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Comparison of Whole Blood Fatty Acid Profiles between Lionfish (*Pterois* spp.) in Wild and Managed Care Environments

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Abstract: Suboptimal nutrition may contribute to lionfish (*Pterois volitans* and *Pterois miles*) health issues in managed care environments. This study's objective was to establish and compare whole blood fatty acid profiles in wild and aquarium lionfish. Whole blood samples were dried onto specialized high-quality paper cards from wild, invasive lionfish harvested off the North Carolina coast ($n = 16$) and lionfish managed by the North Carolina Aquariums ($n = 12$). Blood fatty acid profiles were analyzed from dried blood spots. Aquarium lionfish had significantly ($p < 0.05$) higher linoleic (18:2ω6) and eicosapentaenoic (20:5ω3) acid levels than wild lionfish. Similarly, aquarium lionfish had significantly ($p < 0.05$) lower saturated fatty acids and arachidonic (20:4ω6) to eicosapentaenoic acid (20:5ω3) ratios than wild lionfish. Total omega-3 and omega-6 fatty acids, as well as the ratio of these two fatty acid groups, were similar between wild and aquarium lionfish. Gut content analysis of wild lionfish diets included reef-dependent and schooling fish while aquarium lionfish diets were pelagic fish, crustaceans, mollusks, and commercial gel diets with nutrient supplements. This study reports whole blood fatty acid profiles in lionfish, providing comparative macronutrient data that may be useful for improving their nutrition and welfare in aquariums.

Keywords: fatty acids; lionfish; nutrition; *Pterois volitans*; *Pterois miles*

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1. Introduction

Native to Indo-Pacific reef habitats, ornate lionfish (*Pterois volitans* and *Pterois miles*) have long been popular in private and public aquariums. However, these carnivorous fish have become one of the most successful invasive marine species in the western Atlantic Ocean [1]. First observed in 1985 off the coast of Florida [2], the invasive range of lionfish now spans a diverse array of tropical and subtropical marine habitats across the North Atlantic Basin, Caribbean Sea, Gulf of Mexico, and Guyana Basin [3]. Their rapid establishment and proliferation is facilitated by their unique biology [4], including wide ecologic and physiologic tolerances [5,6], high fecundity, rapid growth and recruitment [7,8], putative resistance to parasites and known fish pathogens in their invaded range [9–11], antipredator defenses (e.g., venomous spines) [4], and generalist diet [12].

The health and welfare of lionfish in managed care environments has garnered little published research, despite their popularity. Morbidity and mortality data are currently lacking, but anecdotally, lionfish often suffer from obesity, increased cuticle shedding, prolonged to terminal anorexia (i.e., starvation syndrome), and 'lockjaw' syndrome [13]. Lionfish 'lockjaw' syndrome is commonly discussed yet enigmatic among hobbyists and

online resources. Affected lionfish mouths stay in an open position for days, weeks, or sometimes permanently [13]. There is widespread speculation in online forums that improper nutrition is a risk factor for—or potentially the underlying etiology of—aquarium lionfish health issues. To date there is no consensus on optimal dietary recommendations for lionfish.

Dietary fatty acids influence health and disease risk through numerous physiological functions, including cell membrane integrity, energy production, organ function, antioxidation, and modulation of inflammatory processes [14]. Consequently, fatty acid profiles provide valuable macronutrient information for the development of evidence-based diets for fish, including lionfish, in aquarium environments. Fatty acid profiles have historically been acquired in animals via postmortem lipid depot (i.e., tissue) samples [15]. However, whole blood fatty acid profiles can be readily acquired through dried blood spot samples, which have become increasingly popular in wildlife research [15–19]. Blood spot samples are allowed to dry on specialized high-quality paper cards designed for laboratory analysis, require only a small volume of whole blood, can be transported easily, and do not require immediate freezing or refrigeration [20]. Whole blood fatty acid profiles provide a representation of an animal's long-term status, given that red blood cell fatty acids reflect the time of erythropoiesis [21,22]. While the lifespan of lionfish red blood cells is unknown, other fish red blood cells can be in circulation for 13–500 days [23]. Thus, whole blood fatty acid profiles may provide information about longer periods, in contrast with plasma or serum fatty acid profiles that reflect an animal's status at the time of or just prior to phlebotomy [21,22].

Although partial fatty acid profiles of wild lionfish fillets have been investigated [24–26], whole blood fatty acid profiles of wild and aquarium lionfish have not yet been reported. The objective of this study was to document and compare whole blood fatty acid profiles between aquarium lionfish and wild, invasive conspecifics. It was hypothesized that differences in blood fatty acid profiles may be more noticeable in the total omega-3 and omega-6 fatty acids, along with saturated fatty acid levels. Previous research in other wildlife species has shown lower total omega-3 fatty acid levels and higher total omega-6 fatty acid levels in animals under managed care [27]. While invasive lionfish may have a more natural diet, it may not be optimal compared to the prey base in their native range.

2. Materials and Methods

Postmortem blood samples were collected in August 2021 from 16 of approximately 100 wild lionfish of unknown sex off the coast of Hatteras, North Carolina, as part of ongoing local lionfish mitigation efforts. Lionfish were collected and culled by divers with pole spears and ZooKeeper Lionfish Containment Units (ZooKeeper, Sunrise, FL, USA) from two shipwrecks: the *Proteus* (34°45′55.08″ N, 75°47′0.6″ W, depth: ~33.5–36.5 m) and the *British Splendour* (34°49′7.54″ N, 75°54′10.91″ W, depth: ~30.5 m). Approximately 40 and 60 lionfish were collected and culled from the *Proteus* and *British Splendour*, respectively. Blood and measurements were gathered from eight lionfish from each shipwreck. To avoid the venomous spines associated with the dorsal, pelvic, and anal fins, lionfish were grasped by their lower jaw or tail. Total (snout to the tip of the tail) and standard (snout to the end of the vertebral column) lengths were measured using a flexible measuring tape. Blood samples were collected using heparinized 25-gauge needles and 1 mL syringes from either intracardiac or caudal hemal arch phlebotomy. Blood was immediately transferred to Perkin-Elmer Spot Saver Cards (Perkin–Elmer, Waltham, MA, USA), which contain proprietary preservatives, using two spots of approximately 80 µL whole blood (approximately 160 µL total per lionfish). Gastric contents were examined by collaborators via necropsy in a subset of 25 lionfish pooled from both shipwrecks to infer diet composition. Lionfish were dissected via ventral midline incision cranial to the cloaca. The stomach was removed, opened, and grossly examined. Gastric contents were not preserved. Postmortem blood samples and gastric content data were collected from different subsets of lionfish.

Aquarium lionfish handling and sampling were performed according to procedures approved by North Carolina State University's Institutional Animal Care and Use Committee (IACUC #21-117). Blood samples were collected in June and September 2021 from lionfish at the North Carolina Aquariums at Fort Fisher, Pine Knoll Shores, and Roanoke Island. Blood samples were collected as part of preventative health examinations. Twelve aquarium lionfish of unknown sex were sampled: two from Fort Fisher, five from Pine Knoll Shores, and five from Roanoke Island. Lionfish were sedated for phlebotomy and examination using 100 mg/L tricaine methanesulfonate (MS-222) buffered 1:1 with sodium bicarbonate immersion to ensure safe handling and minimize stress. Lionfish were measured as described above. Blood samples were collected using heparinized 25-gauge needles and 3 mL syringes from caudal hemal arch phlebotomy. Blood was then transferred to Perkin–Elmer Spot Saver Cards as described above. Lionfish were recovered in anesthetic-free water and returned unharmed to their exhibits. Diet composition was collected through husbandry records and discussion with animal care staff.

Perkin–Elmer Spot Saver Cards were kept dry in a cool container immediately after phlebotomy and later stored at −80 °C for less than 90 days before being shipped on gel ice packs frozen at −80 °C to Lipid Technologies (Austin, MN, USA) for a simultaneous full fatty acid profile analysis including 36 individual fatty acids representing 13 fatty acid groups. Using previously reported methods [20], samples were transmethylated with acidified methanol, and the fatty acid methyl esters were quantified by area percent as analyzed on gas chromatography. Values are provided as a percent of total fatty acids present. Fatty acids are reported using their common names and respective abbreviations using the omega-reference system in parenthesis (e.g., arachidic acid (20:0)).

Using the total and standard length of each lionfish, mean, median, and range of these variables were calculated for wild and aquarium fish. Similar summary statistics for individual fatty acids and fatty acid groups were also calculated. The normality of each fatty acid and fatty acid group was assessed visually and quantitatively via the Shapiro–Wilk test. The homogeneity of variances between wild and aquarium lionfish were assessed quantitatively via Levene's test for each fatty acid or fatty acid group with normal distributions. Individual fatty acids or fatty acid groups with parametric distributions were analyzed via a Student's *t*-test or Welch's *t*-test. A Wilcoxon rank sum test was used to analyze individual fatty acids or fatty acid groups with nonparametric distributions. To account for multiple comparisons, *p* values were adjusted using the Bonferroni correction. For all statistical tests, null hypotheses were rejected when *p* values were less than an alpha level of 0.05. All calculations and analyses were performed in the statistical software R (R Foundation for Statistical Computing, Vienna, Austria) using the 'dplyr' and 'car' packages and their dependents.

3. Results

Wild lionfish median total length was 33 cm (min–max: 23–43 cm) and median standard length was 26 cm (min–max: 19–34 cm). Aquarium lionfish median total length was 37 cm (min–max: 31–38 cm) and median standard length was 29 cm (min–max: 23–30 cm). Body condition of each lionfish used in this study was subjectively estimated to be in fair condition. Gastric contents of necropsied wild lionfish were moderately to heavily digested, but round scad (*Decapterus punctatus*), vermillion snapper (*Rhomboplites aurorubens*), and tomtates (*Haemulon aurolineatum*) could be identified. The diets of aquarium lionfish varied among the three aquariums (Table 1).

Table 1. Diets of lionfish at the North Carolina Aquariums. While diets varied among the three aquariums, lionfish were fed primarily pelagic fish, crustaceans, mollusks, commercial gel diets and nutrient supplements.

Aquarium	Assorted Food Items	Supplement?	Total Amount Per Lionfish	Frequency
Pine Knoll Shores (n = 5)	Krill, sardines, shrimp, silversides, Spanish mackerel	Yes **	12 g	2×/week
Fort Fisher (n = 2)	Capelin, clam, in-house gel diet, * shrimp, Spanish mackerel, squid	No	10 g	2×/week
Roanoke Island (n = 5)	Capelin, clam, Mazuri® Aquatic Gel Diet for Omnivorous Fish, shrimp, squid	Yes ***	10 g	3×/week

* Includes Mazuri® Aquatic Gel Diet for Omnivorous Fish. ** Vitachem Marine (Boyd Enterprises, Fort Lauderdale, FL, USA) supplemented twice weekly. Food items are soaked in supplement for approximately 30 min before feeding per manufacturer instructions. *** Supplemented with one of the following once weekly: AminOmega, Iodion, Vitamarin-M, and Vitamarin-C (Brightwell Aquatics, Fort Payne, AL, USA). Supplements are rotated through in a four-week cycle. Food items are soaked in supplement for approximately 30 min before feeding per manufacturer instructions.

Of the 36 individual fatty acids and 13 fatty acids groups identified, 29 individual fatty acids and 13 fatty acid groups were quantifiable (Tables 2 and 3). Fatty acids that were not in high enough concentration to be quantified or whose antioxidant peak obscured their elution included: lauric acid (12:0), myristic acid (14:0), myristoleic acid (14:1), 9-hexadecenoic acid (16:1ω5), 13-octadecenoic acid (18:1ω5), gondoic acid (20:1ω9), and mead acid (20:3ω9).

Significant differences were found for 48% (14/29) of the detected individual fatty acids and 38% (5/13) of the quantified fatty acids groups. Significant differences were found by Student's t-test between aquarium and wild lionfish for the following fatty acids: palmitic acid (16:0), palmitoleic acid (16:1ω7), margaric acid (17:0), arachidic acid (20:0), behenic acid (22:0), adrenic acid (22:4ω6), lignoceric acid (24:0), and saturated fatty acids (Tables 2 and 3). Significant differences between aquarium and wild lionfish were found by Wilcoxon rank sum test for the following fatty acids: stearic acid (18:0), vaccenic acid (18:1ω7), linoleic acid (18:2ω6), paullinic acid (20:1ω7), eicosapentaenoic acid (EPA, 20:5ω3), erucic acid (22:1ω9), osbond acid (22:5ω6), monounsaturated fatty acids, omega-3 highly unsaturated fatty acids (HUFA), omega-6 HUFA (Table 2), and arachidonic acid (AA)/EPA ratios (Tables 2 and 3). No difference between aquarium and wild lionfish was found in total omega-3 or omega-6 fatty acids, nor omega-3 to omega-6 fatty acid ratios.

Aquarium lionfish had statistically higher median values of: palmitoleic acid (16:1ω7; +1.8%), vaccenic acid (18:1ω7; +2.1%), linoleic acid (18:2ω6; +1.4%), paullinic acid (20:1ω7; +4.9%), EPA (20:5ω3; +2.9%), erucic acid (22:1ω9; +0.2%) monounsaturated fatty acids (+8.8%), and omega-3 HUFA (+12.1%). Aquarium lionfish had statistically lower median values of: palmitic acid (16:0; −3.9%), margaric acid (17:0, −0.2%) stearic acid (18:0; −5.4%), arachidic acid (20:0; −0.1%), behenic acid (22:0; −0.1%), adrenic acid (22:4ω6; −0.3%), osbond acid (22:5ω6; −0.7%), lignoceric acid (24:0; −0.2%), saturated fatty acids (−10%), omega-6 HUFA (−12.1%), and AA/EPA ratio (−0.6).

Table 2. Whole blood individual fatty acid profiles of wild, invasive lionfish harvested off the coast of North Carolina and lionfish at the North Carolina Aquariums. Values are provided as a percent of total fatty acids present. To account for multiple comparisons, *p* values were adjusted using the Bonferroni correction. Fatty acids that significantly differ between wild and aquarium lionfish are in **bold**, with the higher mean, median, and (min, max) in bold.

Individual Fatty Acids	Aquarium Lionfish			Wild Lionfish			Adjusted
	Mean	Median	(Min, Max)	Mean	Median	(Min, Max)	*p* Value
Pentadecylic Acid (15:0)	0.8	0.5	(0.3, 1.3)	1.2	1.1	(0.8, 1.7)	0.093
Pentadecanoic Acid (15:1)	0.2	0.2	(0.1, 0.5)	0.3	0.2	(0.1, 0.7)	1.000
Palmitic Acid (16:0)	29.8	30.4	(25.4, 32.7)	**34.7**	**34.3**	**(30.8, 40.1)**	<0.001
Palmitoleic Acid (16:1ω7)	**10.2**	**10.1**	**(8.1, 12.5)**	8.2	8.3	(5.2, 10.7)	0.013
Margaric Acid (17:0)	1.1	1.1	(0.7, 1.4)	**1.4**	**1.4**	**(1.1, 2.2)**	0.050
Heptadecanoic Acid (17:1)	0.7	0.3	(0.2, 1.3)	0.8	0.8	(0.5, 1.2)	1.000
Stearic Acid (18:0)	5.7	4.7	(3.6, 8.9)	**10.1**	**10.1**	**(8.4, 12.1)**	<0.001
Vaccenic Acid (18:1ω7)	**2.5**	**2.2**	**(0.2, 4.2)**	0.7	0.1	(0.1, 2.3)	0.002
Oleic Acid (18:1ω9)	14.4	12.9	(10, 24.5)	14.4	14.8	(10.5, 18.9)	1.000
Linoleic Acid (18:2ω6)	**2.6**	**2.8**	**(1.3, 5.1)**	1.6	1.5	(1.1, 2.8)	0.035
γ-Linolenic Acid (18:3ω6)	0.1	0.1	(0.1, 0.2)	0.1	0.1	(0, 0.2)	1.000
α-Linolenic Acid (18:3ω3)	0.6	0.6	(0.4, 0.9)	0.6	0.6	(0.5, 1)	1.000
Stearidonic Acid (18:4ω3)	0.5	0.3	(0.2, 0.8)	0.4	0.5	(0.3, 0.6)	1.000
Arachidic Acid (20:0)	0.3	0.3	(0.1, 0.4)	**0.4**	**0.4**	**(0.3, 0.7)**	0.001
Paullinic Acid (20:1ω7)	**5.1**	**5.7**	**(0.7, 10.6)**	0.7	0.7	(0.6, 1)	0.001
Eicosenoic Acid (20:2ω6)	0.2	0.2	(0.1, 0.4)	0.3	0.3	(0.2, 0.3)	1.000
h-γ-Linolenic Acid (20:3ω6)	0.1	0.1	(0.1, 0.2)	0.1	0.2	(0.1, 0.2)	1.000
Arachidonic Acid (AA, 20:4ω6)	1.8	1.4	(0.5, 4.1)	3	2.9	(1.5, 4.5)	0.079
Eicosatrienoic Acid (20:3ω3)	0	0	(0, 0.1)	0.1	0.1	(0, 0.1)	1.000
Eicosatetraenoic Acid (20:4ω3)	0.3	0.3	(0.3, 0.5)	0.3	0.4	(0, 0.6)	1.000
Eicosapentaenoic Acid (EPA, 20:5ω3)	**6.5**	**6.7**	**(2.8, 9.4)**	3.9	3.9	(2.1, 4.9)	0.009
Behenic Acid (22:0)	0.1	0.1	(0.1, 0.2)	**0.2**	**0.2**	**(0.2, 0.4)**	<0.001
Erucic Acid (22:1ω9)	**2.1**	**0.3**	**(0, 7.3)**	0	0	(0, 0.1)	0.020
Adrenic Acid (22:4ω6)	0.2	0.2	(0.1, 0.4)	**0.5**	**0.5**	**(0.3, 0.7)**	<0.001
Osbond Acid (22:5ω6)	0.2	0.2	(0.1, 0.5)	**1**	**0.9**	**(0.5, 1.5)**	<0.001
Clupanodonic Acid (22:5ω3)	1.8	1.8	(1.1, 2.9)	1.3	1.3	(0.7, 2.1)	0.137
Lignoceric Acid (24:0)	0.1	0.1	(0, 0.2)	**0.3**	**0.3**	**(0.2, 0.4)**	<0.001
Docosahexaenoic Acid (DHA, 22:6ω3)	11.2	10.5	(7.5, 18.7)	12.6	12.3	(5.9, 17.1)	1.000
Nervonic Acid (24:1)	0.8	0.8	(0.6, 1.1)	0.7	0.7	(0.4, 1)	1.000

Table 3. Whole blood fatty acid groups of wild, invasive lionfish harvested off the coast of North Carolina and lionfish at the North Carolina Aquariums. Values are provided as a percent of total fatty acids present, except for omega-3 and omega-6 highly unsaturated fatty acids (HUFA), which are provided as a percent of HUFA present. To account for multiple comparisons, *p* values were adjusted using the Bonferroni correction. Fatty acid groups that significantly differ between wild and aquarium lionfish are in bold, with the higher mean, median, and (min, max) in **bold**. EPA: Eicosapentaenoic Acid (20:5ω3), DHA: Docosahexaenoic Acid (22:6ω3), AA: Arachidonic Acid (20:4ω6).

Fatty Acid Groups	Aquarium Lionfish			Wild Lionfish			Adjusted
	Mean	Median	(Min, Max)	Mean	Median	(Min, Max)	*p* Value
Saturated Fatty Acids	36.7	36.3	(30.1, 43.6)	**47**	**46.3**	**(41.7, 53.7)**	<0.001
Monounsaturated Fatty Acids	**25.2**	**25.8**	**(16.1, 31)**	16.9	17	(13.2, 20.8)	<0.001
Polyunsaturated Fatty Acids	26.1	25.6	(19, 37)	25.8	27.7	(14.1, 33.9)	1.000
Highly Unsaturated Fatty Acids (HUFA)	22.1	21.2	(14.7, 32.6)	22.8	24.3	(11.5, 30.6)	1.000
Total Omega-3 Fatty Acids	20.8	19.9	(13.3, 28.4)	19.2	19.5	(9.9, 25.7)	1.000
Total Omega-6 Fatty Acid	5.3	5.8	(2.3, 8.7)	6.6	6.5	(4.2, 8.8)	1.000
Total Omega-9 Fatty Acids	17.3	17.1	(13, 25.4)	15.2	15.42	(11.2, 19.7)	1.000
Omega-3 HUFA	**89.4**	**92.2**	**(82, 95.8)**	79.8	80.1	(74.3, 82.4)	<0.001
Omega-6 HUFA	10.6	7.8	(4.2, 18)	**20.2**	**19.9**	**(17.6, 25.7)**	<0.001
EPA + DHA	17.7	16.9	(10.2, 24.3)	16.5	16.6	(8.0, 21.8)	1.000
Omega-6/Omega-3 Ratio	0.3	0.3	(0.1, 0.4)	0.3	0.3	(0.3, 0.4)	1.000
AA/EPA Ratio	0.4	0.2	(0.1, 0.7)	**0.8**	**0.7**	**(0.6, 1.1)**	0.015
Red Blood Cell O_3 Index	24.6	23.7	(14.3, 33.9)	23	23.2	(11.1, 30.4)	1.000

4. Discussion

Research that promotes the health and welfare of lionfish under managed care is scarce, despite their popularity as aquarium fish. Nutrition is a cornerstone of animal husbandry, and suboptimal nutrition has been anecdotally implicated in managed lionfish health issues including obesity, starvation syndrome, and 'lockjaw' syndrome [13]. This study compared whole blood fatty acid profiles between lionfish at the North Carolina Aquariums and invasive, wild conspecifics offshore of North Carolina. Notable differences included aquarium lionfish having significantly higher linoleic acid (18:2ω6) and EPA (20:5ω3) levels, and significantly lower saturated fatty acids and AA/EPA ratios. Wild lionfish diets included reef-dependent and schooling fish, whereas aquarium lionfish diets included pelagic fish, crustaceans, mollusks, commercial gel diets, and nutrient supplements. It is important to note that the commercial products provided to the aquarium lionfish were supplemented with various fatty acids at the three North Carolina Aquarium locations.

There is limited understanding of the optimal dietary ratio of saturated, monounsaturated, polyunsaturated fatty acids across the vast diversity of fish species [28]. In this study, aquarium lionfish had significantly lower saturated fatty acids (median: 36.3%) and significantly higher monounsaturated fatty acids (median: 25.8%) compared to wild lionfish (median: 46.3% and 17%, respectively). Interestingly, whole blood saturated fatty acid levels from aquarium lionfish were more similar to muscle tissue levels of saturated fatty acids reported from lionfish from the Bay of Bengal [25], Gulf of Mexico [26], and Bahamian Archipelago [29] than their harvested conspecifics. Lionfish have been previously found to have relatively low levels of saturated fatty acids compared to other Caribbean marine reef fish [29]. Thus, aquarium lionfish saturated fatty acid levels may be more normal, whereas the higher levels found in lionfish offshore of North Carolina is notable. Differences in abundance and distribution of lionfish prey base is one possible reason lionfish off the coast of North Carolina have higher whole blood saturated fatty acid levels. Wild lionfish sampled in this study were collected and culled from artificial reefs (i.e., shipwrecks), which may be inhabited by reef communities that differ from natural coral reefs. Although higher than wild lionfish, aquarium lionfish whole blood monounsaturated fatty acid levels fall within the range of previously reported lionfish muscle tissue levels in native and introduced waters [25,26,29]. The higher monounsaturated fatty acid levels in aquarium lionfish could be due to commercial nutrient supplements or commercial omnivorous fish diet. Overall, both aquarium and wild lionfish had higher saturated and monosaturated fatty acids than polyunsaturated fatty acids, typical of fish from warm or temperate regions [25].

Consistent with previous muscle fatty acid assessments in lionfish [29], both aquarium and wild lionfish had higher omega-3 polyunsaturated fatty acids than omega-6 polyunsaturated fatty acids. Total omega-3 and -6 fatty acid levels were similar between wild and aquarium lionfish. However, aquarium lionfish had significantly higher omega-3 HUFA and lower omega-6 HUFA. Aquarium lionfish median omega-3 HUFA and omega-6 HUFA levels were 92.22% and 7.78%, respectively, whereas wild lionfish median omega-3 HUFA and omega-6 HUFA levels were 80.14% and 19.86%, respectively. An important subset of polyunsaturated fatty acids, HUFA have $\geq$20 carbon atoms and $\geq$3 double bonds in their hydrocarbon chain. All HUFA play critical roles in growth, development, and reproduction in marine and freshwater organisms [30]. Omega-3 HUFA, mainly EPA (20:5ω3) and DHA (22:6ω3), directly influence cellular inflammatory pathways in an anti-inflammatory manner, with more recent studies suggesting that they may be involved in the resolution of inflammation [31]. Moreover, omega-3 HUFA can inhibit omega-6 HUFA metabolism to downstream pro-inflammatory eicosanoids helping to prevent chronic inflammation [31]. The difference in omega-3 HUFA and omega-6 HUFA levels between aquarium and wild conspecifics suggests that lionfish offshore North Carolina may have a more pro-inflammatory diet; what implications this has for their physiology and health is uncertain.

Despite considerable research, dietary fatty acid requirements remain one of the least understood areas in fish nutrition [28]. While specific essential fatty acids have

not been elucidated in every species, it is believed that most freshwater fish require the polyunsaturated fatty acids linoleic acid (18:2ω6) and α-linolenic acid (18:3ω3), whereas marine fish require EPA (20:5ω3) and DHA (22:6ω3) [32]. Of these polyunsaturated fatty acids, aquarium lionfish had higher levels of linoleic acid (18:2ω6, 2.82%) and EPA (20:5ω3, 6.74%) compared to wild conspecifics (1.45% and 3.89%, respectively), suggesting a potential excess in their diets. While unexplored in animal nutrition, human nutritional research has shown that excess consumption of EPA (20:5ω3) and DHA (22:6ω3) may impair pathogen clearance during acute infections by decreasing host resistance or interfere with tumor surveillance [33]. Both AA and EPA are precursors of pro-inflammatory eicosanoids (e.g., thromboxanes, prostaglandins, and leukotrienes); consequently, the AA/EPA ratio has been used as an index to evaluate cellular inflammation status [34]. Aquarium lionfish had lower AA/EPA ratios (median: 0.17) than wild lionfish (median: 0.74), suggesting less cellular inflammation potentially due to high EPA (20:5ω3) levels modulating eicosanoid actions. It is possible that an excess of linoleic acid (18:2ω6) and EPA (20:5ω3) may impair the innate immune system of aquarium lionfish and compound the physiologic stress of aquarium environments.

While lionfish have been considered generalist, opportunistic predators, there is growing evidence to suggest lionfish exhibit selective foraging behavior and prey preference, depending on size and life stage [35–37]. Foraging behavior and prey preference are likely further altered depending on where lionfish are found in their native or invasive range. For instance, smaller-bodied benthic fishes (gobies, basslets, and wrasses) were the most important lionfish prey items in the Bahamas [24], whereas typically larger-bodied prey (sea basses, grunts, and parrotfishes) were most important off North Carolina [38]. These differences in prey preference and prey base likely have direct macro- and micronutrient implications that may be difficult, if not impossible, to replicate in aquarium environments. Due to commercial supply chain accessibility and cost, the aquarium lionfish in this study and aquarium lionfish in general are more likely to be fed pelagic fish than benthic, reef-dependent fish that are difficult to collect or to rear in aquaculture. While crustaceans, mollusks, and echinoderms have been found in lionfish stomachs, all measures of prey importance indicate that adult lionfish are essentially piscivorous [38]. Thus, including crustaceans and mollusks in aquarium lionfish diets at best is likely unnecessary and, at worst, may be deleterious to their nutrition in more frequent or larger quantities.

A major assumption of this study was that wild and aquarium lionfish cohorts had a uniform age distribution despite a relatively wide range in total length (min–max: 23–43 cm and 31–38 cm, respectively). Similarly, the sex distribution and reproductive statuses of wild and aquarium lionfish cohorts may influence whole blood fatty acid levels. Lionfish have no obvious external sexual dimorphism, and sex was not determined during necropsy or via ultrasound for wild and aquarium lionfish, respectively. Postmortem blood samples from wild lionfish were collected at sea on a commercial dive boat which did not allow lionfish necropsies on board. Thus, the number of male and female lionfish included in our results is unknown. All lionfish were sampled over a single season (summer), so any seasonal variability in whole blood fatty acid levels is yet to be determined. Coupled with the widely variable diets and nutrient supplementation, the small lionfish population at each aquarium may also skew our understanding of whole blood fatty acid levels in lionfish under aquarium care. Sampling more aquarium and wild lionfish could have strengthened our study design and better accounted for population variability in whole blood fatty acid levels. For aquarium lionfish, it is unclear how much nutritional supplement is absorbed by food items when they are soaked or whether the supplement is further diluted when fed in an aqueous environment. For wild lionfish, gastric contents were moderately to heavily digested in necropsied individuals so key prey species could have been missed.

5. Conclusions

Creating and providing evidence-based diets for all fish, including lionfish, in aquarium environments is important in advancing their health and welfare. This study provides

whole blood fatty acid comparative data for aquarium and wild lionfish to improve the health and well-being of lionfish in captivity. This study also highlights differences in select fatty acid levels, saturated fatty acids, and HUFA between aquarium and wild lionfish, which have implications for their innate immune system and subsequent health.

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Data Availability Statement: The data presented in this study are available from the corresponding author upon reasonable request.

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