2%. This can be observed from Figure 1 that in 1st and 3rd LD Chlorophyceae was dominant in the river Ganga, while in 2nd LD Bacillariophyceae. Contribution of 23% Rotifers in the river Ganga was striking feature; their sudden disappearance in 2nd LD and reappearance in 3rd LD was also noticeable phenomenon. In this period, in the river Ganga total numbers of taxa recorded were 40 among them 7 from Bacillariophyceae, 20 from Chlorophyceae, 3 from Myxophyceae, 1 from Euglenophyceae, 1 from Dianophyceae, 7 from Rotifera and 1 from Protozoa. Dominant taxa were *Melosira*, *Synedra*, and *Nitzschia*, (diatoms), *Scenedesmus*, *Ankistrodesmus*, and *Coelestrum* (green algae), were *Merismopedia* and *Microcystis*, (blue green) *Ceratium*, (Dianophyceae) and *Brachionus* and *Keratella* (Rotifera). In before LD period Bacillariophyceae was 15%, Chlorophyceae 66%, and Myxophyceae 19% with dominance of *Melosira* sps and *Asterionella* sp (Diatoms), *Chlorella* and *Elakatothrix* (Green algae), and *Merismopedia* (blue green). Improvement in water quality was reflected by increase in Bacillariophyceae

contribution from 15% to 38% in LD period with presence of diversified 7 planktonic groups (Figures 1, 2).

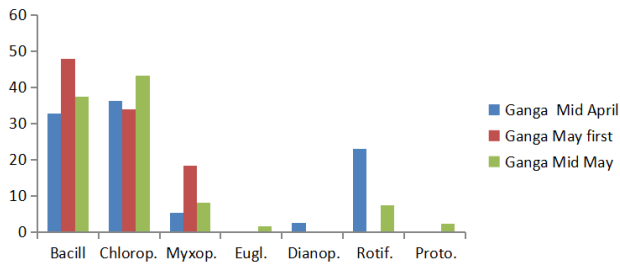


Figure 1. Plankton composition (%) in 1st, 2nd and 3rd LD periods in the river Ganga

Comparative account of percentage contribution of various planktonic communities of the river Ganga and Yamuna before lockdown and in lockdown is presented in Figures 2 and 4.

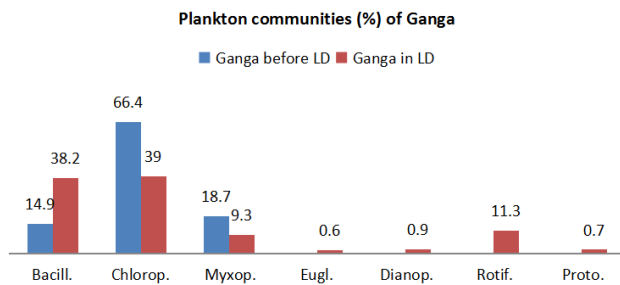


Figure 2. Comparison of average plankton composition (%) in the river Ganga before and during LD period

In the before LD period, total 28 planktonic taxa were recorded from the river Ganga, among them 10 taxa from Bacillariophyceae, 15 from Chlorophyceae and 3 from Myxophyceae. While during LD period total 54 genera with 86 species was recorded (Bacil. 10, Chlorop. 23 Myxop. 9 Eug 2, Dianop. 1, Rotif 7 Proto 2). Various species of green algae were observed in this small period of LD (Plates 1-5). Some species were not observed since a long, like *Pediastrum tetras*, *Scenedesmus abundans*, *Ankistrodesmus fusiformis*, and *Brachionus angularis*. Various species of phytoplankton and zooplankton were in reproductive phase because rivers were flowing silently, without any internal and external disturbance. Most of the species of *Brachionus* was observed in reproductive phase and contributed more for zooplankton abundance. Although factories were closed but sewage channels were open in total lock down period as Rotifers were regarded as pollution or productivity indicator in the river Ganga [11].

In the river Ganga, at higher TDS (257 ppm) *Melosira* sp (Bacillariophyceae) was dominant followed by *Ankistrodesmus* spp. (Chlorophyceae) and *Microcystis* sp. (Myxophyceae). Further at lower TDS (89 ppm Ganga), some morphological changes in *Scenedesmus* sp. were

recorded as reduction in spines (1,3 instead of 2, 4, 6, 8). This may be regarded as adaptation for movement in stress free environment of river water, as the main function of the spines are to maintain buoyancy, reduced TDS may require less efforts for movement. As previously *Scenedesmus* sp with big spines (*S. quadricauda*.) was found dominated, but in lockdown period 90% *Scenedesmus* species were without spines.

3.2 Planktonic Scenario in River Yamuna

Plankton population in the river Yamuna during lockdown period were 750 u/l (1st LD), 610 u/l (2nd LD), 1200 u/l (3rd LD) with 15.8-21.3% Bacillariophyceae, 20-47.5% Chlorophyceae, 31.1-41.3 % Myxophyceae, 4.2% Euglenophyceae, 10-22.6% Rotifera and 6.6% Protozoa (Figure 4). In 1st and 3rd LD phase Myxophyceae was dominant but in 2nd LD Chlorophyceae dominated. Total 23 taxa were recorded among them 4 from Bacillariophyceae, 8 from Chlorophyceae, 5 from Myxophyceae, 2 from Euglenophyceae 4 from Rotifera and 2 taxa from Protozoa. Dominant diatoms were *Melosira*, *Synedra*, and *Nitzschia*, green algae were *Scenedesmus* and *Coelestrum*, blue green were *Merismopedia* and *Microcystis*, and zooplankton were *Brachionus* and *Keratella* (Rotifer). If we compare present studies with that of April 2019, increase in plankton communities and diversity was recorded. Usually in the river Yamuna Rotifers were observed in winters but in LD period rotifers were noticed in April and May 2020. In case of river Yamuna Bacill., Chlorop and Myxop % contribution reduced, as compared to before LD data (Figures 5, 6) but other planktonic groups developed in LD period. Major dominant taxa were *Nitzschia*, *Cyclotella*, (Diatoms), *Actinastrum*, *Coelestrum* (Green algae), and *Microcystis* (Blue green).

Similar to the river Ganga, presence of 22.6% of Rotifers in 1st LD phase, sudden disappearance in 2nd LD and reappearance in 3rd LD was also noticeable phenomenon which may be correlated with high TDS values (Figure 6), as negative correlation of TDS and Rotifers was observed [12].

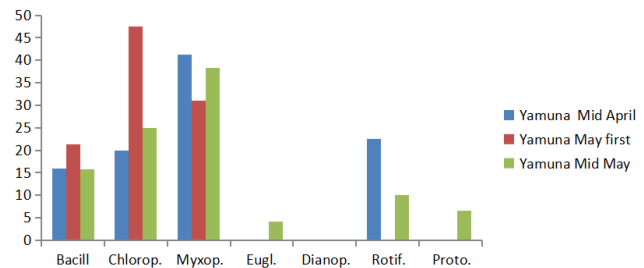


Figure 3. Plankton communities in 1st, 2nd and 3rd LD periods in the river Yamuna.

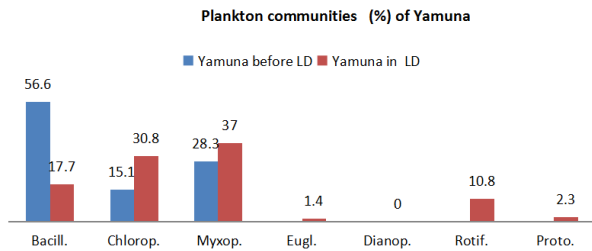


Figure 4. Comparison of average Plankton composition of the river Yamuna before and during LD period

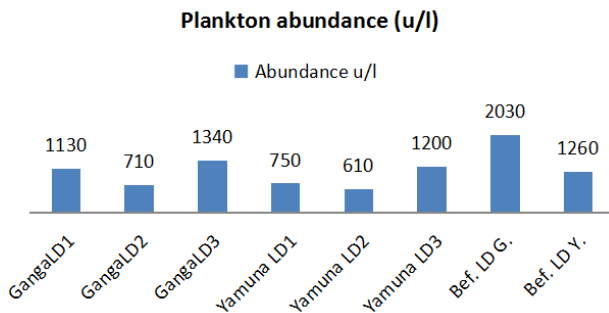


Figure 5. Plankton abundance in Ganga and Yamuna during lockdown, Similar trend of plankton abundance in both the rivers

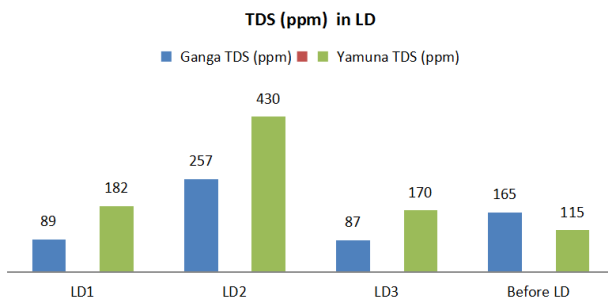


Figure 6. Variation in TDS in Ganga and Yamuna in LD, maximum in LD

Disappearance of Rotifers in 2nd LD may be attributed to the higher (TDS 257 ppm in Ganga and 430 ppm in Yamuna) concentration of TDS as Rotifers were found negatively correlated with TDS in the river Ganga and Yamuna [12]. Our previous studies revealed that, in the river Yamuna, Rotifers peak was recorded in winters [13] but in LD phase Rotifers was recorded in the month of April and May 2020. Although factories were closed but sewage channels were open in total lock down period. At highest TDS (430 ppm) in the river Yamuna Chlorophyceae (*Scenedesmus* sp., and *Chlorella* sp.) was dominant followed by Myxophyceae (*Merismopedia* sp.) and Bacillariophyceae (*Melosira* sp.). While in the river Ganga, at higher TDS (257 ppm) Bacillariophyceae (*Melosira* sp.), was dominant followed by Chlorophyceae

(*Ankistrodesmus* sp) and Myxophyceae (*Microcystis* sp.). In this phase *Melosira* (Diatom) *Scenedesmus* sps, *Chlorella* (green algae) and *Merismopedia* (blue green) were found as salinity tolerant taxa in these rivers. Further at lower TDS (89ppm Ganga), some morphological changes in *Scenedesmus* sp. were recorded as reduction in spines (1,3 instead of 2, 4, 6, 8). This may be regarded as adaptation towards free movement in river water, as the main function of the spines are to maintain buoyancy, reduced TDS may require less efforts for movement because dominant species of *Scenedesmus* was without spines as compared to previously recorded *Scenedesmus* with big spines (*S. quardicauda*.). Therefore species with fewer spines/ no spines, or short spines were observed in water with reduced TDS.

Reduction in total plankton abundance, in comparison to before LD, but gradual increase from 1st to 3rd LD was may be attributed to the reduced eutrophic conditions due to factories shut down and minimum human interference. Yamuna, the big tributary of Ganga also followed the same trend for plankton abundance and composition, CPCB also found that water quality trends of tributaries were similar to the trend observed in the river Ganga during LD period [14]. These environmental conditions were also favourable for reproduction of phytoplankton and zooplankton as recorded in 1st LD. Presence of *Ceratium* in Ganga in the month of April 2020 was also a noticeable feature at the water temp (30.8 °C) and air temp (35.6 °C), as migration of this species has been impacted by global warming. When surface temp of the river water rises, these organisms move to deeper layer of water as they are temperature sensitive. Due to this behaviour species of *Ceratium* are used as bio indicator of global warming [15]. It appeared that this taxa is chemical pollution sensitive also, as during LD factories shut down provided comfortable aquatic environment, even at water temp of 30.8 °C. Similarly various sps of *Ankistrodesmus*, *Elakatothrix*, *Scenedesmus*, and *Pediastrum* etc were recorded in LD period which were not recorded previously. River Ganga revealed improved water quality in LD period, because of lack of anthropogenic activity and factory shut down but River Yamuna exhibited only a slight change in Rotifers contribution only.

These environmental conditions were also favourable for reproduction of phytoplankton and zooplankton as recorded in LD period. Presence of *Ceratium* sp. in Ganga in the month of April 2020 was also a noticeable feature at this water temp (30.8 °C) and air temp (35.6 °C), as migration of this species has been impacted by global warming. When surface temperature of the river water rises, these organisms move to deeper layer of water as

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Ganga was much affected by anthropogenic influence, in comparison to river Yamuna, so by LD zero human interference and factory shut down, resulted in improved water quality which favoured increased diversity and reproduction of planktonic micro organisms in surface water of the river Ganga as compared to before LD period. Ganga was more affected by anthropogenic activity and factory discharge than Yamuna, so lack of chemicals in the water and minimum human interference favoured auto rejuvenation of Ganga in terms of plankton quality, diversity and reproduction behaviour. Increases in percentage contribution of Bacillariophyceae, Rotifera, reduction in Chlorophyceae, Myxophyceae, were recorded in LD period. Various new species of Chlorococcales (green algae) were observed in LD period which were not recorded previously at Prayagraj. Such type of environmental changes may stimulate for origin of new species and disappear or reappear of various aquatic species.

An improved understanding of the inherent natural variability of phytoplankton is therefore important for forecasting the extent of global change impact on aquatic ecosystem functioning. The extent of physical changes and potential for species to adapt to changing environmental conditions will greatly influence food web dynamics as the future climate warms and becomes more variable. Elevated temperature as a stress factor in aquatic environments has received increased attention recently. Population of blue green, green and bacteria, exists naturally at higher temperature, but diatoms cannot tolerate. Decreased temperature during LD improved water quality by increasing diversity and diatoms.

Before considering the effect which pollution may have on the qualities of natural waters, it is necessary to attempt to define just what we mean by natural waters. The change from oligotrophic to eutrophic involves a great increase in the amount of plankton. Oligotrophic water has sparse plant plankton, composed very largely of desmids, and correspondingly few planktonic animals. As fertility increases desmids are replaced by diatoms, and then by various flagellates and other green algae, finally blue green algae appear and these can become so abundant at some

season as to form water blooms. The increase in tiny plants is followed by an increase in the small animals. Different species have different geographical distributions. The factors which control river animals are current speed, the type of substratum, the type of vegetation, the temperature, and the amount of oxygen in river, the hardness of the water and finally the geographic position of the river. All creatures have a limited geographical range so one cannot expect always to find the same set of species in the same type of environment/microhabitat. Most of the above parameters are altered by most type of pollution.

Table 1. Planktonic taxa of river Ganga and Yamuna in lock down phase (April - July 2020)

Plankton species	No.of Species	Plankton species	No.of Species
Bacillariophyceae		Myxophyceae	
Melosira	2	Merismopedia	2
Meridion	1	Microcystis	2
Asterionella	1	Aphanothece	1
Cyclotella	2	Aphanezomenon	1
Tabellaria	1	Phormidium	1
Synedra	2	Nodularia	1
Nitzschia	2	Anabaena	1
Navicula	2	Nostoc	1
Stephnodiscus	1	Lyngbya	1
Epithemia	1	Dianophyceae	
Chlorophyceae		Ceratium	1
Scenedesmus	6	Euglenophyceae	
Pediastrum	3	Euglena	2
Ankistrodesmus	5	Lepocynclis	1
Westella	1	Rotifera	
Coelestrum	1	Brachionus	4
Elakatothrix	3	Asplanchna	1
Chlorella	2	Testudinella	1
Actinastrum	1	Keratella	1
Kirchneriella	1	Polyarthra	1
Schroderia	2	platyias	1
Oedogonium	1	Lecane	1
Protococcus	1	Protozoa	
Eudorina	1	Epistylis	1
Cosmarium	4	Paramoecium	1
Oocystis	2		
Staurostrum	2		
Micrasterias	2		
Desmatractum	1		
Dictyosphaerium	1		
Quadrigula	1		
Selenastrum	1		
Golenkinia	1		
Terubaria	1		

In all rivers there are seasonal changes in abundance, there is a minimum algae in winters and maximum in spring and autumn, the 2 maximal caused by different species. There are different species in oligotrophic and eutrophic waters in the former especially near the source many sp are characteristics, although they may be scarce, but one nearly always finds the diatoms *Eunotia* spp, *Achnanthes* spp and *Diatoma haemale* and often *Ceratoneis* and *Tabellaria* spp and members of *Chaetophorales*. As one proceeds downstream the water becomes more eutrophic and the algal community changes until it becomes dominated by the diatom *Cocconeis placentula*, and the green algae *Chamaesiphon*. Several other species of diatom are also present. These include *Synedra ulna*, *Navicula viridula*, *Surirella ovate*, *Cymbella ventricosa* and *Gomphonema olivaceum*. In our present studies of lockdown, we also noticed presence of phytoplankton species which were noticed previously in the upper stretch of the river Ganga. The controlling factor seems to be the amount of nutrient salts.

4. Conclusions

Ganga was much affected by anthropogenic influence so by lockdown zero human interference and factory shut down, resulted in improved water quality which favoured increased diversity and reproduction of planktonic micro organisms in surface water of the river Ganga as compared to before lockdown period. In the river Yamuna much improvement in water quality, plankton composition and algal diversity could not be recorded in lockdown period, because Ganga was more affected by anthropogenic activity and factory discharge than Yamuna So lack of chemicals in the water and minimum human interference favoured auto rejuvenation of Ganga in terms of plankton quality, diversity and reproduction behaviour. Increase in percentage contribution of Bacillariophyceae, Rotifera, and reduction in Chlorophyceae, Myxophyceae, and presence of 4 more planktonic groups were recorded in lockdown period. Various new species of Chlorococcales (green algae) were observed in lockdown period which were not recorded previously at Prayagraj. Various species of phytoplankton and zooplankton were in reproductive phase because rivers were flowing silently, without any internal and external disturbance. Palmer pollution index was 13 for Ganga and 17 for Yamuna indicating healthy water quality to support aquatic life.

Authors' Contributions

KS drafted the manuscript and analysed the data, JK helped in the identification of the planktons, DNJ helped

in statistical analysis, VRT Edited and revised the MS, VK collected and analysed water parameters and BKD overall supervision.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

Authors are grateful to, the Director ICAR-CIFRI, Barrackpore and Head of ICAR-CIFRI, Allahabad for providing facilities and guidance, and thankful to the staff associated with sample collection.

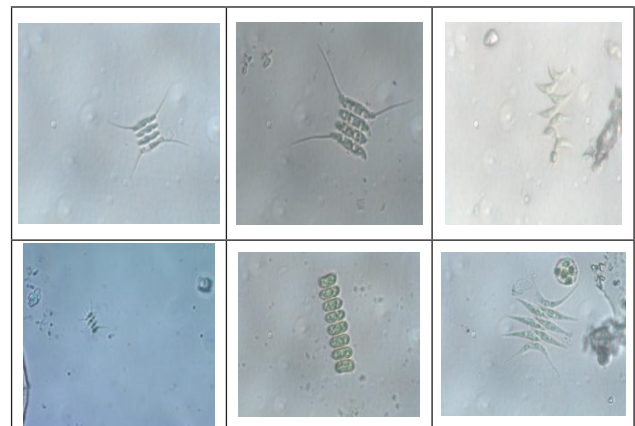


Plate 1. Various sps of *Scenedesmus* with or without spines

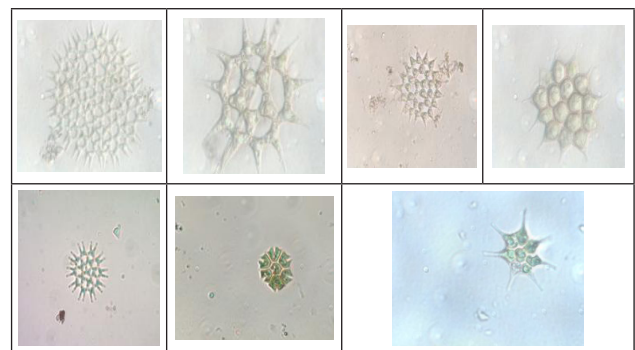


Plate 2. Various sps of *Pediastrum*

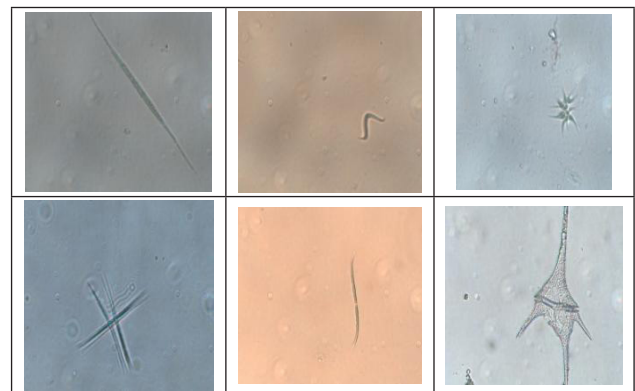


Plate 3. Various sps of *Ankistrodesmus* and rare *Ceratium* sp

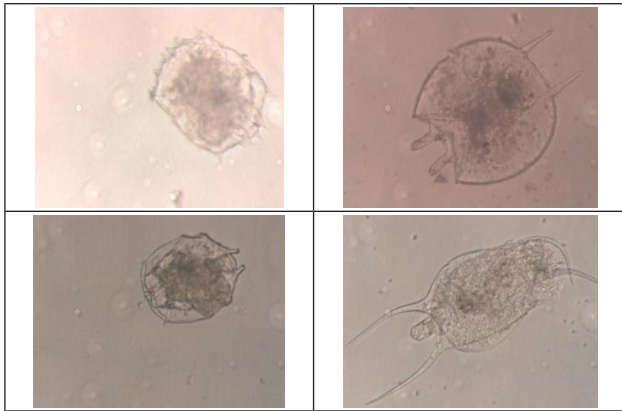


Plate 4. Various sps of *Brachionus*

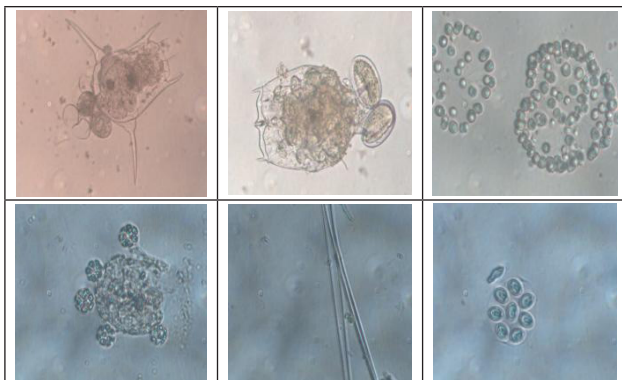


Plate 5. Zooplankton and phytoplankton in reproductive phase.

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ARTICLE

Dietary and Nutritional Value of Fish Oil, and Fermented Products

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ARTICLE INFO

Article history

Received: 31 December 2021

Accepted: 7 February 2022

Published: 14 February 2022

Keywords:

Fish foods

Eicosapentaenoic acid (EPA)

Docosahexaenoic acid (DHA)

Dietary and therapeutic value

ABSTRACT

Present review article explains the dietary and nutritional value of various fish derived natural food products. Fish is a good source of important nutrients such as proteins, fats, vitamins and minerals. Fish oil contains polyunsaturated fatty acids (PUFAs) mainly omega-3 fatty acids, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and eicosanoids. Fish contains high-quality protein (~ 14-16 percent) and is consumed worldwide. This article also emphasizes therapeutic uses of fish nutrients and oil in healing of wounds, hyper pigmentation, dermatitis, and in cardiovascular risks. Fish oil polyunsaturated fatty acids (PUFAs) are highly beneficial in cardiovascular problems and dermatitis. Fish oil is good for skin-related diseases such as photo-ageing and melanogenesis. These also affect anticancer, wound healing and anti-depressant activity. In the present review various local, national, and international processed fish derived food currently available in the market fish dishes have been mentioned.

1. Introduction

Fish is a major source of food for millions of people throughout the globe. Fish derived food has high nutritional value as it is rich in protein, fat, mineral, vitamins, etc.^[1] Fish derived food possess high-quality protein and approximately 16-17% of the animal proteins come from fish eating at global level by the people. Fish protein is a good substitute of livestock protein in various parts of the world. In coastal areas fish eating is significantly much higher than land locked areas. Consumption of marine fish is more than fresh water fishes. Coastal countries

consume fish foods at large scale as fish supplies <10% of animal protein consumed in North America and Europe. It is 17% in Africa, 26% in Asia and China uses 22% fish foods. Fish trade is of very high economic importance. According to an estimate fish export by US\$ is 51 billion per annum alone that has been exceeded much higher in these two decades. Both fishery and aquaculture has social implications as more than 37 million people are directly involved in it, and more than 200 million people seek direct and indirect income from fish production (FAO 2003)^[2]. Global production of aquatic animals becomes 179 million metric tons (mmt) in 2018, valued at US\$401 billion, of

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DOI: <https://doi.org/10.30564/jfs.v4i1.4311>

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which 97 mmt originated from capture fisheries. About 25% of capture fisheries is diverted to non-food uses; mainly fishmeal and fish oil used in aquaculture feeds. Of total fish production 46 percent of the total production comes from aquaculture farming and 52 percent of fish for human consumption. China becomes a major fish producer and it produced 35 percent of global fish production in 2018. Other than China 34 percent production is noted from Asia (34 percent), followed by the Americas (14 percent), Europe (10 percent), Africa (7 percent) and Oceania (1 percent) in 2018. The global average supply of aquatic food (excluding seaweeds) has grown by about 1.5% per annum, to reach 20.5 kg per capita in 2018 (FAO, 2020)^[3]. This high consumption rate is due to easy availability of variety of fish products. For better utilization and usage fish processing and fermentation industries turn out large quantities of fisheries products in the international market. Fish foods consumption is increasing every year because of its high nutritional and health benefits. Both fresh water fishes and marine fishes are major source of high-quality animal protein, oil, vitamins and minerals throughout the globe. Freshwater fishes are more edible and have higher consumption rate in plane areas. There is a large demand of freshwater inland fish species like singhi (*Heteropneustes fossilis*), magur (*Clarias batrachus*), murrels (*Channa sp.*), and koi (*Anabas testudineus*) in India, Nepal, Bangladesh and other coastal south-east Asian countries. These are also famous for their therapeutic properties.

Fish and fish products are of historical, regional, and cultural significance. These are used since ancient times. Nutritionally fish is used an important source of proteins, oils, enzymes, hormone, vitamins, micronutrients and minerals and oils. Fish is, used to prepare the various types of fermented food products. These possess high nutritional value. A variety of fish are used as an ingredient in many curries, food items, and sauces. Besides fish oil, fish food, fish manure, fish glue are the main commercial products. Present review article explains nutritional and therapeutic value of various fish foods used round the world.

2. Nutritional Value of Fish Derived Food

Fish derived foods are a rich source of proteins, amino acids lipids, minerals, lipids, vitamins, and carbohydrates. Fish contains 15 to 20% protein contents that vary according to size, liver body weight and species. The fish derived food products possesses physiologically required amounts of essential amino acids which improve the overall protein quality of a mixed diet and its health benefits. In addition, fishes are good sources of PUFAs as 40% of these possess 5 or 6 double bonds (C14 - C22)^[4]. Fish lipids (oils) are nutritionally much better in quality

than mammalian lipids. Fish liver oils possess vitamin B complex and fat-soluble vitamins A and D.

2.1 Fish Proteins

Fish origin foods possess higher contents of nutritionally important proteins than other terrestrial animal meats^[5]. In addition, aquatic proteins are easily digestible and contain so many peptides and essential amino acids^[6]. Dietary use of fish proteins is highly beneficial for human health it lowers down insulin resistance, leptin and TNF α , it improves hyperglycemia and decreased adipose tissue oxidative stress^[7]. Dietary use of fish protein hydrolysates lowers down tumor necrosis factor α (TNF α) in comparison to casein hydrolysates in human macrophages. PUFA and fish protein hydrolysates synergistically decrease expression levels of TNF α (Arginine an amino acid from fish minimizes the production of superoxide anions by nitric oxide synthase (iNOS) while glycine repress the expression of TNF- α and the pro-inflammatory interleukin-6 (IL6) in cell cultures^[8]. Similarly, taurine, is an amino acid excreted as fish by product, it suppresses the production of TNF α , IL6, and interleukin-1b (IL-1b). Its consumption delays type 2 diabetes through different molecular mechanism^[9]. Sardine is good source of omega-3 fatty acids (EPA & DHA) which have multiple health benefits. Thus, consumption of fish food is highly beneficial as it cut down both glucose and lipid levels, and reduce the chances of cardiovascular problems in man^[10].

Dietary use of salmon fish also lowers down inflammation while consumption of cod protein increases improvement in insulin sensitivity in rats^[11-13]. Fish proteins also promote growth and regeneration of skeletal muscle after trauma compared to peanut protein and casein. Salmon calcitonin, is an 32 amino acid peptide cut-down blood calcium. This is 40 to 50 times more potent than human calcitonin. Supplementation of fish protein in diet improves health of children affected with Kwashiorkor (chronic protein deficiency) and marasmus (chronic deficiency of calories). It is much beneficial in eradication of protein deficiency diseases.

2.2 Micronutrients

Fish supplies important minerals calcium, phosphorus, iron, copper selenium trace elements like fluorine, and zinc. Calcium obtained from fish bones is easily absorbable.^[14] Marine fishes contain zinc in higher quantities. Zinc is also required for activity of several enzymes which participate in catabolism carbohydrates, cell division, growth, development and in making immune defence Zinc is also required for enzymes which assist

in recognition of senses of smell and taste. Fish derived food items contain higher contents of vitamin D. A. E. K and B complex mainly thiamine and B complex mainly thiamine, riboflavin and niacin (vitamins B1, B2 and B3) (Table 2) Fish liver contains higher amounts of vitamin D and oils, both are required for bone growth, calcium absorption and metabolism of. Vitamin D is required for

preparation of immune defence and protection against carcinoma ^[15]. Fish eating is good for health as it supplies few important mineral elements including calcium and phosphorus (Table 2).

3. Fermented Fish Products

Thousands of fermented fish derived food items are

Table 1. The Calories value and nutritional information of different edible fishes provided by the USDA

Fish type	Calories	Fat	Sat. fat	Omega -3	Proteins	Carbohydrates	Sugar	Fibre	Sodium	Mercury levels
Anchovies	111	4 g		1.7 g						
Bass	82	1.7 g, 0.4 g	0.4 g	506 mg	15.7 g	9 g		0 g	58 mg	120 ppb
Catfish	81	2.4 g	0.6 g	309 mg	13.9 g	0 g	0 g	0 g	37 mg	144 ppb
Clams	73	0.8 g	0.2 g	91 mg	12.5 g	3 g	0 g	0 g	511 mg	28 ppb
Cod	70	0.6 g	0.1 g	156 mg	15.1 g	0 g	0 g	0 g	125 mg	70 ppb
Crawfish	61	0.8 g	0.1 g	122 mg	12.6 g	0 g	0 g	0 g	53	34 ppb
Flounder	60	1.6 g	0.4 g	208 mg	10.6 g	0 g	0 g	0 g	252 mg	115 ppb
Grouper	78	0.9 g	0.2 g	210 mg	16.5 g	0 g	0 g	0 g	45 mg	417 ppb
Haddock	63	0.4 g	0.1 g	112 mg	13.9 g	0 g	0 g	0 g	181 mg	164 ppb
Halibut	186	2.7 g	0.6 g	396 mg	37.9 g	0 g	0 g	0 g	139 mg	261 ppb
Lobster	65	0.6 g	0.2 g	145 mg	14 g	0 g	0 g	0 g	360 mg	200 ppb
Oysters	43	1.4 g	0.4 g	263 mg	4.8 g	2.3 g	0.5 g	0 g	71 mg	18 ppb
Rainbow Trout	101	2.9 g	0.6 g	499 mg	17.4 g	0 g	0 g	0 g	26 mg	344 ppb
Salmon	177	11.41 g	2.6 g	1,671 mg	17.4 g	0 g	0 g	0 g	50 mg	26 ppb
Sardines	177	9.7 g	1.3 g	835 mg	21 g	0 g	0 g	0 g	261 mg	79 ppb
Scallops	59	0.42 g	0.1 g	88 mg	10.3 g	0 g	0 g	0 g	333 mg	40 ppb
Shrimp	72	0.4 g	0.1 g	52 mg	17 g	0 g	0 g	0 g	481 mg	52 mg
Snapper	85	1.1 g	0.2 g	264 mg	17.4 g	0 g	0 g	0 g	54 mg	230 ppb
Spanish Mackerel	118	5.4 g	1.6 g	1,140 mg	16.4 g	0 g	0 g	0 g	50 mg	440 ppm
Squid	78	1.2 g	0.3 g	415 mg	13.2 g	0 g	0 g	0 g	37 mg	44 ppb
Swordfish	122	5.7 g	1.4 g	641 mg	16.7 g	0 g	0 g	0 g	69 mg	893 ppm
Tilapia	81	1.4 g	0.5 g	77 mg	17 g	0 g	0 g	0 g	44 mg	19 ppb
Tuna	93	0.4 g	0.1 g	85 mg	20.7 g	0 g	0 g	0 g	38 mg	270 ppb

Table 2. Major vitamins and minerals reported in Salmon, Sardine, Trout and Mackerel per 100 gram flash.

Salmon	Sardine	Trout	Mackerel
Vitamins % of RDAs(Recommended Dietary Allowances)			
Vitamin C-6%	Vitamin B6-10%	Riboflavin-19%	Vitamin D-90%
Vitamin B12-53%	Vitamin D-48%	Vitamin B12-130%	Vitamin B12-145%
Vitamin B6-30%	Vitamin B12-148%	Thiamin-23%	Vitamin B6-20%
Vitamin A-%	Vitamin A2%	Niacin-23%	Vitamin A-3%
Minerals			
Selenium-34%	Iron-16%	Selenium-18%	Selenium-63%
Magnesium-6%	Magnesium-9%	Magnesium-43%	Magnesium-19%
Potassium-10%	Potassium -11%	Potassium-10%	Potassium-9%
Phosphorus-24%	Calcium-38%	Phosphorus-24%	Phosphorus-22%

available in the market having different brand names. Different fish fermentation methods are available in different countries, which have special and unique taste and nutritional value. For development of different tastes salt is added during fermentation processing. The taste of natural fish and digestibility of fish derived food items differs from geographical location, water quality, and fish food preference^[16]. During fermentation a series of desirable biochemical changes are done to generate unique taste of finished product. For making fish products more edible and digestible steaming, drying, pasting and saltation are followed. For fermentation various microorganisms and enzymes are used. Both microorganisms and enzymes make food items soft after acidification, gelation of myofibrillar and sarcoplasmic muscle proteins. These also do degradation of fish proteins and lipids. More specifically, acidification generates some antimicrobial substances, which cause de-contamination of processed food material and extend their shelf life. Due to gelation of protein muscle loss its elasticity, cohesion and hardness. Enzymatic degradation of proteins and lipids provides a flavored taste to the dietary nutrients^[17].

Fermentation is done under strict controlled conditions for preservation of primary and secondary microbial metabolites in final finished. Fermentation has important steps such as washing, degutting, salting, drying, and smoking. These steps are followed to develop unique flavor, texture and color of finished products. Few traditional methods are also used for fish fermentation. These methods have important operation steps i.e. dry salting and brine salting, freezing, and smoking, fermentation^[18]. The major fish products are dried, salted, fermented, and smoked fish. Fermentation significantly enhances the quality of meat such as taste, texture and nutritional value^[19,20].

3.1 Traditional Fermented Fish Products

Various traditional methods of fish fermentation are available throughout the world. For fermentation large size edible fish is chopped in to pieces or a small size fish is used as whole^[21]. Now it is used to make a paste and finally a products fish sauce is prepared^[22]. For increasing the edibility and taste few fermented products are fully changed into a liquid state^[23]. Generally silver carp is used for preparation of fish slurry^[24]. For providing a desirable unique taste, quality and scaling of product fermentation is done by using few selected microorganisms in controlled conditions. More especially bacteriocins or other antimicrobials are used to check the growth of undesirable organisms^[25]. For production of fermented fish paste four commercially available mold

starters are used^[26]. These are few fungal species belong to *Aspergillus* and *Actinomucor* genus which are used for fermentation^[27,28]. These micro-organisms should be nonpathogenic in nature and aid in production of unique taste- and flavor. For fermentation lactic acid bacteria (LAB) are also used as starter culture to obtain final finished fish products^[29-32]. In different countries various methods of fish fermentation are prevalent. Moreover, in Thailand Som-fug, is prepared by using *Lactobacillus plantarum*, *Pediococcus acidilactici*, and *P. pentosaceus*. Similarly, for fermentation of Thai fish. *L. plantarum*, *Lactococcus lactis subsp. lactis* and *L. helveticus* are used. For the production of ferment mackerel mince fermentation is done at 37 °C temperature^[33]. A mixed-strain culture of *L. plantarum* 120, *L. plantarum* 145, and *P. pentosaceus* 220 is used in fermentation of Suanyu, a traditional Chinese fermented fish. For improvement of quality of the whole carp-based product fermentation is done for very short time duration^[34].

Type of substrate used, salt contents, and carbohydrate material is added to generation of aroma/smell and unique taste in final products. For this purpose, generally rice, millet, flour, and even syrup or sugar are used. Millet is used as a major carbohydrate source in Northeastern Asian countries, whereas in Southeastern Asian countries, rice is commonly used as a carbohydrate source^[35]. Carbohydrates addition to a mixture of fish serve as energy source and accelerate the fermentation, it also helps in the absorption of excess moisture and provides a distinctive flavor to the final product^[36]. Salt also inhibits the growth of food spoilage microorganisms and increases the growth of halophilic and salt-tolerant bacteria during fermentation. Addition of salt lowers down effects of water on the finished product, and generates desirable taste. Furthermore, on the basis of addition of salt fermented fish products are classified as high-salt (more than 20% of the total weight), low-salt (3-8%), and no-salt products. Today, fermented fish products are available in the market and become very popular in Asia, Africa, and Northern Europe due to their unique flavor and taste. This making it tasty and maintain its quality non pathogenic microbial starter cultures, crude materials, unique fermentation conditions, are used different parts of the world.

3.2 Fermented Fish in Europe

In many European countries, various traditionally fermented fish derived products are available in the market with different brand names for sale. Fish sauce is very popular in many European countries. A fermented fish sauce “Garum” was used traditionally used by Ancient

Greeks and Romans”^[37]. For the preparation of it both Mackerel and herring fish were used because they possess best edible ingredients. Hakarl, a fish item most popular in Iceland and other European countries are famous for its delicacy. This is prepared from sharks; it can be preserved and used for several years^[38]. The fermented Hakarel provides good energy and quality nutrition to mostly old age people. Many European MNCs are producing fish sauces by following traditional Garum production methods^[49]. Similarly, Surströmming is prepared from herring by fermentation; it is very popular in Sweden because of its special smell and taste. For this herring fish is pre-salted, and saturated in salt solution before fermentation. Only fleshy muscular portion of fish is used for fermentation at 16-19 °C for 21-28 days. After fermentation soup is filled in vessels including brine and finished product. Fermentation is continuously done for about six months. Starter culture used is halophilic bacteria, *Haloanaerobium* and anaerobic is used for generation of Surströmming product with a unique taste and flavor and contains protein (11.8%), salt (8.8%) and fat (3.8%) and the pH ranges between 7.1-7.4^[40]. In Norway Rakfisk a native fish dish is very popular due to its taste and flavor; it is commonly eaten during Christmas season in many European countries. Trout or char freshwater salmon are used to prepare this dish. For obtaining unique taste 3-5% salt is used with little amount of sugar. Fermentation is done at 5-7 °C for more than 120 days. LAB used as dominant bacteria is used in Rakfisk fermentation^[41].

3.3 Fermented Fish in Africa

In African countries, whole fish is preserved after fermentation. For getting fresh fish for dietary utilization, It is treated with salt before drying because of extremely unfavorable climatic conditions. In Africa are Lanhoun, is a salt treated fermented fish item. This is prepared by using condiments and fermentation of whole cassava fish (*Pseudotolithus senegalensis* is used^[42]). In Ghana Momone is a very popular fermented fish product having an unique taste and flavor^[43]. Besides this, fermentation products are produced from different African freshwater fishes. Similarly, in Egypt, Feseekh is salted fermented products prepared from un-dried fish. In African countries fish fermentation is done for a very short period of time and usually done after adding some higher amounts of salt, and the product is used either whole or in pieces.

3.4 Fermented Fish in Asian Countries

In Southeast Asian countries dozens of processed and fermented fish products are traditionally prepared and available in market. These are famous for their

special taste, flavor and differ from the rest of the world. Fish fermentation is based on traditional culture and is relatively done for a longer period from 15 days to several months or even longer. Several fermented dishes and recopies and sauces are prepared by fermentation process and are available in Asian markets. Most of these items are prepared by using traditional methods by local people. Their market is expanding at the global level year after year. Among these items variety of sauces prepared from fish fermentation are sold in different names in different countries for example in Thailand it “Nampla”^[44] in Malaysia “Budus”^[45]; in Philippines “Patis”^[46]; in Indonesia “Bakasang”^[47] and in China, Yu-lu are very famous^[48].

Hundreds of fermented fish items/ products such as Plaa-som a Thai product are very popular because of its salty taste. Its other preparations are such as full fermented fish, fillets and dry salted flakes are also sold. These are prepared by mixing little bit of salt, and cooked with rice and meshed garlic. In some cases, palm syrup and roasted rice are used to in some preparations cooked rice and garlic are replaced by palm syrup and roasted rice for generation of special taste and aroma^[49]. A fermented fish item Plaa-som. Suanyu is made by using very less amount of salt. Fresh water fishes i.e. *P. pentosaceus* and *Z. rouxii* are also used for fermentation. Pekasam is very popular in Malaysia, it is prepared by fermentation of whole fish traces of salt and rice both roasted and uncooked.

In India Shidal a fermented fish product is prepared from dried salt-free punti fish (*Puntius sophore*) and phasa fish (*Setipinna phasa*) Shidal is fermented fish item that is made by using traditional methods. This is very popular in rural and semi-urban India^[50]. It is prepared by using *Staphylococcus* as starter culture, while *Micrococcus* and *Bacillus* are used in phasashidal fermentation. For preparation of Shidal punti and pahsma fish (*Puntius sophore* and *Setipinna phasa*) are dried without adding salt. Similarly, small fish puthymaas (Ticto barb) is fermented with traces of salt and chopped green chilies, tomatoes, ginger, and rice after roasting^[51]. This fish recipe is very popular in North-East India. Another fish product Ngari is also very popular in Manipur is also very popular. People eat it with cooked rice. This is prepared after fermentation for 2-3 days and two days drying in sunshine or in heat^[52]. In Korea, Jeotgal and dried bonito fermented fish product Katsuo-bushi is highly popular dish in Japan. Both are traditionally fermented fish foods. Skipjack is a traditionally prepared fermented fish item by rural people, they use *Aspergillus* and *Eurotium* species as starter culture. It is prepared by fermentation

of mackerel, bonito and tuna fish species are used (*Auxis rochei*, *S. orientalis*, *E. affinis*, *A. thazard*)^[53]. It is famous for its special taste and is much better than raw fish products.

3.5 Health Benefits of Fermented Fish Food

Dietary use of fish provides major ingredients which are essentially required for body growth and metabolism. Fish is an important source of human diet; it is highly beneficial for human health. Fish proteins and fatty acids are easily absorbed and provide energy. Pekasam and shidal are fermented fish food products are highly edible, tasty and are good source of nutrients and natural antioxidants. These were found highly effective in prevention of ROS-related chronic diseases^[54-56]. Jeotgal contains high amount of coenzyme Q10 (CoQ10) (291 mg/g). CoQ10 is a powerful antioxidant and an essential cofactor that assists in energy production and boost up immune function^[57,58]. CoQ-10 deficiency results in several clinical disorders like chronic heart failure^[59], hypertension^[60], Parkinson's disease^[61], and malignant growth^[62]. Fermented fish proteins and peptides show antihypertensive activity and show inhibition of ACE (angiotensin I-converting enzyme) activity. In Japan dried bonito is "Katsuo-bushi," a traditionally prepared fish origin food item, its dietary use regulate blood pressure without any adverse side effects^[63]. This is also used as a nutritional supplement that assists in recovery from fatigue^[64]. In addition, some other fish products

such as Narezushi showed antihypertensive while Kajami-sikhae (flatfish), chuneobamjeot (shad gizzard) anticancer activity. Jeotgals, stops proliferation of liver hepatocellular carcinoma HepG2 cells^[65]. Fish food items contain various peptides, glutamic acid, and glutamine and tripeptides showed immunomodulatory effects^[66]. Fish food contains several ingredients which act as stable fibrin-clotting inhibitors. These show anticoagulant and anti-platelet effects. Fibrinolytic enzymes are used to for maintaining softness in fermented fish such as small cyprinid fish (*Puntius sophore*) fibrinolytic enzymes are used which break fibrin and fibrinogen^[67]. In addition, fish oil is highly beneficial for human health. More often, omega-3 fatty acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) have great therapeutic value in disease prevention (Table 3). These are good nutritional supplements that can be used by any age group^[68,69] and have multiple health benefits against many physiological diseases^[70]. Fish oils are precursors to eicosanoids which lowers down inflammation^[71] and ischemic heart disorders^[72]. The PUFAs in fish oils assist in curing rheumatoid arthritis, psoriasis, ulcerative colitis, asthma, Parkinson's disease, osteoporosis, diabetes mellitus, cardiovascular events, cancers, and depression^[73].

4. Fish Oil

Fish oil is mostly extracted from fishes menhaden (*Brevoortia* sp., *Ethmidium maculatum*); tuna and mackerel; sardine and herring; anchovy; halibut (*Hippoglossus*

Table 3. Principal fatty acids (%) of major commercial fish oil.

Fish oil/s	Myristic C14:0	Palmitic 16:0	Palmitoleic C16:1	Oleic C18:1	Eicosaenoic C20:1	Eurucic C22:1	ω-3 fatty acids	Docosahexaenoic Acid (C22:6)
Anchovy	9	19	9	13	5	2	17	9
Capelin	7	10	10	14	17	14	8	6
Cod liver	3	13	10	23	0	6	11	12
Herring	7	16	6	13	13	20	5	6
Horse mackerel	8	18	8	11	5	8	13	10
Mackerel	8	14	7	13	12	15	7	8
Menhaden	9	20	12	11	1	0.2	14	8
Norway pout	6	13	5	14	11	12	8	13
Pilchard	8	18	10	13	4	3	18	9
Sprat	1	16	7	16	10	14	6	9
Sand eel	7	15	8	9	15	16	9	9
Marine menhaden	7	15	10	15	3	2	17	10

sp.); salmon (*Oncorhynchus* sp. and *Salmon* sp.); and cod (*Gadus* sp.). Whale (Order Cetacea) blubber, seal (clade Pinnipedia) blubber, and shark liver ^[74,75] (Table 3). Its quality depends on fish species, water quality and climate. Fish production also, depends on method of fish culture and technology used. Although many species of fish are used to produce fish oil, a single species is typically used for any single production run of fish oil ^[76]. Fleshy marine fish oil, possess higher contents of oil in their liver hence single species largely used for oil production (Rizliya and Mendis, 2014). Peru is a major producer of most fish oil as it covers one-third of the global market ^[77]. According to FAO, 2020 data Denmark, Japan, and Iceland are also prominent producers of fish oil. Overall, Peru is the world's largest exporter of fish oil; together, Peru and Chile are responsible for 39% of global fish oil exports. Most of the fish oil produced in many countries like Norway, United States, Canada Peru, Chile, and other South American countries. Fish oil extraction is a major source of socioeconomic benefits in small scale coastal communities, it is a ocean based livelihoods of millions of people ^[78]. Enzyme assisted oil extraction is done from whole fish that provides oil in large quantities ^[79]. In few countries more than 70% of fish are used exclusively for the production of fish meal and fish oil. Approximately 58% of total fish oil production comes from non-species-specific.

4.1 Physical Properties of the Fish Oil

Refined fish oil possess triglycerides in the mixture, it partially solidifies because triglycerides have a higher melting point than fish oil. Generally fish oil is liquid at room temperature (20 °C). For dietary use triglycerides are removed to increase EPA and DHA concentrations in the fish oil and its products up to an efficient range ^[80] (Table 4).

Table 4. Physical Properties of fish oil

Property	Value
Appearance	Amber colored oil
Odour	Characteristic fish odour
Molecular weight	EPA :302.45, DHA 328.49, other oils vary
Melting point	10-15 degree C
Flash point(fatty acids)	Approximately 220 degree C
Boiling point	>250 degree C
Specific gravity (30 degree C)	0.91

4.2 Chemical Composition of the Fish Oil

Fish oil contains omega-3 PUFAs, whose concentration depends upon the type of fish, the body part for oil extraction and procedure followed ^[81]. Fish oil contains long-chain fatty like saturated, monounsaturated, polyunsaturated fatty acids of carbon chains ranging

from 14 to 22 carbon atoms. Approximately one-third of the fatty acids present in fish oil are omega-3 long-chain polyunsaturated fatty acids (LC-PUFAs). Omega-3 fatty acids possess carbon chains ranging from 18 to 22 carbon atoms possess a double bond located at the third carbon atom from the end of the carbon chain (i.e., the methyl or omega [ω] end). EPA has a 20-atom carbon chain; DHA has a 22-atom carbon chain. The ratio of fatty acids in fish oil depends on species and type of fish, geographical location of fish culture (Table 4). Important omega-3 fatty acids found in fish oil are EPA, DHA, ALA, stearidonic acid, docosapentaenoic acid (DPA), and arachidonic acid. The omega-3 LC-PUFAs in fish oil are mostly EPA and DHA with some DPA ^[82] have been linked to potential health benefits ^[83] (Tables 5 & 6).

Table 5. Chemical composition of various fish oil

Fatty acids	Lipid Number	% by Weight in Fish Oil
Polyunsaturated fatty acids		
Omega-3 Fatty		
α -Linolenic acid/ ALA	C18:3 (n-3)	6.0%
Stearidonic acid	C18:4 (n-3)	-
Arachidonic acid	C20:4 (n-3)	-
Eicosapentaenoic acid/ EPA	C20:5 (n-3)	27.5%
Docosapentaenoic acid/DPA	C22:5 (n-3)	2.2%
Docosahexaenoic acid	C22:6 (n-3)	8.9%
Omega-6 Fatty Acids		
Linoleic acid	C18:2 (n-6)	-
γ -Linoleic acid	C18:3 (n-6)	1.6%
Eicoatetraenoic acid	C20:4 (n-6)	1.2%
Monounsaturated Fatty Acids		
Palmitoleic acid	C16:1 (n-7)	11.3%
Vaccenic acid	C18:1 (n-7)	2.8%
Oleic acid	C18:1 (n-9)	7.8%
Eicosenoic acid	C20:1 (n-9)	
Cetoleic acid	C22:1 (n-11)	
Saturated Fatty Acids		
Myristic acid	C14:0	6.1%
Heptadecanoic acid	C16:0	7.6%
Palmitic acid	C17:0	2.0%
Stearic acid	C18:0	6.2%

Unrefined fish oil is containing approximately 90 percent long chain fatty acids (EPA, DHA, and others), sterols (including cholesterol), fatty acid esters, cholesterol, and free fatty acids ^[84] (Table 6). Unrefined fish material contains

Table 6. Comparison of LC-PUFAs Concentrations from Herring, Salmon, Cod Liver, eel oil, Shark oil, Tuna oil, Lemuru oil

LC-PUFA	Salmon Oil (mg/g FA)	Herring Oil	Cod Liver Oil	Eel oil	Shark oil	Tuna oil	Lemuru oil
DHA	140	20-62	96-114	0.16	0.28	24.56	4.60
EPA	194	39-88	100-104	0.19	0.05	7.81	14.36
Myristic acid	67	46-84	40-50	5.42	0.12	2	8.80
Palmitic	156	101-150	112-122	10.41	1.21	12.93	15.71
Palmitoleic	82	63-120	74-91	0.17	0.28	2.25	9.76
Oleic acid	140	93-214	238-259	28.25	2.68	11.18	7.78
Eicosenoic acid	18	110-199	71-110	0.17	0.21	1.96	0.23
ALA	8	2-11	12-20	7.77	0.21	0.32	0.39
GLA	3	NR	2	0.56	NR	0.02	0.04
n-6 Linoleic acid	15	6-29	23-42	0.10	NR	0.04	0.28

*NR- not reported

vitamins A, D, E, and some water-soluble amino acids, peptides, and minerals. Shark liver oil contains hydrocarbons such as squalene in more quantity, but commercial fish oils usually contain hydrocarbons less than 0.2 percent. The lipid number takes the form C:D (n-x), where C is the number of carbon atoms, D is the number double bonds, and n-x symbolizes the location of the last (or ω) double-bond. A lipid number ending in n-3 displays an omega-3 fatty acid (Tables 5 & 6).

4.3 Fish Oil Processing

Fish oil is extracted from muscles and liver. Living fishes are caught for maintaining oil quality^[85]. For oil extraction fish are chopped in fine pieces, it is heated and steamed at 100 °C for wet reduction^[86]. This steamed and cooked material is then strained and sent to a press, and oil is separated from the pressed liquid fish mass^[87]. For obtaining fish oil liquid is allowed to centrifuge at high speed for 1-2 hrs. Now separated oil is washed with hot water for polishing^[88]. After separation in pure form oil is filled in tanks for storage. The remaining fish solids are dried and used as fish meal. At this point in the process, the only additions to the fish oil are water, heat, and pressure. The waste streams from this process include emissions of the volatile organic compounds. Net yield obtained is about 20 -80 kilograms/ ton of fish.

Further, fish oil is processed by hardening, and extracted oil is allowed treat with an alkaline solution e.g., sodium hydroxide, potassium hydroxide, or other alkali metal. It reacts with free fatty acids present in the oil and form soaps. The soaps are then removed from the solution by washing with hot water. For dietary use fish oil is filtered through carbon filter (e.g., dioxins/furans,

polybrominateddiphenyl ethers [PBDEs], polychlorinated biphenyl 309[PCBs], polycyclic aromatic hydrocarbons [PAHs]) to reduce contaminants^[89]. For generation of quality fish oil is passed through selective hydrolysis, followed by filtration. For final purification both solvent extraction and supercritical fluid extraction (SFE) method are used.

4.4 Encapsulation of Fish Oil

PUFAs are polyunsaturated fatty acids found naturally in purified fish oils. For pharmaceutical applications fish oil PUFAs are encapsulated with tocopherols and vitamin E. These are considered as packaged in protective capsule (usually made of gelatin) to protect the oils from oxidation^[90-92].

5. Therapeutic Uses of Fish Oil

PUFAs found in fish oil are highly beneficial to human health. PUFAs are important for the development of the nerve conduction, immune defense, visual sensation, and integutary systems in infants^[93-96]. Fish oil supplements rich in DHA and EPA are highly useful in asthma^[97] and lower down in plasma triglyceride concentration, and decrease the chances of hyperlipidemia^[98]. PUFAs significantly lowers down the risks of cardiovascular diseases mainly thrombosis, high blood pressure^[99]. Dietary consumption of PUFAs decreases the risk factor of type 2 diabetes mellitus via enhanced insulin sensitivity^[100]. Daily consumption PUFAs are highly beneficial in cancer prevention and therapy^[101,102]. The PUFAs extracted from cod liver are natural antibacterial and anti-infectious agents^[103]. Intravenous lipid emulsions are used for supplying energy releasing to PUFAs^[104-106]. PUFAs

reduce trans-epidermal water loss (TEWL), maintain epidermal homeostasis and its deficiency (K17) ^[107]. Fish oil is also used in preparation of pharmaceutical products which are highly useful in dermatological therapy ^[108,109] (Table 7).

Table 7. Therapeutic and Cosmetics properties of fish oil

Therapeutic activity	Mode of action	Source
Lowering of cardiovascular risks	Reduction of lipogenesis; decrease triglyceride synthesis and an increase FA oxidation; and the promotion of apolipoprotein B degradation in the liver through the stimulation of an autophagic process.	Balk, E. M.; Lichtenstein, A.H., 2017
Photoprotective activity	Omega-3 PUFAs suppress UV-induced keratinocyte damage via COX-2, NF-Kb, and mitogen-activated protein kinase (MAPK)/extracellular-signal-regulated kinase (ERK) pathways.	(Pilkington et al., 2011)
Anti-carcinogenic	The omega-3 PUFAs act as the inducers of anti-inflammatory IL-10, suppressors of IL-6 and TNF- α to depress cell growth	(Rehman and Zulfakar 2017)
Anti-dermatitis	GLA-rich oil modified fatty acid metabolism and increased the skin barrier function.	(Brosche et al., 2000)
Cutaneous Wounds Healing	Omega-3 and omega-6 PUFAs modulate or enhance local inflammatory response at wound areas, and accelerating the rate of wound healing.	(Kiecolt-Glaser et al., 2014)
Anti-Hyper-pigmentation	DHA ALA and LA cause skin whitening capability through the mechanism of tyrosinase inhibition.	(Ando et al., 1998, Shigeta et al., 1004)
Antidepressant activity	PUFAs cause membrane modification by direct interaction with the plasma membrane and via modification of the G-protein signalling.	Erb et al., 2016)
Antioxidant activity	DHA inhibits oxidative reactions and pro-inflammatory responses in microglia cell.	(Saldeen et al., 1997)

5.1 Lowering of cardiovascular risks

Omega-3 FA plays important role in cardiovascular disease control ^[110]. It lowers down risks of cardiac failure due to blockage of arteries ^[111]. Fish oil possesses low levels of LDL cholesterol that is physiological much safer. It contains typically 4g/d of eicosapentaenoic acid and docosahexaenoic acid and cut down high levels of triglycerides ^[112]. Use of fish oil omega-3 fatty acids lowers down the risk of arrhythmias, myocardial infarction, and heart failure ^[113]. It is highly beneficial in

coronary artery disease, hypertriglyceridemia and diabetes ^[114] (Table 7). Omega-3 fatty acid most likely reduces serum triglyceride levels by modulating very-low-density lipoprotein (VLDL) and chylomicron metabolism ^[115].

Major problems in cardiac flow and pumping are caused by very low density lipoproteins because they mark with high percentage of cholesterol in their structural composition ^[116]. Dietary use of fish oil improves cardio-logical health it decreases the hepatic secretion of VLDL17 that is a major endogenous source of triglycerides. The main reason of lower synthesis of triglycerides and increased rate of fatty acid oxidation is that omega-3 fatty acid as are un-preferred substrates for enzyme diacylglycerol O-acyltransferase, these never interacts with nuclear transcription factors. It massively controls lipogenesis; and apolipoprotein B degradation in the liver by stimulation of a cellular autophagy ^[117]. It regulates formation of VLDL particles and their secretion. Further, dietary use of omega-3 fatty acid accelerates VLDL and chylomicron clearance by inducing lipoprotein lipase activity (Table 7). For treatment of hypertriglyceridemia patients fenofibrate provided with fish oil found highly effective and safe. Omega-3 fatty acid in diet show improvement in mixed lipid disorder mainly those facing metabolic syndrome and/or type II diabetes mellitus ^[118].

5.2 Photoprotective Activity

A repeated ultraviolet (UV) exposure from sunlight elicits both acute and chronic adverse effects on the skin. It activates sunburn, photosensitivity, inflammation, immunosuppression, and also induces photocarcinogenesis and main cause of photo aging in damaged human skin cells ^[119]. This damage in skin cells is caused due to formation of reactive oxygen species (ROS) that lead to the massive infiltration of immune cells such as neutrophils and macrophages in viable skin epidermis and dermis ^[120]. In this process a protein cyclooxygenase-2 (COX-2), proteins mediate the inflammatory signals generated due to UV-induced injury in skin cells. It also catalyzes the biosynthesis process of prostaglandins ^[121]. Omega-3 PUFAs can decrease the production of proinflammatory eicosanoids through direct competition with the metabolism of AA ^[122]. Dietary use of Omega-3 PUFAs suppress the UV-induced keratinocyte damage. It also regulates COX-2, NF-Kb, and mitogen-activated protein kinase (MAPK)/extracellular-signal-regulated kinase (ERK) pathways ^[123]. Interleukin (IL)-8 mediates UVB-induced keratinocyte inflammation. This is proinflammatory cytokine that belongs to C-X-C chemokine subfamily. ^[124] its modulation is done by both

DHA and EPA which are responsible for inhibition of UVB-induced inflammation in keratinocytes and skin fibroblast cells. In keratinocytes. A similar pattern was observed in fibroblasts. Oleic acid showed no influence on IL-8 release [125] explored the ability of DHA to influence the resistance to UV-activated apoptosis in keratinocytes (Table 7). DHA reverted HaCaT cell resistance to UV-induced apoptosis, increasing the Bax/Bcl-2 ratio and caspase-3 activity. It decreases COX-2 by the inhibition of human antigen R (HuR). That is a COX-2 mRNA stabilizer in keratinocytes. For evaluation of inhibitory effect DHA is applied on UVB-induced skin in hairless mice [126,127]. Topical pretreatment of DHA (2.5 and 10 μ mol) significantly decreased COX-2 and nicotinamide adenine dinucleotide phosphate (NADPH): oxidase-4 (NOX-4) in mouse skin. Both COX-2 and NOX-4 are important in evoking oxidative stress and inciting inflammation [128-130]. The molecular mechanisms of this inhibition could be the suppression of UVB-induced NF-KB activation and COX-2/NOX-4 expression by blocking the phosphorylation of stress-activated kinase-1(MSK1), which is a kinase down-stream of ERK and p38 [131-133]. From UV induced injury few photoprotective agents are used to receive photo protection from sunburn and skin damage.

Oral and topical administration of oils enriched with LA and ALA lowered the erythema occurrence in UV-induced hairless mice [134]. But dietary use of ALA displays greater erythema inhibition than LA by the oral route. Hence, both omega-6 and omega-3 PUFAs finish the UVB-elicited lesions. However, PUFAs from fish oil prevent skin aging caused in patients after use of cosmetic products, when these are provided oral or topical application.

5.3 Anti-carcinogenic

UVA causes oxidative stress and continuous inflammation in skin cells, it starts its action as procarcinogenic agent [135] that finally results skin photocarcinogenesis [136]. These skin cancers are either melanoma or non-melanoma skin carcinoma (NMSC). Use of fish oil PUFAs obstructs both initiation and promotion phases of cutaneous carcinogenesis. The cause synergistic inhibition of carcinogenesis and operate premalignant keratinocyte apoptosis [137]. Fish oil DHA and EPA enhance molecular permeation and improve drug delivery into the skin [138] (Table 7). Fish oil combined with imiquimod is used to treat human basal (BCC) and squamous carcinoma cells (SCC) [139]. For this purpose a combined dose of 21% DHA and 42% EPA is administrated. The pure DHA or EPA also generate immunomodulatory effects against the

carcinoma cells. Fish oil PUFAs work as the inducers of IL-10, an anti-inflammatory cytokine, and suppress of IL-6 and TNF- α production to depress cell growth [140]. Topical use of the imiquimod cause significant reduction tumor size by gel (2.07 mm) and the commercial imiquimod cream (1.98 mm) as compared with the sham control (6.48 mm). Fish oil intake could increase the latency to the development of UVB-induced tumor and decrease the size of the papilloma, keratoacanthoma, and carcinoma in mice. Similarly, topical application of fish oil rich in omega-3 PUFAs reduce skin papilloma formation by benzo (a) pyrene and croton oil [141]. Fish oil inhibits binding of benzo (a) pyrene to DNA that causes reduction in mean papilloma number per mouse from 6.0 to 3.1. Dietary use of omega-3 PUFAs represses UVB-induced carcinogenesis and melanoma patients [142,143]. Dietary use of fish oil rich in omega-3 fatty acids also lower down risk of melanoma development. A DHA-paclitaxel covalent conjugate more effectively work against tumors and block its increase in size [144] (Table 7).

5.4 Anti-dermatitis

Dermatitis is severe dehydration of skin. It is also characterized by cutaneous flexure, inflammation and itching. Dry skin occurs due to loss of epidermal water content due to stratum corneum barrier function loss [145]. Use of fish oil in diet was found highly useful for ameliorating dermatitis symptoms [146]. These successfully cause reduction in cutaneous dryness and pruritus when provided as oral supplementation. A regular dietary use of fish oil for 60 days cause a 30% increase in cutaneous hydration in the acetone-induced dry skin animal model. It also finished itch-related scratching behavior after 90 days regular supplementation. It also causes significant reduction in the ear thickness, cutaneous eosinophils, and mast cells. PUFAs also decrease the inducible nitric oxide synthase (iNOS) expression and collagen fibers [147]. Supplementation of both DHA and AA in diet decrease intensity of dermatitis in mice. PUFAs, especially GLA, in diet improve dry skin, dermatitis and reverse hyper proliferation of epidermis [148]. Regular consumption of GLA-rich enhances the skin barrier function and inflammation [149] (Table 7).

5.5 Healing of Cutaneous Wounds

PUFAs were also found curative in healing of skin wounds. These induce skin cell formation, replacement of affected cells and promote synthesis of wound healing metabolites. These were found highly effective in second-degree burns, chronic wounds, and ulcers [150]. These acts

at all three stages of wound healing suppress inflammatory response, proliferation of wounds and maturation^[151]. PUFAs regulate synthesis and activity of cytokines during inflammatory phase of wound healing^[152]. These play a key role in cell membrane structure and anabolic events during skin cell formation and tissue reconstruction. Both omega-3 and omega-6 PUFAs modulate or enhance local inflammatory response at wound areas and accelerate the rate of wound healing^[153,154]. The topical use of DHA (30 μ M) accelerates the skin wound healing through the inflammatory activity modulation in rats^[155]. It may happen due to activation of the G-protein-coupled receptor (GPR) 120, to which DHA binds during anti-inflammatory activity. DHA treatment increases production of pro-inflammatory cytokines at the wound site (Table 7).

5.6 Hyperpigmentation

Melanin is synthesized in melanocytes found distributed in skin cells^[156]. In biosynthesis of melanin an enzyme tyrosinase catalyzes the conversion of tyrosine to 3, 4-dihydroxyphenylalanine (DOPA) and the oxidization of DOPA to dopaquinone that finally changes into melnin^[157]. Skin hyperpigmentation is caused due to long term repetitive exposure of UV light. It stimulates melanogenesis and by inducing synthesis of endothelin-1, α -melanocyte stimulating hormone (α -MSH), growth factors, and cytokines^[158]. Dietary use of DHA ALA and LA stop skin whitening via mechanism of tyrosinase inhibition^[159,160] (Table 7).

5.7 Antidepressant Activity of Fish Oil

Dietary uses of fish oil show antidepressant effects and exert anti-inflammatory response in neural cells. Fish oil contains omega-3 polyunsaturated fatty acids (PUFA), and there are several mechanisms by which PUFAs are thought to induce an antidepressant effect, including anti-inflammatory action and direct effects on membrane properties^[161]. Both EPA and DHA to prevent decrease, the incidence of IFN α -induced depression^[162,163]. This is the main reason that fish oil is used to treat depression and inflammation effect. Fish oil use in the treatment of depression sites of action of PUFAs at the cell membrane with special attention being placed on lipid rafts and G-proteins^[164]. Omega-3 fatty acids showed antidepressant effects due to their association with rafts, they modify raft structure, and/or release raft-associated proteins into non raft membrane sections. There are two ways by which the PUFAs might be affecting the membrane: by DHA's preference to localize into non-raft membrane samples might create a DHA-rich domain

capable of altering conformation of both membrane domains and signaling proteins^[165]. In such circumstance, PUFAs could affect neurotransmitter signaling and second messengers. PUFAs also facilitate the coupling between the estrogen GPCR, GPER1, G α s, and adenylyl cyclase. These specially regulate the palmitoylation of several different proteins^[166,167].

5.8 Antioxidant Activity of Fish Oil

Studies in experimental models suggest that n-3 polyunsaturated fatty acids (PUFAs) improve metabolic and anti-inflammatory/antioxidant capacity of the heart. PUFA-treated patients compared with untreated, interestingly, PUFA patients had greater nuclear transactivation of peroxisome proliferator-activated receptor- γ (PPAR γ), fatty acid metabolic gene expression, and enhanced mitochondrial respiration supported by palmitoyl-carnitine in the atrial myocardium, despite no difference in mitochondrial content. Myocardial tissue from PUFA patients also displayed greater expression and activity of key antioxidant/anti-inflammatory enzymes. PUFAs enhance mitochondrial fatty acid oxidation and antioxidant capacity in human atrial myocardium, and that this preoperative therapeutic regimen may be optimal for mitigating oxidative/inflammatory stress associated with cardiac surgery^[168].

Fish oil EPA- and DHA-rich oil diets lower down blood lipid levels and increase blood glucose levels^[169]. It may be due to increased lipid peroxidation^[170,171] in the pancreatic cells that also decreased the insulin production^[172,173]. DHA also peroxidizes the blends of different fatty acids, inhibits oxidative reactions and pro-inflammatory responses in microglial cells. DHA is anti-inflammatory and anti-oxidative in nature^[174].

6. Conclusions and Future Directions

More than 37% of world trade is related to various fish derived food items and more than 50% has been captured by fish products. For the economic growth of any country fish and fisheries become highly important. Fish derived food possess immense nutritional value, its consumption in daily diet is beneficial for the skin and muscles. Fish food is good for mental health and improves neural complications. It assists in relieving stress and depression. Omega-3 fatty acids found in fish oil restore eye sight, photoreception, remove sleep deprivation, replace harmful fats and lowers down LDL levels or "bad" cholesterol levels in blood. Use of PUFAs restores blood pressure and cause a significant decrease in risks of cardiovascular problems, brain strokes and type

1- diabetes. Fish foods are good source of minerals such as calcium, iron, phosphorus and vitamin D. These are highly important for body physiology, immune functions and metabolism. Fermented fish products are easily digestible and absorbable and show a positive effect on body metabolism. Fish foods possess high therapeutic value as these lower down risk of fatty liver diseases and cancer. Both vitamin D and fish PUFAs accelerate metabolic rates during exercise, fat oxidation and energy supply. Fish in daily diet restores premenstrual symptoms in young women. Dietary use of fish oil alleviates the swelling and pain in rheumatoid arthritis and relieves from chronic inflammation. Fish food also lowers down risk of Alzheimer's disease. Fish oil has many possibilities of new human health-related products which can be used in cosmetology and dermatology. Fish consumption is beneficial for removal of fatigue and assists in muscle regeneration.

Authors' Contributions

Ravi Kant Upadhyay and Shweta Pandey were responsible for conception, literature review, writing and revising the manuscript.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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ARTICLE

Physical Characteristics of Functional Indigenous Farm-made Feeds Using Crude or Gelatinized Tapioca Starch as Sources of Energy

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ARTICLE INFO

Article history

Received: 20 January 2022

Accepted: 18 February 2022

Published Online: 25 February 2022

Keywords:

Physical properties

Tapioca

Indigenous

Farm-made

ABSTRACT

The experiment compared the physical characteristics of aqua feed with crude or gelatinized tapioca starch as sources of energy. The bulk density (BD), water absorption index (WAI), water solubility index (WSI), pellet durability index (PDI) and water stability (WS) were measured in both experimental diets. The results showed significant variations ($p < 0.05$) in BD and WAI in diet with crude tapioca starch while non-significant variations ($p > 0.05$) were recorded for WSI and WS in both diets. The higher BD of a diet, the better its ability in resisting external forces that can cause disintegration. A high BD also reduces ability to the feed material shrinking, thereby preventing loss to feed dust and fines. The results of WSI, WS and PDI of diets denotes that both pellets were water stable and could spend about same time in water but diet with gelatinized starch had a better water absorption index and pellet durability index. Furthermore, proximate composition of diets showed that diets with gelatinized starch had low moisture (9.04%), low fibre (5.24%), and higher ash (13.61%) and lipid (9.64%) contents. It can be concluded from this experiment that diets with gelatinized starch stands the chance of being a better functional feed for small-scale fish farmers in Sub-Saharan Africa.

1. Introduction

Fish is an essential source of animal protein and other nutrients in human diets, especially in the sub-Saharan Africa ^[1]. With the geometric rise in world population and consciousness about healthy living, people prefer fish-based protein diets, hence, an increase in fish farming either at subsistence or commercial levels. Aquaculture

has continued to be the fastest-growing animal-food-producing sector, from a production of 61.8 million tons in 2011 to 80 million tons in 2016 ^[2]. In an attempt to provide good quality feeds for farmed fish species, farmers combine various feedstuffs to cater for the chemical and nutritional compositions of aqua feeds with an unintentional oversight into the physical characteristics of such feeds. The introduction of functional farm-made

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DOI: <https://doi.org/10.30564/jfs.v4i1.4369>

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feeds epitomizes a progressing concept in farmed animals' diets. The use of these feeds is beyond satisfying the basic nutritional requirements of species but to also reduce the effect of stress on the animal to the barest minimum by providing a conducive aquatic environment ^[3,4]. Feeding cost accounts for the lump sum of expenses in any viable aquaculture venture, hence, the need to give it special attention. Farm-made aqua feeds depend on a "basket" of conventional ingredients such as soybean, maize, fish oil, rice bran and wheat for which it competes in the marketplace with other feed production sectors - man and livestock. Most of the major feedstuffs used in the production of both farm-made and commercial feeds are also subjected to global market shocks and volatility as other edible feed ingredients ^[1].

Nutritional requirement (protein, lipid, vitamins and minerals) of most fish species are not different from those required by man; protein (amino acids), lipids, energy, vitamins and minerals in meeting physiological needs of growth, reproduction, maintenance and tissue repairs. Dietary nutrient levels for optimum growth performance are dependent on fish species, stage of development, sex, maturation, state, season, locality, environment, management practices and other external factors. The value of supplementary feeds depends on the chemical composition and digestibility of individual feed stuff, therefore, adequate combination of various individual feed components that would improve the digestibility and utilization of compounded feeds ^[5]. Good quality feeds is a major setback to the development of aquaculture in Sub-Saharan Africa as fish farmers depend largely on importation at a premium price.

Cassava (tapioca) is a multipurpose plant that thrives well in the tropics (Nigeria inclusive) with the extract from the root referred to as 'starch'. Its roots/tubers are cheaper sources of dietary energy compared with grains but the extent of the practical use in aqua feeds is limited ^[6]. Researchers have indicated that cassava tuber meal can replace conventional energy feedstuffs such as maize, broken rice and sorghum in Africa ^[7]. Binders are increasingly on the use by feed manufacturers to produce good quality pellets would not crumble upon handling. However, the high cost of conventional synthetic binders makes farm-made aqua feed production difficult for the local small-scale farmer in Sub-Saharan Africa ^[8]. The high starch content in tapioca root makes it an excellent aqua feed binder which is a promising potential alternative to eliminate expensive artificial binders ^[9]. This agricultural product will provide an appropriate local substitute to feed manufacturers and low-scale indigenous fish farmers to compound their own feeds ^[10]. Although,

farmers have since embraced the use of cassava tapioca starch as a binder, but an excellent performance can be enhanced by gelatinization. Gelatinization is a process of breaking down the inter-molecular bond present in starch by applying heat and water. Starch has semi-crystalline structure with two components-amylose and amylopectin. The bond between semi-crystalline structures irreversibly breaks down as granules swells and burst with the application of heat and water. This process makes amylose molecules to leach out of the granules giving enzymes access to glycosidic linkage and consequently increase digestion ^[11]. Gelatinized starch is a readily available source of energy because gelatinization increases starch digestibility, as well as protein and energy retention by catabolizing gelatinized starch for growth and energy instead of protein and energy ^[12]. When starch is cooked, it is believed to improve the physical quality of the feed by increasing binding properties between particles in the ingredient mix. Tapioca starch is also water stable, hence, its use in this experiment by comparing the physical characteristics in its crude or gelatinized forms.

2. Materials and Methods

The experiment was carried out at the nutrition laboratory of Department of Fisheries and Aquaculture Technology, The Federal University of Technology, Akure, Ondo State, Nigeria. The experiment lasted for 14 days from the preparation of diets to the experimental procedure and analysis of results.

2.1 Preparation of Experimental Diets

Experimental feed ingredients (fish meal, soybean meal, palm and fish oil, tapioca starch, vitamin and mineral premix) were purchased from Animal Feed Concept in Akure, Ondo State, Nigeria, and prepared using the concept of ^[13]. Two iso-nitrogenous diets containing 40% crude protein of same basal ingredients were prepared and designated as D1 and D2 (Table 1) with diets D1 and D2 containing crude and gelatinized tapioca starch respectively. The tapioca starch was divided into two parts; one part was added to the basal feed mix in its crude form (D 1) while the other was gelatinized by mixing with boiling water and allowed to cook to obtain a sticky and transparent dough (D 2). The gelatinized starch dough was poured as a thin film into a tray and oven-dried at 60 °C for 3 days, after which it was pulverized into powder using a milling machine. The tapioca powder was then added into the basal ingredients to form a homogenized dough which was pelletized through a 2 mm die opening using a Hobart A-120 pelletizer (London, UK). Resultant pellets were sun

dried at 30 °C for 72 hours, cooled under room temperature for another 24 hours and stored prior to use.

Table 1. Gross composition (%) of experimental diets

Ingredients (g)	D 1 (crude tapioca starch)	D 2 (Gelatinized tapioca starch)
Fish meal	42.30	42.30
Soybean meal	21.15	21.15
Tapioca	22.55	22.55
Fish oil (g/vol.)	3.00	3.00
Palm oil (g/vol.)	4.00	4.00
Vit. & min. premises	5.00	5.00
Cellulose	2	2

2.2 Physical Characteristics of Experimental Diets

The experimental diet pellets were subjected to some physical properties tests such as bulk density (g/L), water absorption index (%), water solubility index (%) and water stability (%).

2.2.1 Bulk Density (BD)

Bulk densities of experimental diets were carried out in triplicates following the methods of [14]. Pellets was introduced into a 1,000 mL measuring cylinder and the corresponding weight was recorded. BD was calculated as the ratio of the weight of pellets to the volume of the cylinder and expressed as g/L.

$$BD = \frac{\text{Weight of feed (g)}}{\text{Vol. of Cylinder (ml)}}$$

2.2.2 Water Absorption Index (WAI)

Pellets from each treatment was grinded and sieved through a 200 µm sieve. 2.5 g of the fine powdered pellets (W_{ds}) was suspended in 30 mL distilled water in a 50 mL centrifuge tube at 30 °C for 30 minutes and stirred intermittently. The suspended sample was later centrifuged at 3,000 *rpm* for 10 minutes. The supernatant was poured into a known weight of an aluminum disposable dish and oven-dried at 135 °C for 2 hours. The weight of gel (W_g) remaining in the centrifuge tube was calculated as WAI from the following equation:

$$WAI = \frac{W_g}{W_{ds}}$$

2.2.3 Water Solubility Index

The weight of dried solid supernatant (W_{ss}) that was recovered after evaporation from the aluminum disposable dish was calculated as WSI following the method of [15].

$$WSI = \left(\frac{W_{ss}}{W_{ds}} \right) \times 100$$

2.2.4 Water Stability

This parameter was calculated from the ratio of original sample to that of the sample after drying.

$$WS = \frac{\text{Original sample}}{\text{Sample after drying}} \times 100$$

2.2.5 Pellet Durability Index

$$PDI = \frac{\text{weight of sample after fines removal}}{\text{Weight of original sample}} \times 100$$

2.3 Proximate Analysis

The proximate analysis of the experimental diets was carried out following standard methods as described by [16]. Crude protein was determined using micro-kjeldahl distillation method, crude lipid extraction was carried out with the aid of Soxhlet's apparatus, moisture content of dry matter was determined using a conventional oven at 105 °C, crude ash content was measured by placing dry samples in a furnace at 550 °C, crude fibre content was determined using the method by Tecator Fibretec System of 1995.

2.4 Statistical Analysis

All data generated were subjected to one way analysis of variance (ANOVA) using SPSS 22 (Statistical Package for Social Sciences) and means were separated by Duncan's Multiple Range Rest (DMRT) at 5 % confidence level.

3. Results and Discussion

3.1 Proximate Composition of Experimental Feed and Starch

The proximate composition of experimental diets is presented in Table 2. Crude protein of experimental diets ranged between 42.12% and 42.93%, this did not show any significant variation ($p > 0.05$) observed in other parameters. The basal feed mix had 40% crude protein, the slight increase recorded in this experiment could be from other feed ingredients in the diet. Crude protein of 40% is adjudged adequate for the supply of nutrients for most farmed tropical fish species especially, the African catfish. Crude protein of 35%-40% in the diets of cultivable tropical fish species was recommended by [17] for optimum growth. Dry matter, crude ash and lipid contents in diets were significantly different ($p < 0.05$) as diet with crude tapioca starch was consistently low in

the fore-mentioned parameters. The percentage of dry matter in diets signified low moisture content especially in diet with gelatinized starch which is a good parameter to consider in farm made feeds. Low moisture content is important for good storage and also to prolong shelf life of feed ingredients and prepared feeds, therefore, low moisture content recorded in diet D2 points to a longer shelf life and keeping ability. A moisture content of about 10% or less was suggested in fish diets to prevent prepared feeds and feed ingredients from spoilage and optimal ^[18]. Fish diet with crude tapioca starch had a lower crude ash and lipid contents when compared with the gelatinized diet. Tapioca starch is known to be a good source of minerals (calcium and iron) and gelatinization makes the starch readily available, hence, the probable reason for a higher ash content in the latter diet. Lipids are primarily included in formulated diets to maximize their protein sparing effect being a source of energy. Crude lipid contents recorded in this experiment were within the recommended limits (5%-10%) of inclusion into the diets of fresh water fish species ^[17]. Lipid of 10%-20% was opined by ^[19] in diets for most freshwater fish species. Crude fibre and nitrogen free extract (NFE) contents in experimental diets was lower in gelatinized tapioca starch diet but were all within the recommended dietary limits for most freshwater fishes. Fish diets with high fibre content of more than 12% would result in low nutrient quality, as such, not desirable in fish feeds due to low digestibility ^[20]. Chemical constituents or composition (%) of the starch (crude) used in the experiment are; moisture (7.60±0.18), crude protein (1.71±0.21), crude ash (1.56±0.13), crude fibre (0.68±0.02) and nitrogen free extract - NFE (88.45±0.28).

Table 2. Proximate composition (%) of experimental diets

PARAMETER (%)	D 1	D 2
Dry matter	87.94 ±0.07 ^a	90.96±0.03 ^b
Crude ash	6.26±0.03 ^a	13.61±0.06 ^b
Crude lipid	4.19±0.00 ^a	9.64±0.23 ^b
Crude fibre	6.24±0.07 ^b	5.24±0.07 ^b
Crude protein	42.93±0.26 ^a	42.12±0.09 ^a
NFE	28.31±0.29 ^b	20.36±0.37 ^a

Values with the same superscripts on the same row are not significantly different

3.2 Physical Properties of Experimental Diets

The result of physical properties of the experimental feed is illustrated in Table 3. There were significant

variations ($p<0.05$) in bulk densities (BD) and water absorption indices (WAI) of both diets. On the other hand water solubility indices (WSI) and water stability (WS) did not indicate any significant ($p>0.05$) variation. Physical characteristics of feeds such as the above mentioned play important roles in the overall feed efficiency to obtain optimum result. The diet with gelatinized tapioca starch had a lower BD (0.48%) compared with the crude tapioca starch diet. Low BD observed in the microstructure of feed was linked to pore formation by ^[21]. BD is an important factor that determines inter particulate bond that facilitates closer better performance during preparation. Extruded diets having higher BD require less space which facilitates transportation of more products in smaller containers thereby reducing the total cost of shipment ^[22].

The WAI and WSI explain the interaction between extruded diets and water which involves the conversion of starch granules to a homogenous lump ^[23]. The result showed that there were significant variations ($p<0.05$) in the WAI of experimental diets, with the gelatinized tapioca starch having a higher numerical value of 4.65%. This corroborates the study of ^[24] that increased WAI values indicated that the pellets underwent a high degree of gelatinization while a decrease on the other hand connotes starch degradation ^[25]. Water absorption and solubility indices are parameters that measure swollen gelled particles which maintain their integrity in aqueous diffusion and the degradation of molecular components ^[26,27]. The WSI depends largely on the volume of soluble matter which increases due to the breaking down of starch molecules ^[28,29]. Although, there was no significant variation ($p>0.05$) in the WSI of both diets, however, the diet with crude tapioca starch had a slightly higher numerical value. At high die temperatures, extruded pellets were able to maximize the degree of starch gelatinization leading to a reduction in starch degradation ^[22]. This result showed that there was no significant variation ($p>0.05$) in water stability of experimental diets. Water stability between 15.65%-16.12% was attributed to the presence of starch ^[29] and its duration in water. The degree of a diet's stability is directly related to the degree of gelatinization during steam condition ^[30,31]. Diets with good water stability might be due to the presence of gelatinized binder in the feed ^[31]. Pellet durability index (PDI) is used to determine the amount of fines that will exist in pellets at feeding time. Pellet durability indicates the ability of the pellet to resist attrition during storage and transport ^[32]. This was developed as a predictor of pellet fines produced during mechanical handling. PDI of diet with gelatinized starch was higher, corroborating the assertion of ^[31]. Also, ^[33]

reported that pellet durability is an effective means of reducing fines which can be improved by steam pelleting and the use of raw materials with good binding ability.

Table 3. Physical properties of experimental diets

PARAMETER	D 1	D 2
Bulk density (BD)	0.57±0.01 ^b	0.48±0.01 ^a
Water absorption index (WAI)	2.62±0.26 ^a	4.65±1.85 ^b
Water solubility index (WSI)	2.77±0.45 ^a	2.63±0.81 ^a
Water stability (WS)	18.81±0.55 ^a	18.76±0.59 ^a
Pellet durability index (PDI)	60.04±0.25 ^a	68.71±0.16 ^b

Values on the same row with same superscripts are not significantly different ($p>0.05$)

4. Conclusions

The aqua feed technology is a moving tandem with aquaculture growth through production of feeds for improved digestibility. One goal the industry tends to achieve is compounding functional feeds that would enhance digestibility. Gelatinized starch improves physical quality of feed by increasing binding between particles in the ingredient mix. Physical characteristics such as bulk densities, water absorption and water solubility index are good parameters for evaluating the efficiency of feeds. From this experiment, there were significant variations in some physical characteristics such as BD and WAI. The crude tapioca starch had a higher BD which is a measure of the packing characteristics of the diet's particulate solids. The higher the BD of a diet, the better its ability to resist external forces that can disintegrate it, hence, a higher durability which reduces shrinkage that gives way to dustiness. It is a known fact that gelatinized starches improves pellet quality and have better water stability as shown in this experiment. Starch concentration also gives the feed more compactness, decrease water deterioration and helps in effective monitoring of feeding. Furthermore, chemical compositions of both experimental feeds were within recommended ranges for successful culture of tropical fish species. This study provides some facts that the local or small-scale farmer may be unaware of, since the essence of research is to proffer solution to farmers' immediate challenges and bridge the gap between research and stake-holders. Feed ingredients used are indigenous and also conventional, hence, the fish farmers can easily lay hands on them. Also, method used in gelatinizing starch is not complicated as such, does not require any form of expertise prepared diets can be replicated in any part of the world using same method.

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ARTICLE

Comparative Analysis of Three Types of Fishing Gear Marking for Anchored Fish Aggregating Devices in Purse Seine Fishery in Thai Waters

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ARTICLE INFO

Article history

Received: 15 February 2022

Accepted: 16 March 2022

Published Online: 21 March 2022

Keywords:

AFAD

ALDFG

Fishing gear marking

Ownership

Gulf of Thailand

Andaman Sea

ABSTRACT

Anchored fish aggregating devices (AFADs) have been widely used for fishing in Thai waters. However, abandoned, lost, and discarded fishing gears (ALDFGs), including lost AFADs, may cause environmental impacts. Fishing gear marking (FGM) is considered as a tool to help identification of ALDFGs. The main objective of this study is to compare the durability represented by the percentage of remaining condition (R-value) of three material types of FGM applied for AFADs, *i.e.*, stainless steel (SS), colored acrylic (CA), and polypropylene (PP). This study was carried out using 50 AFADs deployed in the Gulf of Thailand (GOT) and the Andaman Sea (ANS) between July and October 2020 in cooperation with 10 fishers. The AFADs were deployed in similar habitat (bottom depth and type) between the GOT and the ANS. The three material types of FGM were assumed to be sufficiently durable to last for the lifespan of the AFADs in both the GOT and the ANS (within 2 months and 3.5 months, respectively) though some FGMs in the ANS were detached from cable ties or broken before AFADs were lost. The loss of AFADs and FGMs was mainly caused by adverse weather condition (rough sea). Only data from the ANS was included in comparative analysis due to the insufficient variance data obtained from the GOT. The analysis revealed that SS had the higher durability than CA and PP when the AFADs lasted for less than 3.5 months. As a result of our study, some recommendations were made. For example, the cable ties can be replaced by ropes or threads to improve the installation method. This study serves as a basis to develop FGM and to support responsible fisheries. Beneficiaries of the study include fisheries policy makers, managers, and fishers.

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DOI: <https://doi.org/10.30564/jfs.v4i1.4446>

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1. Introduction

Purse seine fishery shares a great portion of the world's total catch from marine capture fisheries targeting pelagic fish resources, *e.g.*, tunas, mackerels, sardines, and anchovies^[1]. Instead of searching fish schools, fish aggregating device (FAD) has been used by fishers to attract pelagic fish resources in purse seine fishery for several decades^[2,3]. FAD can be categorized into two main types; namely, drift FAD (DFAD) and anchored FAD (AFAD). In the main oceans (*i.e.*, Atlantic, Indian, and Pacific Oceans), DFADs are principally deployed in the open seas or oceans, while AFADs are deployed in both inshore and offshore areas^[4].

There are many types of AFADs deployed in the three main oceans^[5], and the main structures are anchor (weight or sinker), anchor (mooring) line or rope, and float^[5-7]. AFADs often have mid-water aggregators attached to the float or the upper mooring line; moreover, the aggregators are frequently made of rope, fishing net, plastic strapping, plastic mesh, or mussel rope^[6] as well as fiber-reinforced plastic or coconut/palm fronds^[5]. AFADs with only a small float as a position marker but without any tracking device are difficult to detect at sea^[8]. Fishers sometimes attach light, steel buoy, and radar reflector on AFADs to locate the position^[5-7]; in addition, electronic devices (*e.g.*, satellite buoy) has been recommended to attach on AFADs to enhance fishing operations, but its implementation has low feasibility for small-scale fisheries due to its cost^[6].

As benefit of AFADs for fishers, the attraction of pelagic fish resources continued when AFADs remained in the sea without being lost for a sufficient time period^[6].

The lifespan of an AFAD varied by areas from few to several months depending on their designs, materials, maintenance, and environmental factors. For example, AFADs usually lasted for two months or less in Indonesia^[9], 3-5 months in Thailand^[10], 1-33 months in Vanuatu, 4-12 months in Martinique, and up to 65 months in La Reunion^[6]. The loss of AFADs was common and fishers regularly replaced AFADs in the fishing ground. Moreover, lost AFAD is one of the several types of abandoned, lost, and discarded fishing gear (ALDFG) which is the major component of sea-based marine litter^[11]. The loss of AFADs occurred when the structures are deconstructed from the anchor parts. Floats with/without mooring line and mid-water aggregators are assumed to have a similar function to lost DFADs with a wide range of environmental impacts from beaching^[11-13], such as contact with marine habitats (*e.g.*, coral reefs) and entanglement of marine animals (*e.g.*, bony fishes, sharks, and turtles).

The Sustainable Development Goals (SDGs) has been established by the United Nations (UN), and its 14th goal (SDG 14) or the “Life below water” is to conserve and sustainably use the oceans, seas, and marine resources for sustainable development, including the preventing and reducing marine pollution, the sustainably managing and protecting marine ecosystems, and enhancing conservation and sustainable use of marine resources^[15]. The Code of Conduct for Responsible Fisheries of the Food and Agriculture Organization of the United Nations (FAO)^[16] mentioned the fishing gear marking (FGM) as a measure for identifying the ownership of fishing gears, which supports the SDG 14 to address ALDFG. For Thailand fisheries, FGM has been mandated to commercial fishers operating outside Thai waters^[17] but has not been applied to the fisheries in the Thailand's EEZ. The FAO has also developed the Voluntary Guidelines on the Marking of Fishing Gear (VGMFG) as a tool for combatting, minimizing, and eliminating ALDFGs and for facilitating the identification and recovery of such gears^[18]. The FAO^[19] also recommended several types of FGM, for example, coded wire tag, electronic tag, barcode tag, metal or steel tag, band tag, marker tape, and rogue yarn. Aside from identifying the ownership of fishing gear, the benefits of FGM include providing information on the origin of fishing gear entangled on marine animals and indicating the position to reduce gear conflicts and improve safety at sea^[20].

For the application of FGM in some fisheries, plastic tags were found effective from the pilot study with small-scale gillnet fishers in Java, Indonesia^[21]; plastic bottles and polyurethane foam sheets with coding were used by artisanal gillnet fishers in Kerala, India^[22]; stainless steel clamps were applied by Thai trawlers operated in the area of the Southern Indian Ocean Fisheries Agreement^[23]. Furthermore, the use of unique identification code was suggested to provide the encrypted information on the FGM that can be read by a machine^[22]. In DFAD fishery, tuna purse seiners in the Indian Ocean put identification codes as physical marks on the surface of their satellite buoys for ownership; besides, tuna purse seine fishers suggested that the physical mark should be sufficiently durable to last for the lifespan of a DFAD^[24]. However, there is a lack of information on the application of FGM for AFADs.

As AFADs are similar to other static gears, this study focused on the suitable materials for FGM to identify the ownership, which was still needed to develop FGM for AFADs in the purse seine fishery. The main objective of this study was to compare the durability of three material

types of FGM applied for AFADs in purse seine fishery in Thai waters. This study is expected to serve as basis for developing the practice of FGM to address ALDFG and support responsible fisheries; moreover, it would benefit fisheries policy makers, managers, and fishers.

2. Materials and Methods

2.1 Study Area

The exclusive economic zone (EEZ) of Thailand covers 420,280 km² (304,000 km² in the Gulf of Thailand (GOT) and 116,280 km² in the Andaman Sea (ANS)) [25]. This study was carried out in two fishing grounds in Thai waters (*i.e.*, offshore areas of the GOT and the ANS) where AFADs were regularly deployed for purse seine fishery (Figure 1).

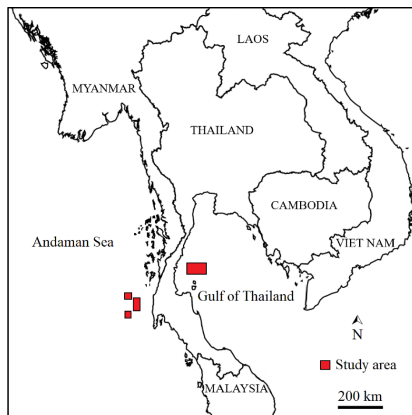


Figure 1. Study areas where anchored fish aggregating devices were deployed in the Gulf of Thailand and the Andaman Sea

2.2 Anchored Fish Aggregating Devices (AFADs)

Fishers deployed AFADs for purse seine fishing operations to capture associated schools of pelagic fishes. The AFADs were anchored in the fishing grounds with the distance of at least 1 nm between AFADs as the regulation of the Department of Fisheries (DOF), Thailand [26]. The structure of AFADs in this study was similar between the GOT and the ANS which were constructed using concrete blocks as the anchor, rope as the mooring line, coconut fronds as the aggregator, and Styrofoam blocks as the float (Figure 2). In this study, 10 voluntary fishers represented the experimental units or cases (five in the GOT and five in the ANS) were recruited, and 50 AFADs (five AFADs or replications for each fisher) were used in our experiment.

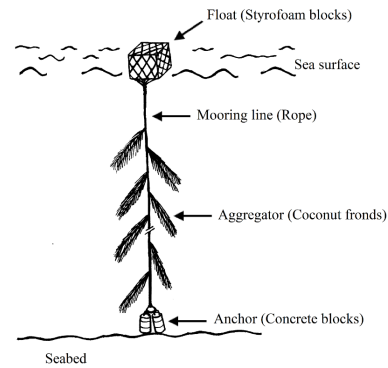


Figure 2. Typical anchored fish aggregating devices deployed by fishers in purse seine fishery in the Gulf of Thailand and the Andaman Sea

2.3 Fishing Gear Marking (FGM)

We adopted the physical tag with coding which was one of the marking technologies for FGM [20] for the identification of origin and ownership of AFADs. The physical tags used in our experiment were made of three material types, including stainless steel (SS), colored acrylic (CA), and polypropylene (PP) (Figure 3). SS and CA were 30 mm wide and 60 mm long, while PP was 210 mm wide and 297 mm long. The physical tags had holes of 5 mm diameter. Moreover, each physical tag was labeled with 10 alphanumeric code with the first seven characters as simulation of the fishing vessel marking in Thailand [26,27] and the last three characters as the order of FGM. The code was 6 mm high for SS and CA, and 20 mm high for PP. For each AFAD, we prepared one set of FGM consisted of one piece (unit) of each material type, which was attached to the rope using cable ties and installed on the float of an AFAD. For 50 AFADs, we installed 50 sets of FGM in combination of the three material types.

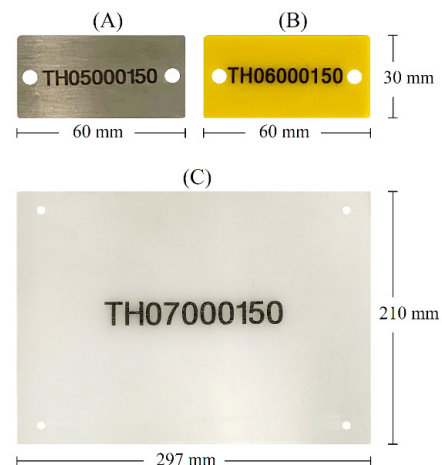


Figure 3. Three material types of fishing gear marking (stainless steel (A), colored acrylic (B), and polypropylene (C)) labeled with alphanumeric code

2.4 Data Collection

The participation of stakeholders, particularly fishers has been considered as an important mechanism in marine fisheries management^[29]. The data were collected in cooperation with the 10 fishers who closely observed the FGMs installed on their AFADs between July and October 2020. We interviewed each fisher and collected information on their fishing vessel and AFADs structures as well as the information on their fishing ground such as bottom depth and type in areas where the AFADs were deployed. Each fisher was also inquired about the remaining condition of each unit of FGM via field surveys and telephone calls. The remaining condition of each unit of FGM for four categories: FGM was broken or lost; all characters were removed, some characters were remained, and all characters were remained. The FGMs were monitored and recorded the remaining condition after installation at five different times at 0.5 month, 1.0 month, 1.5 months, 2.5 months, and 3.5 months (or shorter if all FGMs were broken or lost). For the FGM broken or lost, the information on its cause was also inquired from the fishers.

In addition, the information on the characteristics of particular fishing vessels, including length overall or LOA (m), gross tonnage (GT), and engine power (kW) was acquired from the Marine Department (MD), Thailand^[30] and the DOF, Thailand^[31].

2.5 Data Analysis

For each unit of FGM installed on any AFAD, the value of remaining condition for material type i (C_i) was given, *i.e.*, 0 (zero) for FGM broken or lost, 1 (one) for all characters removed, 2 (two) for some characters removed, and 3 (three) for all characters remained. In the other words, C -value was between zero and three. The percentage of remaining condition (%) for each material type of FGM installed on AFADs deployed by each fisher each time was calculated using Equation 1;

$$R_i = \left(\sum_{j=1}^n [(C_{ij}/3) \times 100] \right) / n_i \quad (1)$$

Where R_i is the percentage of remaining condition for material type i of FGM used by a fisher; C_{ij} is the value of remaining condition for material type i of FGM installed on the j^{th} AFAD; n_i is the total number of AFADs with material type i of FGM; and j is the 1st, 2nd, 3rd, ..., n^{th} AFAD with material type i of FGM. Due to five AFADs applied for each fisher in this study, n_i was equal to five, and j -value was between 1 and 5.

In the cases of FGM broken or lost, the percentage of

each cause for material type i or L_i (%) was calculated for each fishing ground at the end of our experiment using Equation 2;

$$L_i = (M_i/n_i) \times 100 \quad (2)$$

Where M_i is the number of FGM made of material type i lost by the particular cause; and n_i is the number of AFADs with material type i of FGM.

For statistical analyses, only data obtained from fishers in the ANS were utilized because of insufficient variance data obtained from the GOT. There were five experiment units (cases) represented by five fishers (fishing vessels) in the ANS. The material types of FGM and times were defined as the independent variables, and the R -value was set as the dependent variable. To avoid the R -value of 0% for all cases of each material type observed at the same time, the only four consecutive times between 0.5 month and 2.5 months were included in the analyses. The R -value distribution seemed like a binomial rather than a normal, because the characteristics of data distribution were mostly in small percentages (0% to 30%) or large percentage (70% to 100%). To have the data distribution nearly normal, the data of R -value (0% to 100%) was transformed prior to analyses using the angular transformation to $\arcsin[(R/100)^{1/2}]$ which gave the transformed R -value (R') from zero to 1.5708. The comparative analysis among the three material types and the four consecutive times on the value of R' were performed by the Two-way Repeated Measures Analysis of Variance (Two-way RMANOVA) followed by the Least Significant Difference (LSD) for the post-hoc test. In addition, the Mauchly's (W) test was also performed to examine the sphericity assumption. The SPSS Statistics for Windows, version 15.0 (SPSS Inc., Chicago, Ill., USA) was used, and the significant level (α) of 0.05 was applied for all statistical analyses.

3. Results and Discussion

3.1 Characteristics of Fishing Vessels

The characteristics of 10 fishing vessels in this study are shown in Table 1. The Vessels A-E operated in the GOT and the Vessels F-J operated in the ANS. The 10 fishing vessels had the LOA ranging from 17.4 m to 29.5 m; gross tonnage between 41.12 GT and 234.85 GT; and main engine power of 92-473 kW. These fishing vessels were used to regularly observe AFADs and monitor fish schools in the vicinity of AFADs for purse seine operations. Therefore, the fishers were able to closely observe the remaining condition of each unit of FGM installed on AFADs.

From interview, the bottom depth of deployed AFADs

in each fishing ground was 35-60 m in the GOT and 50-80 m in the ANS; moreover, the bottom type was muddy sand for the both fishing grounds. The fishers in each fishing ground also responded that the purse seiners performed AFADs operations about 24 fishing days per month in the GOT, while it was about 22 fishing days per month in the ANS.

Regarding Thai standard for commercial fishing vessels ^[27], the 10 fishing vessels were categorized in medium size (30.00 GT to 59.99 GT), large size (60.00 GT to 149.99 GT), and extra-large size (150.00 GT and above). The size composition of these fishing vessels reflected the size composition of purse seiners in Thai waters acquired from the licensing system ^[31] where most (about 70%) of purse seiners was large size, followed by medium size (20%) and extra-large size (6%). From our results, we assumed that the AFADs deployed by fishers in this study were in similar habitat or condition between the two fishing grounds (the GOT and the ANS).

3.2. Remaining Condition of Fishing Gear Marking

From the observation of FGMs installed on AFADs in cooperation with fishers, we found that most FGMs were lost together with the float of AFADs, while some FGMs was lost or broken before the loss of AFADs (Figure 4). Figure 5 shows the trend lines of average *R*-value in both the GOT and the ANS along the different times (from 0.0 month to 3.5 months) for the three material types of FGM applied for AFADs. The durability of the three material types of FGM installed on AFADs was less than 3.5

months. In the GOT, the three material types of FGM had the same trend lines of average *R*-value along the different times. After the initial installation of the FGMs on AFADs, the average *R*-value was 100% for the three material types of FGM at 0.5 month and 1.0 month. The average *R*-value decreased to 0% for all material types of FGM at 1.5 months. In the ANS, the three material types of FGM had the similar trend lines of average *R*-value along the different times though the average *R*-value of FGM made of SS appeared to be in higher value than CA and PP between 0.5 month and 2.5 months. After the initial installation of the FGMs on AFADs, the average *R*-value decreased to about 90%, 80%, and 70% for SS, CA, and PP, respectively between 0.5 month and 1.0 months; the average *R*-value continually decreased to about 80%, 60%, and 50% for SS, CA, and PP, respectively at 1.5 months; the average *R*-value was about 20% for SS and CA, while it was about 10% for PP at 2.5 months; and the rest of FGM was already lost at 3.5 months.

For the FGM broken or lost, the interviewed fishers indicated that the causes include adverse weather condition (*i.e.*, rough sea) and fishing gear conflicts (*i.e.*, bottom trawls) (Table 2). From our interviews, the fishers in the GOT responded that the major cause for the loss of FGM was due to rough sea which resulted in AFADs lost or float removal between 1.0 month and 1.5 months. It should be noted that the towing of a bottom trawl in the AFADs area was a minor threat and caused damage on one AFAD in this study. The fishers in the ANS specified that the only main threat to the loss of FGMs installed on their AFADs was the rough sea which damaged the FGMs

Table 1. Characteristics of 10 fishing vessels (Vessels A-J) in this study to observe fishing gear marking installed on anchored fish aggregating devices for purse seine fishery in Thai waters

Fishing ground	Vessel	Length overall (m)	Gross tonnage (GT)	Size ¹	Engine power (kW)
Gulf of Thailand	A	21.5	68.64	L	235
	B	21.0	59.38	M	92
	C	23.9	114.83	L	278
	D	21.1	69.95	L	278
	E	17.4	41.12	M	278
Andaman Sea	F	23.3	89.87	L	278
	G	23.8	83.35	L	178
	H	29.5	234.85	X	473
	I	24.0	136.41	L	444
	J	22.1	76.60	L	235

¹ Size categories of Thai standard for commercial fishing vessels (M: medium size or 30.00 GT to 59.99 GT; L: large size or 60.00 GT to 149.99 GT; X: extra-large size or 150.00 GT and above) ^[27]

and the AFADs. This was similar to the lost AFADs in the GOT caused by the same reason; consequently, most FGMs was lost together with AFADs, and some FGMs was detached from the cable ties or broken (*i.e.*, two units of CA) during the rough sea.

The results indicated that the life span of AFADs deployed in the GOT was less than in the ANS due to adverse weather condition which was the main cause of the loss of FGMs. Based on the results from the ANS, the average *R*-value of the three material types appeared to be different and needed to be compared to clarify the durability among the three material types.

The lifespan of deployed AFADs in this study was only a few months. This was similar to the other AFADs used in adjacent waters reported by Yusfiandayani *et al.* [9] and Boonjorn *et al.* [10]. The interviewed fishers mentioned that they regularly deployed new AFADs to replace the old ones to maintain their fishing operations. We assumed that the short lifespan of AFADs was mainly a result of deconstruction of the materials between float or mooring line and anchor due to environmental forces. To increase the lifespan of AFADs, the structure should be improved like the AFADs in La Reunion [6], Japan [7,32], and Maldives [33], besides, maintenance is also needed for deployed AFADs for longer lifespan.

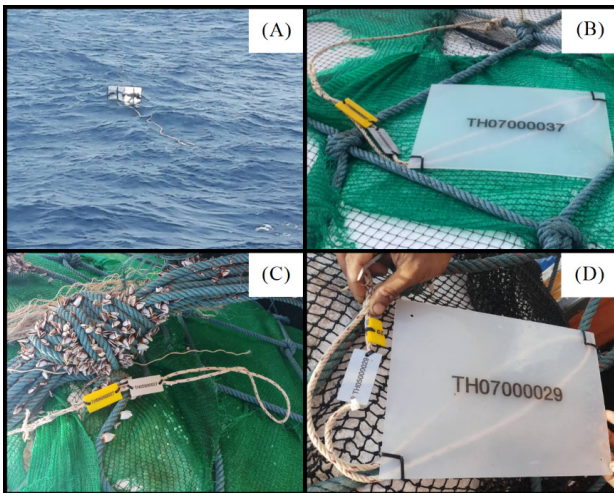


Figure 4. Observation of fishing gear marking (FGM) installed on anchored fish aggregating devices during the study in cooperation with fishers: float of an AFAD on the sea (A); three material types of FGM made of stainless steel or SS, colored acrylic or CA, and polypropylene or PP (B); remaining FGMs with lost PP (C); and remaining FGMs with broken CA and replacement of a cable tie on SS (D)

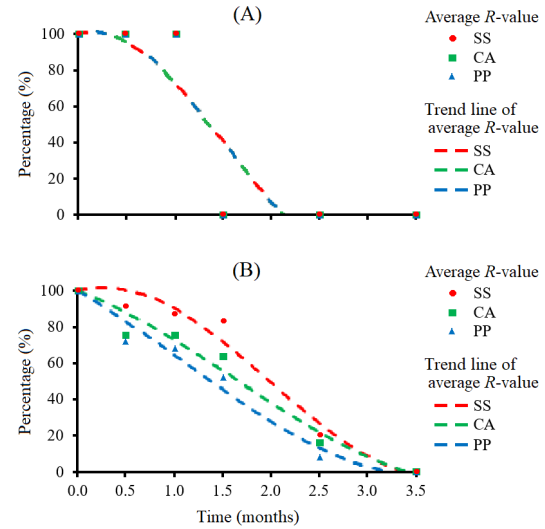


Figure 5. Average percentage of remaining condition (*R*, %) for three material types of fishing gear marking installed on anchored fish aggregating devices at different times (from 0.0 month to 3.5 months) in the Gulf of Thailand (A), and the Andaman Sea (B) (SS: stainless steel, CA: colored acrylic, PP: polypropylene)

Table 2. Causes of fishing gear marking (FGM) broken or lost (%) in the Gulf of Thailand and the Andaman Sea indicated by fishers who closely observed the FGMs installed on their anchored fish aggregating devices (SS: stainless steel, CA: colored acrylic, PP: polypropylene)

Cause of FGM broken or lost	Gulf of Thailand (n = 25)			Andaman Sea (n = 25)		
	SS	CA	PP	SS	CA	PP
Adverse weather condition	96	96	96	100	100	100
Fishing gear conflict	4	4	4	0	0	0

3.3. Comparative Analysis on Percentage of Remaining Condition

Since the three material types were lost at the same time for each set of FGM installed on AFADs in the GOT, the variance data obtained from the GOT were insufficient for the comparative analysis. Consequently, only data obtained from the ANS were included in our comparative analysis for material types and times. The analysis results revealed that the Mauchly's test was not significant for both material types ($\chi^2_{(2)} = 1.653$, $p = 0.438$) and times ($\chi^2_{(5)} = 5.938$, $p = 0.339$); hence, the Mauchly's test did not show any violation of sphericity. For the Two-way RMANOVA results, Table 3 indicates that there was no interaction between material type and time ($F_{(6,24)} = 0.749$, $p = 0.616$); moreover, there were significant differences among the three material types of FGM ($F_{(2,8)} = 5.402$, $p =$

0.033) and among the four consecutive times ($F_{(3,12)} = 56.505, p < 0.001$). Figure 6 shows the results of the post-hoc test for the three material types as well as the four consecutive times. For the three material types, SS had the higher R' than CA ($p = 0.036$) and PP ($p = 0.042$). Besides, there was no significant difference ($p = 0.302$) between CA and PP. For the four consecutive times, R' at 0.5 month, 1.0 month, and 1.5 months, there were no significant differences among them ($p > 0.081$); however, R' at the three times were higher than 2.5 months ($p < 0.002$). The results inferred that the percentage of remained FGM made of SS was higher than CA and PP, and the percentages of remained FGMs were not different from 0.5 month to 1.5 months but lowest at 2.5 months.

For the material types of FGM, the FAO [19] recommended several types of FGM to identify the ownership of AFADs, and we also adopted the steel tag as SS in this study; in addition, we applied plastic tag (*i.e.* CA) and plastic plate (*i.e.*, PP) to our study. Our results suggested that SS had the highest durability among the three material types of FGM. Due to the short lifespan of deployed AFADs in our study, almost all FGMs were lost together with the float of AFADs. We recommend that the improvement of AFADs lifespan is needed for enhancing the particular fishery, and the development of FGMs (*e.g.*, materials and installation) is also necessary for longer use along with the AFADs lifespan. To improve the installation method by using cable ties to install FGMs on the rope in our study, the ropes or threads should be considered instead of the cable ties. Furthermore, the production and effective cost of FGMs should be taken into consideration for adoptability for the implementation [23]. Also, the FGM system should be established, including registry and database, related measures, and retrieval program for lost AFADs. This is supported by the VGMFG of the FAO [18] to address ALDFGs and facilitate the identification and recovery of AFADs.

Material type (A)			
Stainless steel	Colored acrylic	Polypropylene	
a	b	b	
Time (B)			
0.5 month	1.0 month	1.5 months	2.5 months
a	a	a	b

Figure 6. Post-hoc test on average percentage of remaining condition among three material types (A) and four consecutive times (B). Material types and times with same letter (a or b) under the same horizontal line were not significantly different.

4. Conclusions

Among the three material types, all FGMs used in the GOT were durable to last for the lifespan of deployed AFADs, while some FGMs used in the ANS were detached from the cable ties or broken before AFADs were lost. The loss of AFADs and FGMs was mainly caused by adverse weather condition. Therefore, the three material types of FGM were assumed to be sufficiently durable to last for the lifespan of the AFADs both in the GOT and the ANS within 2.0 months and 3.5 months, respectively. For the ANS, the comparative analysis suggested that SS had the higher durability than CA and PP when the AFADs lasted for less than 3.5 months. Besides, ropes or threads should be further considered instead of the cable ties. The FGM system is also required to support the implementation of FGMs. This study would benefit fisheries policy makers, managers, or fishers as a basis to develop FGM to address ALDFG and support responsible fisheries.

Author Contributions

Watcharapong Chumchuen: Conceptualization, Methodology, Investigation, Validation, Formal analysis, Writing-original draft and Writing-review & Editing. Jirawut Kumpirod: Investigation, Validation, Writing-

Table 3. Summary of Two-way Repeated Measures Analysis of Variance from data of fishing gear marking installed on anchored fish aggregating devices in the Andaman Sea

Source	Sum of Square	df	Mean Square	F-ratio	p-value	Partial η^2
Material type	0.993	2	0.496	5.402	0.033*	0.575
Time	7.572	3	2.524	56.505	< 0.001***	0.934
Material type x Time	0.097	6	0.016	0.749	0.616 ^{ns}	0.158

^{ns} $p > 0.05$ * $p < 0.05$ *** $p < 0.001$

original draft. Sahaphat Duerasor: Investigation, Validation, Writing-original draft. Chanont Nualsri: Resources, Investigation. Thassanee Suppruek: Resources, Investigation. Kraison Krueajun: Resources and Investigation. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

Authors declare that there is no conflict of interest.

Funding

This research received no external funding.

Acknowledgments

Authors would like to thank all fishers for their cooperation in data collection. We also extend our gratitude to the staff from the Chumphon Marine Fisheries and Research Development Center and the Phuket Marine Fisheries Research and Development Center for their assistance during the field surveys. We are thankful to Dr. Shiela Villamor Chumchuen for the English review.

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RESEARCH ARTICLE

Parasitic Copepods on Common Carp (*Cyprinus carpio*, L. 1758) from Gradche Reservoir (Macedonia)

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ARTICLE INFO

Article history

Received: 16 February 2022

Accepted: 25 March 2022

Published Online: 01 April 2022

Keywords:

Parasites

Copepods

Common carp

Reservoir

ABSTRACT

During the parasitological examinations of the common carp (*Cyprinus carpio*, L. 1758) from the Gradche Reservoir (Macedonia), a total of 126 fish samples were examined, from which parasite infestation was determined in 87 fish (69.05%). In this research, the following parasitic copepods were identified: *Ergasilus sieboldi*, *Ergasilus briani*, and *Lernaea cyprinacea*. *Ergasilus sieboldi* was found on gills of common carp, with a prevalence of 1.461%, while the mean intensity was 2.357. *Ergasilus briani* was found on fins of common carp, with a prevalence of 1.879%, while the mean intensity was 38.274. *Lernaea cyprinacea* was found on the fins of common carp, with a prevalence of 0.552%, while mean intensity was 2,000. Our findings of *Ergasilus sieboldi*, *Ergasilus briani*, and *Lernaea cyprinacea* in common carp are considered as first records for Macedonia. At the same time, common carp is a new host for *Ergasilus briani* in Macedonian waters.

1. Introduction

There is a growing interest in fisheries and aquaculture development around the world because of the increasing human needs for food of animal origin, as well as the fact that over 70% of the globe is covered with water. Fish is the subject of much research, not only due to its great importance in nutrition but also as a means of improving the economic situation in a country ^[1]. The degree of activity of ectoparasites and endoparasites of the body

surface and/or inside the fish body depend on the degree of water purity in combination with other environmental factors. Poor hygienic aquaculture facilities' conditions have a major contribution to the development of the parasitic disease ^[2].

There is a close relationship between parasitic communities and the level of water pollution. In polluted environments, the degree of parasites' prevalence and intensity can be an indicator of environmental quality. Because the level of water contamination can directly

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DOI: <https://doi.org/10.30564/jfs.v4i1.4448>

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or indirectly affect the aquatic ectoparasites through the action of their intermediate host, ectoparasites that are directly exposed to water may be more sensitive to contaminants, thereby reducing their rates of survival and reproduction. The presence of ectoparasites of genders *Trichodina*, *Argulus*, and some *Dactylogyrus* mainly arises as a result of poor water quality, poor zoo - hygienic conditions, increased water temperature, or excessive settlement of fish in ponds^[3]. The parasites may be present on the skin, fins, gills, and internal organs of fish without major consequences for the host. However, under the influence of certain conditions, such as changes in aquatic parameters, stress and the introduction of exotic pathogens may increase the host acceptability to parasites and cause an imbalance of the system host/parasite/environment.

Copepods have arthropods body, composed of several segments. The segments are grouped into three parts: head, chest, and abdomen. Each segment, except the last, carries one pair of limbs. They have accessories on the head: antennae, maxilla, and mandible. There is a maxilla and 4-5 pairs of thoracic scissors on the chest. The last chest segment has a sex opening, and in females, this is where the two ovarian sacs begin. There are more than 1,500 species of copepods parasitize in fish^[4]. Gills parasites of the genera *Ergasilus* and *Lamproglana* in many high infestations can cause great damage to the fish population in fish ponds and cage farming systems. Copepods cause destruction and necrosis of tissues, feeding on the epithelium and blood of fish and causing injury and compressions of organs (skin, gills, muscles, eyes, kidneys, liver, intestines, skeleton, etc.)^[5]. Due to this, the normal function of organs, anemia, exhaustion, and weight loss occurs. Especially copepods cause big damage in young fish. A variety of different parasitic copepods can cause external infestations of freshwater and marine fish. Some members of the group are commonly referred to as fish lice. They are frequently found on the body, around the mouth, and on the gills. Parasitized fish may act lethargic. Mechanical abrasion due to the attachment and/or feeding by the copepods is common resulting in frayed fins, gill hyperplasia, and patchy epidermal damage and necrosis. Infections with secondary pathogens often occur. Most of these organisms have a direct life cycle involving several free-living and larval stages. Transmission is through contact with an infective free-swimming stage of the organism in the water column. The infective stage attaches to the fish where it goes through several larval stages before becoming

an adult.

2. Materials and Methods

This study aimed to determine the parasite fauna in common carp (*Cyprinus carpio*, L. 1758) from the Gradche Reservoir (Macedonia) (Figure 1). The Gradche Reservoir is located on Kochanska River near the village Dolno Gradche, 6 km north of Kochani. The dam is reinforced concrete, with a height of 32 m, length of the crown of 150 m, and elevation of 467 m above sea level. The Gradche Reservoir is 3.5 km long, 0.2 km wide, with a maximum depth of 29 m. The area of the Reservoir is 0.19 km², with a volume of 2.4 million m³ of water used for the water supply of the population of Kochani and irrigation of about 5761 ha of arable land in Kochansko Pole.



Figure 1. Gradche Reservoir
(web site of Kochani Municipality)

This parasitological study was carried out by seasons, during 2018. A total of 126 specimens of common carp from Gradche reservoir were examined for parasitological investigations. Only fresh fish were subjected to routine identification, dissection, and observation. Cleaned parasites were separated, put in appropriate fixatives, and prepared for determination using determining techniques of staining and clearing^[6-9]. Parasites on native smears were observed under a light microscope at the magnification of $\times 200$ and $\times 400$. The parasite specimens were identified using the reference keys^[7,10,11]. During the examinations at the Department for Fish Diseases in the Institute of Hydrobiology in Ohrid (Macedonia), Zeiss stereomicroscopes (Stemi DV4), as well as a light Reichart microscope, were used. Classical epidemiological variables (prevalence and mean intensity) were calculated^[12].

Data of prevalence and mean intensity (by seasons) with determined parasite species are given in Table 1.

Table 1. Prevalence (P) and mean intensity (I) with parasitic copepods in common carp (*Cyprinus carpio*, L. 1758) from Gradche Reservoir, by season

Parasite species	Spring		Summer		Autumn		Winter	
	I	P (%)	I	P (%)	I	P (%)	I	P (%)
<i>Ergasilus sieboldi</i>	2.889	1.794	/	/	/	/	1.400	0.441
<i>Ergasilus briani</i>	/	/	/	/	38.274	1.879	/	/
<i>Lernaea cyprinaceae</i>	/	/	/	/	/	/	2.000	0.552

3. Results and Discussion

During the parasitological examinations of the common carp from the Gradche Reservoir (Macedonia), a total of 126 fish samples were examined, from which parasite infestation was determined in 87 fish (69.05%). In this research, the following parasitic copepods were identified: *Ergasilus sieboldi*, *Ergasilus briani*, and *Lernaea cyprinaceae*.

Ergasilus sieboldi was found on gills of 14 specimens of common carp in winter, with a prevalence of 1.461%, while the mean intensity was 2.357. The prevalence with *Ergasilus sieboldi* by seasons was as follows: spring is 1.794% and winter is 0.441%, while the mean intensity: spring is 2.889 and winter is 1.400.

The Ergasilidae family is one of the largest families of Copepods that parasitize fish. Only adult females are parasitic, with a larval stage, while adult males are plankton^[13]. *Ergasilus* is the largest genus of the Ergasilidae family that includes more than 180 species^[14]. *Ergasilus sieboldi* is an ectoparasite that lives in large numbers on the gills of freshwater fish, causing a disease called ergasillosis. *Ergasilus sieboldi* parasitizes the gill filaments of fish from families: Cyprinidae, Salmonidae, Percidae, Siluridae, and others and it is very common in marine and freshwaters throughout Europe and Asia^[11].

The body of *Ergasilus sieboldi* has a pear-shaped form, with a length of 1.1 mm - 1.5 mm and width 0.4 mm - 0.7 mm. There is a pair of hooks on the head with which it is fastened to the gill filaments (Figure 2). Females have two elongated eggs bags on the body back, of approximately the same length as the body, which is especially pronounced in the summer period and contains 50 eggs - 140 eggs.

Larvae emerge from the eggs that immediately attack other fish, and reach the adult stage in 2 - 8 weeks^[15]. After fertilization, males die. During one year, under favorable conditions, several generations of shrimp are developing. This is especially pronounced in the summer period.

The females usually find both infest their hosts after mating and are then susceptible to metamorphosis in which adult forms change the shape of the body and increase before egg production begins^[16].

The presence of this copepod in fish gills causes mechanical tissue irritation, so in the beginning, there is epithelial hyperplasia and fusion of gills, and later lead to inflammation and necrotic changes. Severely injured fish are anemic, have pale gills, and weaken. The parasite constricts blood vessels in the gills, causing difficulty breathing. Deaths are quite common in infected offspring. These parasites are more common in lakes, where the disease can reduce natural fish production by up to 50%^[17].

According to data from the previous parasitological research in Macedonia, *Ergasilus sieboldi* was identified in *Alburnus albidus alborella* from Lake Ohrid^[8]. *Ergasilus sieboldi* was identified in the following fish species of natural lakes in Macedonia: *Leuciscus cephalus albus*, *Barbus meridionalis petenyi*, *Alburnus albidus alborella*, *Scardinius erythrophthalmus*, *Rutilus rubilio*, *Pachychilon pictus*, *Barbus cyclolepis prespensis*, *Alburnus alburnus belvica*, *Alburnoides bipunctatus prespensis* and *Tinca tinca*^[17-19]. *Ergasilus sieboldi* was found in common carp from Tikvesh Reservoir^[20]. According to literary reviews from surrounding countries and the world, the presence of *Ergasilus sieboldi* in common carp was determined in waters in Serbia^[21], in fishponds in the Czech Republic^[22], in fishponds in Iraq^[23], and in fishponds in Iran^[24]. In Turkey, data on the appearance of *Ergasilus sieboldi* in common carp were published from Lake Izmir^[25,26], from the Dalyan Lagoon^[27], and from the Lake Karacaören Dam^[28].



Figure 2. *Ergasilus sieboldi* (whole parasite) on gills in common carp (*Cyprinus carpio*) from Gradche reservoir (original)

Ergasilus briani was found on fins of 18 specimens of common carp in autumn, with a prevalence of 1.879%, while the mean intensity was 38.274. Our findings of *Ergasilus briani* in common carp are considered as the first record for Macedonia. At the same time, common carp is a new host for *Ergasilus briani* in Macedonian waters.

The length of *Ergasilus briani* ranges from 0.76 mm - 0.98 mm, and the width from 0.22 mm - 0.24 mm (Figure 3). The segment of the V pair of legs sometimes is not separated from the sex segment. Antenna II has short members, and the penultimate one is wide, so the length exceeds the width by about 2.5 times. The last member is a strong curved. There is a thorn on the basal limb, which is usually absent in other species. On the extremity of the exopodite, on an IV pair of legs, there are 4 hairs (others species have 5). Egg bags do not exceed the length of the copepod.

This widespread species differs from the companion species *Ergasilus sieboldi* in the shorter last member (claw) of the antenna II, in the wider and shorter limbs, the absence of hairs on the abdominal segments, and the micro-location of the gills. *Ergasilus briani* is usually found among gill filaments, compared with *Ergasilus sieboldi* that usually parasitizes on the outer surface of the gills.

The life cycle of *Ergasilus briani* includes several free-swimming stages before the adult female becomes a parasite. These larval stages can live for several weeks in water and feed on algae. Females – parasites live approximately one year and overwinter on fish. The life cycle of *Ergasilus briani* is temperature-dependent, with reproduction beginning in spring at a temperature of about 8 °C, continuing until late autumn, and ending in winter.

Ergasilus briani is found on the gills of cyprinid fish, but may also be found in other fish species, often together with *Ergasilus sieboldi*. Its life cycle covers multiple stages, but only adult females parasitize. This parasite is attached between the gill filaments of its host, using both special antennas that serve this purpose.

The presence of a large number of parasites of this species can cause serious gill damage leading to respiratory disorders and normal gill function, resulting in hyperplasia and necrosis. Infested fish become less tolerant of changes in the environment. It leads to poor condition, weight loss, and in the offspring even to mortality. The insertion of the antennae deep into the gill tissue also can cause blood vessels to constrict and rupture.

According to the literary data, the infestation with *Ergasilus briani* is seasonal and generally occurs during the summer or late autumn, which is in correlation with

our findings on this parasite in the autumn. *Ergasilus briani* attacks the smaller fish, with a body length of less than 10 cm. The transmission of *Ergasilus briani* can occur through infected water, plants, or fishing equipment.

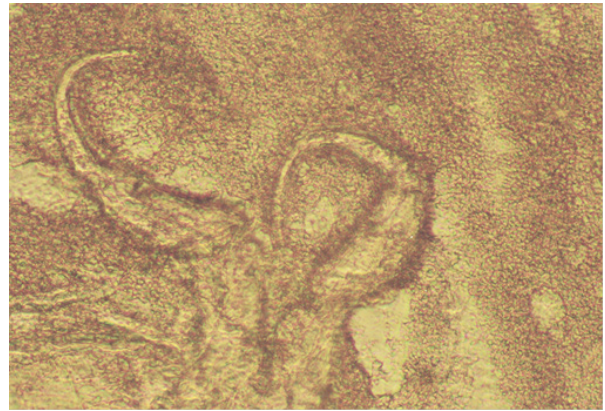


Figure 3. *Ergasilus briani* (hooks) on gills in common carp (*Cyprinus carpio*) from Gradche reservoir × 200 (original)

Lernaea cyprinacea was found on the fins of 5 specimens of common carp in winter, with a prevalence of 0.552%, while the mean intensity was 2.000. Our findings of *Lernaea cyprinacea* in common carp are considered as the first record for the Gradche reservoir.

The female's body reaches a length of 9 mm - 21 mm, while the male is smaller and moves up to 0.7 mm. There are four so-called cephalic horns on the head, of which the anterior two are cylindrical and the other two are T-shaped. The parasite is fixed to the muscles of the host with their help. It can be found on the surface of the whole fish body, but most often parasitizes on the fins and around the oral cavity.

Parasitic copepods of freshwater fish, cause a disease called lerneosis. About 110 species of parasites are described, from 14 different genera belonging to the family Lernaeidae^[13]. The genus *Lernaea* includes more than 40 parasite species. *Lernaea cyprinacea* is the most common species, found in waters across North America, Europe, Asia, South Africa, and Eastern Australia^[29]. *Lernaea cyprinacea* is a cosmopolitan copepod parasite in many species of freshwater fish^[30]. This parasite has a wide range of hosts and is registered in more than 100 fish species, from 25 families and 10 orders. *Lernaea cyprinacea* has been identified in more than 45 cyprinid fish species, which in Europe are major hosts of this parasite^[29].

The life cycle of *Lernaea cyprinacea* consists of nine stages, including three free-floating stages of nauplius, five copepod stages, and one stage of the adult form. As soon as in the body of the host fish are formed male

and female adult forms, the male dies and the female metamorphoses.

The front of the body of the metamorphosed adult female attaches to the host tissue, where it lays eggs, while the rest of the body is released into the water. Free-swimming larval spawns hatch from the eggs, which are about four days pass into infectious copepod larvae, which are usually attached to the gills of the host. Depending on the temperature, for about one week the copepods turn into an adult form, with optimal development of T from 28 °C - 36 °C and poor development of T below 20 °C. Adult males die within 24 hours, and the females remain attached to the same host or swim in search of a new host ^[29].

Infection with these parasites can cause serious pathological effects on their host - fish. The most common histopathological changes in infected adult cyprinids are manifested by severe degeneration and necrosis of the skin and muscles. Extensive edema, hemorrhage, leucocyte infiltrations, and melano-macrophages occur on the dermis and hypodermis. One of the consequences is damage and necrosis of the gill epithelium, while adult females usually cause hemorrhage, muscle necrosis, and severe inflammation, which are the entry point for secondary bacterial and fungal infections ^[31]. The larval stage usually infests the gills of the host, while the main pathogenic effect is caused by adults females, which use their claws to penetrate deep into the skin and body muscles causing sores, blood clots, and a decline in the scales. In severe cases, changes are seen throughout the skin, and the parasite can penetrate deep into the muscles and even into the body cavity. Fish weaken, and deaths occur because of anemia and secondary infections with bacteria and mycoses. Once lernosis occurs, it is very difficult to eradicate ^[13].

According to the data from previous parasitological research in Macedonia, the presence of *Lernaea cyprinacea* was first established in common carp from the fish farm Zabenj – Bitola ^[32]. Also, the presence of *Lernaea cyprinacea* was determined in common carp in waters in Macedonia ^[18]. According to the literary reviews from surrounding countries and the world, data on the presence of *Lernaea cyprinacea* in common carp has been published in waters in Serbia ^[21], in fishponds in Romania ^[33], in rivers in Spain ^[34], in waters in Mozambique ^[35], in fishponds in Iran ^[36,37], in fishponds in Iraq ^[23,38], in fishponds in Egypt ^[39] and in waters in southern California ^[40].

Lernaea cyprinacea causes high mortality in freshwater fish. The pathological effect of *Lernaea cyprinacea* is greater in smaller fish because the attachment organs penetrate much deeper into the fish body, often causing damage to internal organs ^[29]. This is in correlation with

our research, where we concluded that adult forms of *Lernaea cyprinacea* are particularly harmful to young fish due to their relatively large size and the method of attachment and feeding. The presence of *Lernaea cyprinacea* complicates consuming food and slowing down the growth of diseased fish ^[41].

4. Conclusions

Parasites are important components in an ecosystem through their diverse effects on the dynamics of the host population, interactions with the community, and the structure of the biocenosis. Many diseases in fish prevent their productivity and fertility, leading to catastrophic consequences in fisheries and aquaculture. The health of a population depends on disease control and maintaining a healthy relationship between living organisms and their environment.

In terms of intensive fish farming, there is a very high density of susceptible fish in a limited space, and their physiological and health status is not always satisfactory, so there are a large number of susceptible individuals, with excellent conditions for the spread of mass parasitic infections. On the other hand, the fish in open waters regularly has a large number of parasites species, especially with the complex life cycle, as animals that occur as intermediate hosts of parasites (snails, worms, and crabs) are present in the wild.

Preventive measures for the elimination of parasitic diseases consist in the destruction of the parasites themselves or destroying those aquatic organisms that appear as their transitory hosts. Regarding this, permanent ichthyoparasitological controls of the fish health status should be made.

In most parasitic diseases, if stressful moments are avoided and optimal conditions of the water environment are ensured, as well as adequate health and physiological status, the fish may tolerate the invasion of parasites. Severe forms of parasitic infections are often resulting from the adverse influence of the environment.

Author Contributions

Conceptualization, analysis and writing - Dijana Blazhekovikj - Dimovska

Writing - review - Stojmir Stojanovski

Conflict of Interest

There is no conflict of interest.

Funding

This research received no external funding.

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ARTICLE

A Baseline Study on the Quality and Safety of Consumption of a Pest Species (*Sarotherodon melanotheron*) in Bataan, Philippines: Basis for Its Productive Utilization in the Fisheries Sector

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ARTICLE INFO

Article history

Received: 28 February 2022

Accepted: 21 March 2022

Published Online: 01 April 2022

Keywords:

Tilapia

Black-chin Tilapia

Sarotherodon melanotheron

Proximate composition

Heavy metal load

Microbiological quality

Orani

Bataan

ABSTRACT

The baseline study profiled Black-chin Tilapia (*Sarotherodon melanotheron*), a fish farm pest species in Bataan, Philippines, in terms of yield (processing and fillet), proximate composition (moisture, ash, crude fat, and crude protein), heavy metal load (cadmium [Cd], lead [Pb], arsenic [As], and mercury [Hg]), and microbial count (aerobic plate, *Escherichia coli*, and *Staphylococcus aureus* counts). The purpose was to establish the species' safety and quality for consumption and potential utilization in the processing of higher value fishery products. A completely randomized experiment using two factors, fish size (standard and small sizes) and collection season (dry and wet seasons), was employed. The collected data were also compared against food consumption and processing standards and/or previous reports on more valuable species. The results showed that the species has a comparable yield and mineral load with the more popular farmed Nile Tilapia (*Oreochromis niloticus*). It has high moisture and protein compositions. It is a lean fish that can serve as a cheaper functional raw material for processed fishery products. Moreover, the results showed that the species have no As, Cd, and Pb contamination, although traces of Hg, far below the permissible limits, were detected. The Hg load varies across collection season and fish maturity suggesting its manageability. For the microbial contents, the species' aerobic plate, *Escherichia coli*, and *Staphylococcus aureus* counts were far below the standard limits, although best post-capture practices are still suggested due to the kind of microbial parameters measured. It was concluded that the *Sarotherodon melanotheron* infesting Bataan farm ponds can be consumed safely and has the quality of potential raw material for processed fishery products. However, further information is still needed to establish the best post-capture handling on the species. Also, more studies must be done to determine the impact of storage and processing on its stability.

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DOI: <https://doi.org/10.30564/jfs.v4i1.4483>

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1. Introduction

Black-chin Tilapia (*Sarotherodon melanotheron*) is a species native to tropical East Africa, originally occurring from Senegal to Zaire and southern Cameroon. It was introduced to several countries across Asia, North America, and Europe decades in the past ^[1]. In the Philippines, their introduction was believed to be through the country's illegal aquarium trade ^[2]. Naturally, *Sarotherodon melanotheron* can achieve sexual maturity at a very small body size, making it highly adaptive. Being a salt-tolerant and hardy fish, it can spread very rapidly in both saltwater and brackish water environments. These traits make for a very opportunistic invasive species, and its incursion to fish farm areas can be very damaging.

Sarotherodon melanotheron was reported to be infesting Philippine brackish water farm ponds ^[3]. Particularly in the province of Bataan, the "pest" has been causing losses to commercial fish farms. It was able to proliferate in the farm ponds from Manila Bay through its tributaries. It has been rapidly multiplying and competing with cultured fish species for food ^[4,5], compromising the carrying capacity of the aquacultural areas meant for higher-value species. The infestation has triggered calls from fish farm operators to curb their population and/or for their productive utilization in the fishery and food sectors ^[4,6].

While it is locally believed to be edible, literature on the fishery of *Sarotherodon melanotheron* is in paucity. The species was recently investigated for processing ^[7], growth and feeding behavior ^[8], breeding and culture ^[2], and sex-determination mechanisms ^[9] but the safety of its consumption and its nutritional profile has not been reported extensively, particularly those infesting Philippine fish farms. Recognizing these, the present study worked on profiling *Sarotherodon melanotheron* infesting Bataan fish farms in terms of yield, proximate composition, heavy metal load, and microbial counts. The purposes were to establish the species' safety and quality and to have baseline data for its potential utilization in the processing of higher value fishery products. Ascertaining the aforesaid information for any prospective fish raw material is important to fish processors for them to design and apply appropriate processing and storage methods.

Specifically, the baseline study's objectives were the following: measurement of yield indices of *Sarotherodon melanotheron* infesting Bataan farm ponds, analysis of its proximate composition (moisture, ash, crude fat, and

crude protein), and determining of its heavy metal load (cadmium, lead, arsenic, and mercury) and microbial count (aerobic plate, *Escherichia coli*, and *Staphylococcus aureus* counts). The data obtained were compared across fish size (standard and small sizes) and collection season (dry and wet seasons). All data obtained were also compared against food consumption and processing standards and/or against previous reports for more valuable tilapia species.

2. Materials and Methods

2.1 Materials

Fresh *Sarotherodon melanotheron* fish of standard and small sizes caught from infested farm ponds in Bataan, Philippines.

2.2 Material Sourcing, Post-harvest Processing, and Yield Computation

All fish samples used were sourced from two 2 m - 4 m deep tilapia grow-out ponds located in Orani, Bataan (14.811898, 120.537822). The town of Orani is known to be the province's aquaculture center being a major tiger prawn, milkfish, tilapia, and mud crab producer through brackish water fishery ^[10]. The pond sites were selected primarily due to their apparent infestation by *Sarotherodon melanotheron* and hence their capacity to supply the needed number of samples for the investigation. The same sourcing and post-harvest processing procedures were performed for acquiring samples for the yield, proximate composition, heavy metal load, and microbial count measurements. Samples were collected in two seasons: wet season (July-December 2019) and dry season (January-June 2020).

Fish samples were caught using net traps and gill nets. The caught fish were kept in insulated plastic coolers with a fish/ice ratio of 1:1 (w/w) to slow post-mortem decomposition and then were transported immediately to the laboratory in BPSU-Orani (20-30 minutes travel time) for sorting and post-harvest processing. The fish were sorted into standard size (4 pcs/kg-5 pcs/kg) and small size (13 pcs/kg-14 pcs/kg) samples and then were weighed per piece (see Figure 1 for reference sizes). Weighed fish were then scaled, de-headed, eviscerated, filleted, and then de-skinned by hand. The separated parts were weighed for each sample. Yield indices were then computed for both sample groups using the formula below. Fifteen replicates for each group were used.



Figure 1. Standard size and small size *Sarotherodon melanotheron* samples

$$\% \text{ Processing Yield} = \frac{\text{Weight of Whole Fish} - \text{Weight of Head, Skin, and Viscera}}{\text{Weight of Whole Fish}} \times 100$$

$$\% \text{ Fillet Yield} = \frac{\text{Weight of De-skinned Fillet}}{\text{Weight of Whole Fish}} \times 100$$

After the yield measurements, the samples were immediately kept in frozen storage (-15°C) until further analysis.

2.3 Proximate Compositional Analysis

Using standard methods, the *Sarotherodon melanotheron* samples were examined for moisture content (AOAC 952.08A), ash content (AOAC 938.08), crude fat content (acid hydrolysis [Soxhlet]), and crude protein content (automated Kjeldahl method [Buchi]). Values were expressed in % w/w. Three replicates for both standard and small size samples were used.

2.4 Heavy Metal Analysis

Heavy metal loads in the fish samples were analyzed using standard methods. The following were tested: cadmium (Cd) in ppm (AOAC 999.10), lead (Pb) in ppm (AOAC 999.10), arsenic (As) in ppb (AOAC 986.15), and mercury (Hg) in ppb (EPA 7473). Three replicates for each group were used.

2.5 Microbial Testing

The fish samples were examined for the following microbial parameters using standard culture techniques: aerobic plate count (FDA BAM-3, 2001, pour plate method), *Staphylococcus aureus* count (FDA BAM-12, 2001, spread plate method), and *Escherichia coli* count (FDA BAM-4, 2002, MPN method). All methods were acquired from the Bacteriological Analytical Manual, Online 2001 of the US Food and Drug Administration

(FDA). Aerobic plate count and *Staphylococcus aureus* count were expressed in CFU/g, while *Escherichia coli* count was expressed in MPN/g. The analyses were done in triplicates.

2.6 Statistical Analysis

The present study utilized a completely randomized experimental design where two factors, collection season and fish size, were investigated on their effect on yield, proximate composition, heavy metal load, and microbial count. Data for the yield, proximate composition, and heavy metal load were presented as mean \pm standard deviation. The microbial load meanwhile was presented in the mean total count. The tests for significant differences were done using One-way Analysis of Variance at $\alpha=0.05$. Post hoc analyses were performed using Fisher's Least Significant Difference Test. Statistical analyses were performed using IBM SPSS Version 20.0 for Windows.

3. Results and Discussion

3.1 Yield Indices of *Sarotherodon melanotheron* Samples

Knowledge of the yield of edible portions from fish is essential for its maximum utilization. As indicated in Table 1, the yield characteristics for *Sarotherodon melanotheron* revealed a fillet yield of $26.02\% \pm 0.23\%$ (dry season) and $25.88\% \pm 0.17\%$ (wet season) for the standard size samples and $25.15\% \pm 0.61\%$ (dry season) and $24.99\% \pm 0.40\%$ (wet season) for the small size samples versus the whole percentage of total fish weight. No significant difference ($P \leq 0.05$) was found in the values based on the statistical test. The processing yield of the present samples on the other hand were $66.65\% \pm 0.74\%$ (dry season) and $67.13\% \pm 0.66\%$ (wet season) for the standard size samples and $66.62\% \pm 0.66\%$ (dry season) and $66.96\% \pm 0.17\%$ (wet season) for the small size samples. No significant difference ($P \leq 0.05$) was found in the said values based as well based on the statistical test. The processing yield is the total weight of a whole fish minus the weight of its head, skin, and viscera. The fillet yield values for all present samples were slightly higher than that for farmed Nile Tilapia or *Oreochromis niloticus* (25.4%) in the previous study by Clement and Lovell^[11]. This suggests that the yield for *Sarotherodon melanotheron*'s edible portion can be comparable to that of the more commercially popular species.

Table 1. Yield indices of standard size and small size *Sarotherodon melanotheron* samples for dry and wet seasons

Form	Farmed <i>Oreochromis niloticus</i>	<i>Sarotherodon melanotheron</i>			
		Dry Season		Wet Season	
		Standard Size Samples	Small Size Samples	Standard Size Samples	Small Size Samples
Whole	100.00%	100.00%	100.00%	100.00%	100.00%
Processing Yield (whole fish weight minus weight of head, skin, and viscera)	51.00% ^[11]	66.65%±0.74%	67.13%±0.66%	66.62%±0.66%	66.96%±0.17%
Fillet	25.40% ^[11]	26.02%±0.23%	25.15%±0.61%	25.88%±0.17%	24.99%±0.40%

- Values are expressed as mean ± standard deviation of 15 measurements
- Values for *Sarotherodon melanotheron* in the same row were found to be not significantly different from each other at $\alpha = 0.05$

3.2 Proximate Composition of *Sarotherodon melanotheron* Samples

Determining the basic nutrients of the fish can justify its consumption and guide its utilization as a raw material for fish product processing. In this present study, the basic nutrients were described in terms of proximate composition. The corresponding mean percentage values for the *Sarotherodon melanotheron* samples are reported in Table 2.

As shown, the crude protein contents of the standard size samples were 19.30%±0.35% (dry season) and 16.54%±0.55% (wet season), while those of the small size samples were 19.23%±0.67% (dry season) and 15.93%±0.13% (wet season). The statistical test revealed that the samples collected during the dry season had values significantly higher ($P \leq 0.05$) than those collected in the wet season. The present figures for the dry season were also far higher than the values for farmed *Oreochromis niloticus* in the previous studies of Desta et al.^[12] and Anani and Agbeko^[13], which reported 14.77% and 16.37%-17.87% respectively. Meanwhile, the present values for the wet season were comparable to the figures from the same previous studies cited. All present samples had protein contents typical for raw tilapia (20.08%) as per the USDA^[14]. Based on the results, we can safely state that *Sarotherodon melanotheron* can be a rich protein source. Its high protein content may be due to its diet^[13,15,16]. For the present samples, such could have come from feeds intended for cultured fish in the ponds they have been infesting.

For crude fat, the standard size samples had mean percentages of 0.63%±0.06% (dry season) 0.50%±0.01% (wet season), while those for the small size samples had 0.57%±0.06% (dry season) and 0.50%±0.01% (wet season). There was no significant difference ($P \leq 0.05$) found among the values. All present values were lower than

that for farmed *Oreochromis niloticus* in the other studies cited earlier. Desta et al.^[12] and Anani and Agbeko^[13] reported 2.39% and 3.14%-4.20% for the species' crude fat respectively. Comparison of the present and previous results using the categories for fish by fat content outlined by Ackman^[17] tells that *Sarotherodon melanotheron* is a lean fish (<2%). Low fat, medium fat, and high fat fish has 2%-4%, 4%-8%, and >8% fat content respectively. Also, the present samples were lower than the average fat content (1.7%) for raw tilapia as per the USDA^[14].

The present crude protein and crude fat figures suggest that *Sarotherodon melanotheron* can be a cheaper alternative fish meat source and a functional raw material in the processing of various fishery products. For instance, the species has so much potential as a raw material for surimi-based goods which depend highly on the amount and strength of fish protein for quality. A high protein and low-fat fish flesh are a well-established surimi raw material standard^[18]. Surimi-based products are growing in demand locally^[19] and abroad^[20]. Apparently, due to its reputation as a pest and its flesh's characteristic bland taste, *Sarotherodon melanotheron* is sold very modestly at local markets in Bataan. A kilogram of the fish will cost only 0.2-0.4 USD.

As seen in Table 2, the major component of all *Sarotherodon melanotheron* samples was moisture. The moisture content of the standard size samples were 79.50%±0.26% (dry season) and 79.76%±0.54% (wet season), while those for the small size samples were 79.23%±0.12% (dry season) and 78.99%±0.94% (wet season). There was no significant difference ($P \leq 0.05$) between the four values. All were within the acceptable range of 60%-80% for tilapia previously mentioned by Tsegay et al.^[16] and were far higher than that for farmed *Oreochromis niloticus* in the previous work by Desta et al.^[12] and

Table 2. Proximate composition of standard size and small size *Sarotherodon melanotheron* samples for dry and wet seasons

Proximate Composition (% w/w)	Typical Values for Raw Tilapia	Farmed <i>Oreochromis niloticus</i>	<i>Sarotherodon melanotheron</i>			
			Dry Season		Wet Season	
			Standard Size Samples	Small Size Samples	Standard Size Samples	Small Size Samples
Crude Protein	20.08 ^[14]	14.77-17.87 ^[12,13]	19.30±0.35 ^a	19.23±0.67 ^a	16.54±0.55 ^b	15.93±0.13 ^b
Crude Fat	1.7 ^[14]	2.39-4.20 ^[12,13]	0.63±0.06	0.57±0.06	0.50±0.01	0.53±0.07
Moisture	78.08 ^[14] ; 60-80 ^[16]	73.62-76.65 ^[12,13]	79.50±0.26	79.23±0.12	79.76±0.54	78.99±0.94
Ash	0.93 ^[14]	1.5-1.96 ^[12,13]	1.30±0.10 ^b	1.23±0.06 ^b	2.25±0.19 ^a	1.05±0.00 ^b

▪ Values for *Sarotherodon melanotheron* are expressed as mean ± standard deviation of triplicate measurements
 ▪ Values for *Sarotherodon melanotheron* in the same row with different superscripts are significantly different at $\alpha = 0.05$

Anani and Agbeko^[13] where 73.61% and 75.61%-76.65% respectively were reported. The present moisture values were also close to the typical figure of 78.08% for raw tilapia^[14].

The high moisture content for the present samples may be attributed to the quality of their proteins and largely to their very small fat content. This is because moisture in fish flesh is associated with the myofibrillar proteins' capacity for water retention, while fat content in meats is proven to be inversely related to water content^[21]. Moisture content is a key quality measure of fishery products because it is linked to their important functional properties^[5]. Also, weight losses in fishery products during frozen storage are related to their capacity for water retention^[22].

In terms of ash content, the present standard size samples figured at 1.30±0.10% (dry season) and 2.25±0.19% (wet season). Meanwhile, the ash content of the small size samples had 1.3±0.06% (dry season) and 1.05±0.00% (wet season). The value obtained for standard size samples collected during the wet season was found to be significantly higher ($P \leq 0.05$) than the other values. Even then, all present values were comparable to the 1.5%-1.96% range for farmed *Oreochromis niloticus* previously reported by Desta et al.^[12] and Anani and Agbeko^[13]. Also, they were higher than the typical ash percentage of 0.93% for raw tilapia set by the USDA^[14]. Ash content is a measure of the samples' mineral load which may include but is not limited to magnesium, calcium, potassium, and zinc^[14]. It can be said that *Sarotherodon melanotheron* may be similar to *Oreochromis niloticus* by mineral load, and this must be confirmed by more specific mineral compositional analyses.

All in all, the proximate compositional results for the *Sarotherodon melanotheron* samples support the

reports that the chemical characteristics of fish flesh vary according to species, season, and size^[23,24]. The variations in protein and ash compositions by species and fish size found in the present study may be due to the combination of several morphological and physiological factors. Meanwhile, the variations by season may be linked to food availability and quality as well as the physical conditions of water where the pest has been thriving.

3.3 Heavy Metal Load of *Sarotherodon melanotheron* Samples

Heavy metals are elements of high densities and atomic weights that are hazardous even at very low concentrations^[25,26]. They can be introduced to the aquatic ecosystem through various anthropogenic and non-anthropogenic means^[27,28]. Because of their high degree of toxicity, As, Cd, Pb, and Hg rank among the priority heavy metals of public health significance^[28,29]. The presence of these heavy metals in the *Sarotherodon melanotheron* samples is indicated in Table 3. Data show that Pb, Cd, and As were not detected in all samples. While Hg was detected in both standard size (16.39±0.02 ppb and 14.16±0.08 ppb for dry and wet seasons respectively) and small size samples (11.57±0.46 ppb and 17.90±1.71 ppb for dry and wet seasons respectively), the levels were way below the permissible limits of 500 ppb outlined in FAO 210^[30]. Because Hg is ubiquitous in the environment^[28], its presence in the samples could have come from several sources, but it may be largely attributed to Hg additives in pesticides and fertilizers used in agricultural areas close to the sample collection site and to domestic discharges from nearby communities.

Also, it is worth noting that there were significant

Table 3. Heavy metals load of standard size and small size *Sarotherodon melanotheron* samples for dry and wet seasons

Heavy Metal	Permissible Limits for Fish Flesh	<i>Sarotherodon melanotheron</i>			
		Standard Size Samples		Small Size Samples	
		Dry Season	Wet Season	Dry Season	Wet Season
Pb (ppm)	0.2 ^[30]	ND	ND	ND	ND
Cd (ppm)	0.05 ^[30]	ND	ND	ND	ND
As (ppb)	10000-400000 ^[31]	ND	ND	ND	ND
Hg (ppb)	500 ^[30]	16.39±0.02 ^a	11.57±0.46 ^c	14.16±0.08 ^b	17.90±1.71 ^a

- Values are expressed as mean ± standard deviation of triplicate analysis
- Values with a different superscript in the same row are significantly different at $\alpha = 0.05$
- ND – Not detected

differences ($P \leq 0.05$) in the Hg loads across collection season and fish size, with the values for the wet season small size samples and dry season standard size samples figuring to be the highest. The seasonal variation can be explained by the different effects a season can bring on a water body's pollutant load, and hence on its heavy metal concentrations. The wet season for example can lower the heavy metal concentration via the dilution effect of rainwater run-off, while it can raise the concentration through the flushing-effect of run-off from areas with significant heavy metal sources ^[32]. The water in the present study's collection site largely comes from the connecting rivers that directly receive run-offs from many sources. On the other hand, the variation by fish size may be due to the difference in swimming patterns and feeding behavior sometimes observed among different sizes or maturities of the same aquatic species ^[33,34].

Overall, the results for the heavy metal load suggest that the *Sarotherodon melanotheron* collected from Bataan farm ponds has no risk of heavy metal contamination and therefore can be consumed safely. The results may also be an indication that Bataan's fish farms have no serious heavy metal concentrations.

3.4 Microbial Count of *Sarotherodon melanotheron* Samples

Analysis of the microbiological quality of fish is important to public health as it determines any presence of pathogenic bacteria. This becomes more necessary if *Sarotherodon melanotheron* will be considered for consumption and utilization as the microbial association is common in tilapia fish ^[35–37].

The microbial load of the present study's samples is shown in Table 4. In terms of aerobic plate count, the

standard size samples had a mean count of 5,100.00 CFU/g (dry season) and 4,300.00 CFU/g (wet season), while the small size samples had a mean count of 25,333.33 CFU/g (dry season) and 16,333.33 CFU/g (wet season). All values were not significantly different ($P > 0.05$) from each other, and all were way below the permissible limits of 500,000 CFU/g set in FAO 210 ^[30]. The aerobic plate count indicates the bacterial populations in a sample ^[38]. Their presence in fish flesh can be due to pre- and post-capture contamination ^[29]. Similarly, for *Escherichia coli* and *Staphylococcus aureus* counts, the figures were far below the standard limits. All four samples had a mean *Escherichia coli* count of <3 MPN/g and a mean *Staphylococcus aureus* count of <10 CFU/g, which were way below the corresponding permissible counts of 11 MPN/g and 1,000 CFU/g as per FAO 210 ^[30]. The four values for each parameter were also found to be not significantly different ($P > 0.05$) from each other. *Escherichia coli* and *Staphylococcus aureus* are two of the most common causes of fish spoilage and seafood-borne diseases ^[37,39]. Their occurrence in fish flesh is often associated with post-harvest handling ^[29].

The present results suggest that the *Sarotherodon melanotheron* infesting Bataan fish farms have a minimal microbial load, hence, the risk of contracting microbial diseases upon consuming and processing the fish is low. This however still hinges on the way the fish will be handled as the occurrence of the microbial parameters measured depends highly on post-capture activities. According to Eltholth ^[29], the post-capture contamination of fish could come from contaminated fishing tools, water, and ice; soiled surfaces and containers; and unhygienic handling practices. Nonetheless, further microbial risk assessments for the pest are recommended to further support the present results, considering bacterial growth

Table 4. Microbial load in standard size and small size *Sarotherodon melanotheron* samples for dry and wet seasons

Bacterial Culture	Permissible Limits for Fish Flesh	<i>Sarotherodon melanotheron</i>			
		Standard Size Samples		Small Size Samples	
		Dry Season	Wet Season	Dry Season	Wet Season
Aerobic Plate Count (CFU/g)	<500000 ^[30]	5100.00	25333.33	4300.00	16333.33
<i>Escherichia coli</i> Count (MPN/g)	11 ^[30]	<3	<3	<3	<3
<i>Staphylococcus aureus</i> Count (MPN/g)	1000 ^[30]	<10	<10	<10	<10

▪ Values are presented as a mean total count of triplicate analysis
 ▪ Values with a different superscript in the same row are significantly different at $\alpha = 0.05$

upon storage and their inactivation through processing, cooking, and consumption.

4. Conclusions

This study worked on profiling *Sarotherodon melanotheron* infesting Bataan fish farms in terms of yield, proximate composition, heavy metal load, and microbial count. The results showed that the species has a comparable yield and mineral load with the more commercially used farmed *Oreochromis niloticus*. The species has high moisture and protein contents but is very low in fat making it a lean fish. These characteristics offer a cheaper fish meat alternative and a potential functional raw material in the processing of fishery products. Meanwhile, results for the heavy metal load tests showed that the species have no As, Cd, and Pb contamination, although negligible traces of Hg were found. The Hg load varies across collection season and fish maturity, which suggests its manageability. Similarly, for the microbial contents, the species was found to be very minimal in microbial counts, although best post-capture handling is still suggested due to the kind of microbial parameters measured. It is concluded in this baseline investigation that the *Sarotherodon melanotheron* infesting Bataan farm ponds can be consumed safely and has quality potential for raw material in processed fishery products. However, further information is still needed to establish the best post-capture practices on the species. Also, more studies must be done to determine the impact of storage and processing on its stability.

Author Contributions

The first author was the one responsible for the study's conception and design and was the one who drafted the article and revised it critically for important intellectual content. All listed co-authors meanwhile contributed significantly through data collection, and analysis and interpretation of results. All authors reviewed the results and approved the final version of the manuscript.

Conflict of Interest

All authors hereby declare no conflict of interest for this article's content.

Funding

The study was funded by the Philippine Council for Industry, Energy and Emerging Technology Research and Development, one of the three sectoral planning councils of the country's Department of Science and Technology (DOST) through its Grants-in-Aid Program.

Acknowledgments

The authors would like to acknowledge the Bataan Peninsula State University's Institute of Fisheries and Aquatic Sciences and its Center for Research on Aquaculture and Aquatic Resources in Brackishwater Systems both in Orani, Bataan, Philippines for all the support and assistance in the conduct of this study. The authors would also like to acknowledge the Standards and Testing Division of the DOST-Industrial Technology Development Institute and the DOST-Food and Nutrition Research Institute for the technical assistance in the sample analyses.

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