

Effect of water soluble fractions (WSF) of Mobile oil on nutritional value of marine prawn *Peneaus indicus*

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Abstract

The water-soluble fractions (WSF) of petroleum products represents the most immediately toxic and sublethal component of oil pollution in marine environments. *Peneaus indicus* (Indian white shrimp) is a commercially significant penaeid species in the Indo-Pacific region, valued for its high-quality protein, essential amino acids, and long-chain polyunsaturated fatty acids. However, the effects of WSF exposure on the **nutritional quality** (as opposed to mere survival) of this species remain poorly characterized.

This proposal aimed to systematically evaluate the effects of sublethal concentrations of Mobile oil WSF on key nutritional parameters in *P. indicus* muscle tissue, including proximate composition, amino acid profiles, and fatty acid profiles, while elucidating the biochemical mechanisms underlying any observed changes.

Adult *P. indicus* were exposed to GC-MS-characterized WSF gradients using static-renewal bioassays. Following 96-h acute toxicity testing to determine LC₅₀, a 28-day sublethal exposure was conducted. Muscle and hepatopancreas tissues were sampled at days 7, 14, 21, and 28. Proximate composition was determined by Kjeldahl (protein), Soxhlet (lipid), and phenol-sulfuric acid (carbohydrate) methods. Amino acid and fatty acid profiles were analyzed by HPLC and GC-FID, respectively. Hepatopancreatic glutathione S-transferase (GST) activity and malondialdehyde (MDA) levels are biomarkers of detoxification and oxidative stress.

During present research work, WSF exposure induced dose- and time-dependent reductions in muscle protein and lipid content, with decrease in n-3 polyunsaturated fatty acids (EPA, DHA). Carbohydrate content showed biphasic responses. GST activity was initially induced then suppressed, while MDA increased progressively.

This study will provide a quantitative framework for assessing petroleum pollution impacts on the **nutritional quality** of marine fishery resources, with implications for seafood safety and coastal fisheries management.

Keywords:

Water-soluble fraction; Mobile oil; *Penaeus indicus*; Nutritional value; Amino acid profile; Fatty acid profile; Oil pollution; Food safety

1. Introduction**1.1 Ecological and Toxicological Significance of WSF in Marine Oil Pollution**

Global marine oil pollution introduces approximately 1.3 million tonnes of petroleum hydrocarbons annually, with roughly half originating from anthropogenic activities. Among petroleum pollutants, the **water-soluble fraction (WSF)** occupies a unique ecotoxicological niche: it comprises low-molecular-weight, relatively water-soluble components—principally monocyclic aromatics (BTEX: benzene, toluene, ethylbenzene, xylenes), polycyclic aromatic hydrocarbons (PAHs, especially naphthalene and alkyl naphthalenes), and minor heterocyclic compounds (e.g., dibenzothiophene). Unlike surface oil slicks, dissolved WSF components are directly bioavailable through gill and integumentary exposure, posing heightened risks to free-swimming organisms including penaeid prawns.

The toxicity of WSF involves multiple mechanisms: low-molecular-weight aromatics disrupt membrane fluidity and ion transport; PAHs undergo metabolic activation via cytochrome P450 systems, generating oxidative and genotoxic intermediates. Japanese Environment Agency studies established 24-h LC_{50} values for prawns of 9.7 $\mu\text{L/L}$ for benzene, 12.6 $\mu\text{L/L}$ for toluene, and 3.98 mg/L for naphthalene. Dibenzothiophene showed lower acute toxicity (24–96 h LC_{50} : 0.47 mg/L) but notable bioconcentration factors (BCF: 180 in prawns) and environmental persistence.

Crucially, **acute lethality** represents only one dimension of oil pollution impact. For fishery resources, **sublethal effects**—particularly those affecting the **nutritional quality** of edible tissues—carry direct food safety and human nutrition implications that remain inadequately addressed in existing literature.

1.2 Nutritional Significance and Commercial Importance of *Penaeus indicus*

Penaeus indicus (currently classified as *Fenneropenaeus indicus*) is among the most commercially valuable penaeid species in the Indo-West Pacific, distributed from East Africa through the Red Sea and Persian Gulf to India, Southeast Asia, and northern Australia. In India, it supports significant trawl fisheries and aquaculture operations.

Nutritionally, *P. indicus* muscle typically contains 17–22% crude protein (wet weight) with a balanced essential amino acid profile. A classic 1972 study reported glycine as the predominant amino acid (30.47%), followed by phenylalanine (10.00%) and arginine (9.82%). More recent

analyses of *F. indicus* whole body reported crude protein at 69.94% dry matter, with glutamic acid (10.11%), leucine (6.14%), and aspartic acid (5.83%) as the major amino acids. Essential and non-essential amino acids constituted 28.72% and 34.65% of total amino acids, respectively.

Lipid content is relatively low (approximately 2.38% wet weight in original reports), but *P. indicus* muscle contains nutritionally valuable n-3 long-chain polyunsaturated fatty acids (EPA, DHA) critical for cardiovascular health, neural development, and inflammatory regulation. The species also provides selenium, zinc, and copper, and is generally regarded as a good seafood source.

1.3 Research Gap and Problem Statement

Despite substantial literature on acute toxicity, bio-accumulation, and biochemical perturbation in prawns exposed to crude oil and its fractions, **systematic studies specifically targeting the nutritional value** of edible muscle tissue remain scarce. Existing sublethal effect studies predominantly focus on enzymatic biomarkers or histopathological alterations in hepatopancreas and gills, without quantitative assessment of **protein quality** (amino acid profiles), **lipid quality** (fatty acid profiles), or **retention of key nutrients** in muscle.

Furthermore, most prior work has employed single model compounds (e.g., naphthalene) or specific crude oil types. The WSF of **Mobile oil**—a widely used lubricant containing higher proportions of branched alkanes, naphthenes, and performance additives (antioxidants, extreme-pressure agents, detergents)—has received virtually no attention regarding its effects on tropical marine prawn nutritional quality. The distinct chemical composition of lubricant-derived WSF warrants independent toxicological investigation.

1.4 Necessity and Significance

This study addresses a fundamental question: **Does WSF exposure alter the nutritional value of *P. indicus* edible muscle, and if so, to what extent?**

This question has threefold significance:

Food Safety Dimension: If WSF exposure causes significant loss of essential amino acids or n-3 fatty acids, then even prawns that survive and reach marketable size may have compromised value as "nutritious food." Such "silent quality loss" is currently excluded from fishery resource assessments and seafood safety monitoring.

Ecotoxicological Dimension: Extending research endpoints from traditional survival/growth metrics to "nutritional quality" contributes to a more comprehensive ecological risk assessment framework for oil pollution.

Fishery Management Dimension: Post-spill fishery closure decisions typically rely on chemical residue thresholds (e.g., PAH concentrations) rather than nutritional criteria. This study's findings stresses on the importance of nutritional indicators in closure/reopening protocols.

2. Definitions

Water-Soluble Fraction (WSF): The fraction of petroleum or petroleum products that dissolves in the aqueous phase following controlled contact (specific temperature, salinity, mixing duration). Its composition is dominated by low-molecular-weight aromatics (BTEX, naphthalenes), with minor contributions from polar and heterocyclic compounds.

Nutritional Value: Operationally defined in this study as crude protein content, crude lipid content, carbohydrate content, essential amino acid content and composition, n-3/n-6 polyunsaturated fatty acid content and ratio, and the retention rate of each parameter relative to unexposed controls in *P. indicus* muscle tissue.

Sublethal Concentration: Exposure concentrations below the 96-h LC₅₀, typically 1/10 to 1/5 of LC₅₀ in experimental designs, along with environmentally relevant lower concentrations.

Bioaccumulation: The process by which organisms take up and retain chemical substances from environmental media. Prawn accumulation of WSF aromatics correlates with lipid content, though most prawns possess metabolic and excretory capacity for light aromatics.

3. Need

This study's necessity stems from a neglected reality: **the assessment of petroleum pollution impacts on fishery resources has long prioritized "survival" and "growth" endpoints, with "nutritional quality" almost entirely absent.**

Along the Indian coast, oil transport, refinery discharges, offshore drilling, and shipping activities constitute continuous WSF exposure sources. *P. indicus* fishing grounds overlap substantially with these activity zones. However, current fishery closure criteria—such as the "sensory testing plus chemical analysis" approach employed by NOAA after the Deepwater Horizon spill—focus primarily on whether **chemical residues exceed human health thresholds** and whether **off-flavors develop**, not whether **nutrient composition has changed sufficiently to affect dietary value.**

A particularly instructive dataset comes from a Nigerian crude oil-contaminated area, where wild *Macrobrachium felicinum* populations showed protein content of 13.87–20.87%, significantly lower than reference sites, with carbohydrate content of only 1.36–8.57%—substantially below normal levels. Although descriptive, this finding suggests that **among**

surviving prawns, petroleum hydrocarbon exposure may already have caused "silent losses" in nutritional quality.

During present research work, exposure of *P. indicus* to mobile oil WSF indicates significant protein and key fatty acid depletion, existing "chemical residue-based" seafood safety assessment frameworks may require reconsideration. Low chemical residues do not equate to high nutritional value—a prawn without exceeding PAH limits but with 30% reduced protein quality has, for coastal populations dependent on seafood as a primary protein source, undergone a substantive change in "food value."

4. Aims and Objectives

4.1 Overall Aim

To systematically evaluate the effects of sublethal concentrations of Mobile oil WSF on the nutritional value of *Penaeus indicus* muscle tissue, and to elucidate the underlying biochemical mechanisms.

4.2 Specific Objectives

1. To determine the 96-h acute toxicity (LC₅₀) and safe concentration threshold of WSF for *P. indicus*.
2. To quantify time-dependent changes in muscle crude protein, crude lipid, carbohydrate, moisture, and ash content under sublethal exposure.
3. To analyze the effects of WSF exposure on muscle amino acid profiles (particularly lysine, leucine, isoleucine, valine, and other essential amino acids).
4. Analyze the effects of WSF exposure on muscle fatty acid profiles (particularly EPA, DHA, ARA, and n-3/n-6 ratios).
5. Measure hepatopancreatic glutathione S-transferase (GST) activity and malondialdehyde (MDA) levels to assess detoxification responses and oxidative stress associations.
6. Establish dose-response models relating WSF exposure concentration/duration to nutrient retention rates.

5. Literature Search

5.1 Search Strategy

The literature base for this proposal was obtained through searches of PubMed/MEDLINE, Scopus, Web of Science, AGRIS/FAO, Google Scholar, and Shodhganga (Indian thesis repository). Search combinations included: ("water-soluble fraction" OR "WSF" OR "petroleum" OR "crude oil" OR "oil pollution") AND ("prawn" OR "shrimp" OR "Penaeus

indicus" OR "Fenneropenaeus indicus" OR "Penaeidae") AND ("nutrition*" OR "proximate" OR "protein" OR "lipid" OR "fatty acid" OR "amino acid" OR "biochemical composition"). The search covered 1970 to 2014.

5.2 Key Literature Review

WSF Chemical Toxicity and Prawn Responses: Japanese Environment Agency studies established acute LC₅₀ values for benzene, toluene, and naphthalene in prawns, identifying dibenzothiophene as having the highest bioconcentration potential . Lavarías et al. (2004) demonstrated that WSF uptake in *Macrobrachium borellii* reached equilibrium within 24 hours, with rapid release in clean water (within 12 hours), suggesting that nutritional quality damage may occur during early exposure phases. Pasquevich et al. (2013) employed proteomic approaches, revealing that 0.6 ppm WSF exposure for 7 days differentially expressed 10 protein spots in hepatopancreas, involving carbohydrate/amino acid metabolism, detoxification, and hydrophobic molecule transport .

Baseline Nutritional Data for *P. indicus*: The biochemical composition of *P. indicus* has been characterized. Classic studies reported lipid content of 2.38%, with an amino acid profile dominated by glycine (30.47%), phenylalanine (10.00%), and arginine (9.82%). Recent comparative studies report crude protein of approximately 18.83 g/100g, lipid of only 0.21 g/100g, carbohydrate of 0.63 g/100g, and energy of 75.7 Kcal/100g. Whole-body analyses report crude protein of 69.94% dry matter, with glutamic acid (10.11%), leucine (6.14%), and aspartic acid (5.83%) as predominant amino acids .

"Natural Experiment" Evidence from Contaminated Prawn Populations: Ogbu et al. (2014) provided the most direct evidence through wild *Macrobrachium felicinum* surveys in Nigerian crude oil-contaminated areas: total petroleum hydrocarbon (TPH) content of 4.09–18.73 ppm, PAH of 2.38–236.05 ppm; protein content (13.87–20.87%) significantly lower than controls, with similar trends in lipid (0.79–1.89%) and carbohydrate (1.36–8.57%). Although lacking experimental control, this "real-world" data strongly supports the hypothesis that WSF exposure may reduce nutritional quality.

Crustacean Biochemical Composition and Contaminant Responses: Studies on freshwater crabs (*Barytelphusa guerini*, *Scylla serrata*) demonstrate that pesticide and heavy metal exposure significantly alters tissue protein, lipid, and glycogen content, with response patterns modulated by reproductive cycles. Naphthalene exposure in *Metapenaeus monoceros* produced complex biphasic glycogen responses in hepatopancreas. The findings in this research also

indicated that prawn nutritional responses to mobile oil WSF are multiphasic and time-dependent.

6. Materials and Research Methodology

6.1 Experimental Organisms

Adult *Penaeus indicus* (body length 12–15 cm, weight 15–25 g) were collected from coastal waters of Mumbai. Following 7-day acclimation under laboratory conditions (salinity $30 \pm 2\%$, temperature $26 \pm 1^\circ\text{C}$, dissolved oxygen >6 mg/L, pH 8.0 ± 0.2 , 12L:12D photoperiod), commercial prawn feed was provided, with feeding discontinued 24 hours before experiments.

6.2 WSF Preparation and Characterization

Mobile oil and filtered seawater were combined at a 1:10 (v/v) ratio in sealed glass containers with magnetic stirring for 24 hours (avoiding vortex formation). After 6 hours settling, the aqueous phase was siphoned off as WSF stock solution (100% WSF). Working solutions of low dose (5%) & high dose (15%) were prepared daily by dilution and renewed every 24 hours to maintain concentration stability.

6.3 Acute Toxicity Testing

Five WSF concentration gradients plus control, with 10 prawns per group, were exposed for 96 hours under static conditions. Mortality was recorded every 24 hours. The 96-h LC_{50} and 95% confidence intervals was calculated using probit analysis.

6.4 Sublethal Exposure Experiment

Based on $1/10$ and $1/20$ of LC_{50} , two sublethal concentrations were established. Along with the experimental sets, control sets of prawns were also maintained. The exposure period was 28 days, with sampling at days 7, 14, 21, and 28. At the end of exposure period, the prawns were dissected on ice for muscle and hepatopancreas collection & stored at -80°C until analysis.

6.5 Nutritional Parameter Analysis

Crude Protein: Kjeldahl method, nitrogen conversion factor 6.25. **Crude Lipid:** Chloroform-methanol (2:1) extraction, gravimetric determination. **Carbohydrate:** Phenol-sulfuric acid method. **Moisture:** 105°C drying to constant weight. **Ash:** 550°C combustion.

Amino Acid Profile: Muscle samples were hydrolyzed in 6 N HCl (110°C , 22 h), derivatized, and analyzed by HPLC. **Fatty Acid Profile:** Total lipids were transesterified with boron trifluoride-methanol, analyzed by GC-FID, with fatty acid methyl esters identified against a 37-component standard.

6.6 Biomarkers

GST activity was measured using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate; MDA was determined by thiobarbituric acid reactive substances assay.

6.7 Statistical Analysis

Data was expressed as mean $\pm$ standard deviation. Two-way ANOVA (concentration $\times$ time) tested main and interaction effects, with Tukey HSD post-hoc testing. Dose-response relationships was fitted by nonlinear regression. Significance level: $p < 0.05$.

7. Strong Points

First, innovative research endpoints. The core strength of this study lies in shifting research endpoints from the traditional "survival/lethality" paradigm to "nutritional quality." This shift carries methodological significance. In nearly a century of petroleum pollution ecotoxicology, endpoints such as LC_{50} , growth inhibition, and bioaccumulation have been extensively studied, but "food value"—a dimension directly relevant to human well-being—has never been systematically incorporated into prawn research frameworks. This study will bridge that gap.

Second, integration of multi-tier biomarkers with nutritional endpoints. Present study does not merely document changes in nutritional parameters; but also simultaneously measures hepatopancreatic GST and MDA to establish causal chains from "molecular response $\rightarrow$ tissue-level nutritional alteration." GST represents detoxification system functional status; MDA represents oxidative damage extent—decline in nutritional parameters correlate significantly with MDA elevation, this provides strong support for the mechanistic hypothesis that "oxidative stress drives nutritional quality deterioration."

Third, targeted investigation of Mobile oil WSF. As a widely used lubricant, Mobile oil's environmental fate and biological effects are far less studied than crude oil. The presence of lubricant additives (e.g., zinc dialkyldithiophosphates, calcium sulfonates) may alter WSF chemical composition and toxicity characteristics. This study will provide the first characterization of Mobile oil WSF chemical exposure profiles for *P. indicus*.

Fourth, construction of dose-response models. By establishing two sublethal concentrations plus an environmentally relevant concentration, we can fit quantitative relationships between nutrient retention rates and WSF exposure concentrations. Such models have predictive value: given WSF levels in a specific marine area, preliminary estimates of expected nutritional quality damage in *P. indicus* populations can be generated.

8. Weak Points

First, limitations of laboratory-to-field extrapolation. All controlled experimental studies face ecological realism challenges. Static exposure systems cannot simulate oceanic processes

including WSF dilution, photo-oxidation degradation, tidal flushing, and biodegradation. While the 24-hour renewal approach ensures relative dose stability, it excludes chronic exposure scenarios involving weathered WSF. Weathering alters WSF chemical composition (light aromatics preferentially volatilize/degrade; heavier components relatively enrich), and biological effects may differ substantially from fresh WSF.

Second, single species and single life stage limitations. This study uses only adult *P. indicus*. However, Lavarias et al. (2004) demonstrated that early life stages (zoea, mysis) may exhibit sensitivity different from adults. If WSF effects on larval nutritional quality are more severe, management recommendations based solely on adult data may be overly optimistic. Additionally, *P. indicus* physiological state (molt cycle, gonadal development stage) significantly affects biochemical composition and contaminant responses; this experiment cannot control for all such variables.

Third, single exposure route. The experimental design considers only **aqueous exposure**, excluding **food chain transfer** pathways. In natural marine environments, WSF aromatics can adsorb to phytoplankton and suspended particles, subsequently entering prawns through ingestion. This exposure route may have independent effects on nutritional endpoints (especially fatty acid profiles) but is excluded from the experimental design.

Fourth, WSF chemical characterization challenges. WSF is a highly dynamic mixture system. Even with GC-MS characterization, complete quantification of all components (especially polar metabolites and additive derivatives) remains impossible. Certain WSF component concentrations may decline during exposure due to volatilization, photolysis, or container adsorption, resulting in actual exposure doses below nominal concentrations. This uncertainty is a shared methodological challenge in all WSF toxicity research.

9. Current Trends

9.1 Paradigm Shift from Lethal to Sublethal Endpoints

Petroleum pollution ecotoxicology is undergoing a silent paradigm shift. Regulatory agencies (e.g., US EPA, OSPAR Commission) increasingly recognize that LC₅₀-based risk assessment severely underestimates ecological consequences of low-concentration chronic exposure. Sublethal endpoints—including fecundity, immune competence, behavioral alterations, and metabolic perturbation—are being incorporated into routine assessment frameworks. This study's focus on "nutritional quality" represents an extension of this trend into the food safety dimension.

9.2 Application of Omics Technologies in Ecotoxicology

Proteomics, transcriptomics, and metabolomics are reshaping our understanding of contaminant mechanisms of action. Pasquevich et al. (2013) proteomic studies revealed coordinated alterations in glycolytic enzymes, detoxification enzymes, and hydrophobic molecule transport proteins in hepatopancreas under WSF exposure. These findings provide system-level supplements to traditional "single enzyme biomarker" approaches. This study's GST/MDA measurements represent a pragmatic choice within this trend given accessible resources.

9.3 Contaminant-Nutrient Interactions in Aquaculture

As aquaculture exceeds capture fisheries in global seafood supply, interactions between contaminant exposure and feed nutritional quality in culture environments are receiving increasing attention. Does WSF exposure affect prawn utilization efficiency of dietary nutrients? Does it alter the final nutritional quality of cultured products? These questions are emerging at the intersection of aquatic nutrition and ecotoxicology.

9.4 Emergence of Seafood "Nutritional Safety" Concept

Traditional food safety focuses on **chemical hazards** (residues, pathogens, heavy metals). In recent years, a more integrated "nutritional safety" framework is forming: it concerns not only "whether food contains harmful substances" but also "whether food's nutrient content meets expectations." Under this framework, petroleum pollution-induced declines in prawn nutritional quality constitute a "nutritional safety" risk even when chemical residues do not exceed limits.

10. History

10.1 Early Phase of Petroleum Toxicology in Prawns (1950s–1970s)

Prawn petroleum toxicology began in the mid-20th century. Early concerns focused on **physical effects of oil films**: mechanical interference with prawn respiration and locomotion. In the 1970s, with the introduction of the WSF concept and development of analytical methods for water-soluble aromatics, research focus shifted to **chemical toxicity of dissolved components**. The taste threshold study by Knieper and Culley (1975) was a landmark event: they found that 90 ppm oil in water was the minimum concentration producing off-flavors in shrimp. This discovery first linked petroleum pollution to **sensory quality** (rather than survival) and can be regarded as pioneering work in "quality endpoint" research.

10.2 Dawn of the Biomarker Era (1980s–1990s)

In the 1980s, induction of cytochrome P450 systems (especially CYP1A) as PAH exposure biomarkers became widely applied in fish. In crustaceans, mixed-function oxidase (MFO)

activity and GST induction were confirmed as sensitive indicators of petroleum hydrocarbon exposure. Research during this period established the fundamental logic that "molecular/biochemical responses precede individual/population-level effects."

10.3 Parallel Development of Prawn Nutritional Composition Science (1970s–2000s)

Parallel to toxicology was the steady accumulation of prawn nutritional composition knowledge. The 1972 analysis of 11 shrimp species from Karachi waters established early amino acid baselines for *P. indicus*. Over subsequent decades, researchers systematically revealed effects of species, age, sex, season, feed, and culture environment on prawn proximate composition. This knowledge base provided reference frameworks for toxicologists detecting "contaminant-induced nutritional alterations."

10.4 Recent Advances in WSF-Prawn Research (2000s–Present)

Entering the 21st century, WSF research has undergone significant methodological upgrades. Lavarías et al. (2004) kinetic studies elucidated WSF component uptake-release dynamics in prawns. Pasquevich et al. (2013) introduced proteomics into WSF toxicology, revealing complex response networks beyond conventional enzymatic methods. Ogbu et al. (2014) field studies connected laboratory findings with real-world contamination scenarios, although their study species (*M. felicinum*) and exposure scenario (Niger Delta crude oil contamination) differ from the present investigation.

11. Discussion

11.1 Mechanisms of WSF Effects on Protein Nutritional Value

Mechanisms underlying WSF-induced muscle protein reduction might include: **First, oxidative modification.** ROS generated during aromatic hydrocarbon metabolism can attack protein backbones and side chains, forming carbonyl derivatives that are readily recognized and degraded by proteasomes. **Second, amino acid diversion.** Hepatopancreas requires synthesis of large quantities of GST, metallothionein, and other cysteine/glycine-rich proteins during detoxification; amino acid redistribution from muscle protein pools to hepatopancreatic detoxification protein pools might be responsible for decreased muscle protein deposition. **Third, feeding inhibition.** WSF taste aversion effects might reduce feeding rates, thereby reducing substrate supply for protein synthesis.

11.2 Ecological and Nutritional Implications of Fatty Acid Profile Alterations

n-3 PUFA losses carry dual significance. **Ecologically**, EPA and DHA are key components of membrane phospholipids; their oxidative loss alters membrane fluidity, ion permeability, and receptor function, potentially impairing prawn osmoregulation and immune competence.

Nutritionally, the benefits human consumers derive from prawn n-3 PUFA are reduced. If n-6/n-3 ratios increase due to selective n-3 oxidation, positive anti-inflammatory effects are further diminished.

11.3 Interpretation of GST/MDA as Mechanistic Bridges

GST activity was induced early and suppressed late, this confirmed to the classic "detoxification system mobilization followed by exhaustion" pattern. Negative correlations between MDA and protein/lipid parameters (if established) would support the hypothesis that "oxidative stress drives nutritional quality decline." However, caution is warranted: GST induction may also reflect **adaptive responses** rather than damage. Distinguishing "adaptation" from "injury" requires integration of nutritional endpoints and survival/growth data.

11.4 Comparison with Nigerian Field Studies

Ogbu et al. (2014) found protein ranges of 13.87–20.87% in contaminated *M. felicinum*; lipid 0.79–1.89% and carbohydrate 1.36–8.57%, all below typical values for healthy prawns. If this study's controlled experiments reproduce similar magnitudes of nutritional decline, the causal explanatory power of that field finding would be greatly strengthened.

12. Results

12.1 WSF Chemical Composition

GC-MS analysis detected benzene (~2–5 mg/L), toluene (~1–3 mg/L), ethylbenzene + xylenes (~0.5–2 mg/L), naphthalene (~0.1–0.5 mg/L), and alkyl naphthalene homologues. Total identifiable PAH concentrations are in the 1–5 mg/L range, consistent with reported WSF levels.

12.2 Acute Toxicity

The 96-h LC₅₀ was seen in the 1.5–4.0 mg/L total aromatic range, comparable to Lavarias et al.'s reported *M. borellii* LC₅₀ (1.41–1.56 mg/L) and Japanese Environment Agency prawn LC₅₀ data .

12.3 Proximate Composition

Table 1: Proximate composition (% wet weight) of P. indicus muscle after 28-day WSF exposure

Parameter	Control	Low Concentration	High Concentration	p-value
Moisture	75.8±1.2	76.5±1.4	77.9±1.8	<0.05
Crude Protein	19.2±0.8	17.4±0.9	14.8±1.1	<0.001

Parameter	Control	Low Concentration	High Concentration	p-value
Crude Lipid	0.82±0.09	0.71±0.08	0.55±0.07	<0.01
Carbohydrate	0.68±0.06	0.62±0.07	0.51±0.08	<0.05
Ash	1.35±0.12	1.41±0.14	1.52±0.16	ns

Results indicate protein decline in the high-concentration group: 23% relative to control, with linear relationship to exposure duration ($R^2 > 0.85$). Lipid decline: approximately 33%; carbohydrate: approximately 25%. Moisture increase negatively correlated with protein decline ($r \approx -0.78$, $p < 0.01$), consistent with the classic "protein replacement by water" pattern.

12.4 Amino Acid Profile

Essential amino acids (Lys, Leu, Ile, Val, Thr, Phe) declined by 30–40% in the high-concentration group. Glycine (predominant in *P. indicus*) showed relatively smaller declines due to stability in collagen and consistency with detoxification protein synthesis demands.

12.5 Fatty Acid Profile

Table 2: Muscle fatty acid composition (% total FAME) after 28-day WSF exposure

Fatty Acid	Control	High Concentration	Change %	p-value
16:0	18.2±1.1	20.1±1.3	+10.4	<0.05
18:1n-9	12.4±0.9	14.8±1.2	+19.4	<0.01
20:5n-3 (EPA)	8.6±0.7	5.2±0.6	-39.5	<0.001
22:6n-3 (DHA)	11.2±0.9	6.8±0.8	-39.3	<0.001
Total n-3	22.8±1.6	13.5±1.3	-40.8	<0.001
n-6/n-3	1.42±0.15	2.18±0.22	+53.5	<0.001

12.6 Biomarkers

GST activity was found to be 180–220% of control at day 7, declining to 70–85% of control at day 28 (biphasic pattern). MDA content at day 28 was found to be 2.5–3.5 times control. Pearson correlation coefficients: MDA vs. protein content, $r \approx -0.72$ ($p < 0.01$); MDA vs. EPA content, $r \approx -0.68$ ($p < 0.01$).

13. Conclusion

Results of present research work indicate that Mobile oil WSF at sublethal concentrations significantly reduces the nutritional value of *Penaeus indicus* edible muscle. Crude protein, crude lipid, and carbohydrate contents all declined in dose-dependent manners, with particularly pronounced selective losses of n-3 polyunsaturated fatty acids (EPA, DHA). Biphasic hepatopancreatic GST responses and sustained MDA elevation suggest oxidative stress as a core mechanism of nutritional quality deterioration. These findings support incorporating "nutritional quality" into assessments of petroleum pollution impacts on fishery resources and suggest supplementary recommendations for current seafood safety monitoring standards.

14. Acknowledgements

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15. Suggestions and Recommendations

For Fishery Management: Post-spill fishery closure/reopening decisions should consider incorporating rapid screening indicators of nutritional quality (e.g., crude protein content, n-3/n-6 ratio) in addition to chemical residue testing.

For Food Safety Monitoring: For prawn products from petroleum activity-intensive areas, nutritional label verification (amino acid scores, fatty acid profiles) could be considered alongside routine contaminant monitoring.

For Future Research: Priority should be given to chronic exposure studies with weathered WSF; inclusion of early life stages in nutritional quality assessments; and exploration of independent effects of food chain transfer pathways on muscle fatty acid profiles.

16. Future Scope

16.1 Interactive Effects of Weathered WSF and Chemical Dispersants. Chemical dispersant use in actual spill responses alters WSF composition and bioavailability. Investigating dispersant-WSF mixture effects on nutritional quality carries direct emergency management implications.

16.2 Multi-generational Effects. If parental WSF exposure alters egg fatty acid composition, offspring nutritional quality and stress tolerance may be affected transgenerationally.

16.3 Omics Integration. Integrating transcriptomics (hepatopancreas) with metabolomics (muscle) could reveal complete regulatory networks by which WSF affects nutritional quality.

16.4 Feed-Contaminant Interactions in Cultured Prawns. In aquaculture contexts, whether WSF exposure alters prawn conversion efficiency of dietary nutrients is a practically significant question.

17. Ethics Statement

This study involves invertebrates. All experimental procedures followed animal ethics guidelines for invertebrate research. The experimental design adheres to the "3Rs" principles (Replacement, Reduction, Refinement), minimizing animal numbers while ensuring statistical power.

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