FISHERIES & AQUATIC LIFE (2023) 31: 1 - 14
Archives of Polish Fisheries
DOI 10.2478/aopf-2023-0001

sciendo

RESEARCH ARTICLE

Comparison of fatty acid composition of the eggs of wild and farmed *Coregonus maraena* and the influence of feed

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Received – 15 July 2022/Accepted – 21 February 2023. Published online: 31 March 2023;
Citation: Bochertm, R., Bernolle, D., Grunow, B. (2023). Comparison of fatty acid composition of the eggs of wild and farmed *Coregonus maraena* and the influence of feed. Fisheries & Aquatic Life 31, 1-14

Abstract. The fatty acid (FA) compositions of eggs from wild whitefish and captive broodstocks were investigated to estimate the FA requirements of *Coregonus maraena*. The aim of this study was to increase basic knowledge on the nutritional needs of broodstock. Whitefish eggs from two natural spawning grounds were compared with that of captive broodstocks that were fed three commercially available feeds used in fish farms in Mecklenburg-Vorpommern, Germany. Wild fish eggs differed significantly in crude protein content and FA profiles from the eggs of experimentally farmed fish groups. The percentage of monounsaturated FAs (MUFA) were significantly lower in the wild fish eggs. The most common MUFA was oleic acid in all groups, although in the wild fish eggs the value of it was almost half of that in farmed fish eggs. Wild fish eggs had significantly higher values of total polyunsaturated FAs (PUFA), especially of n-3 PUFA, which was nearly double that of the eggs of the farmed fish groups. Additionally, it was shown that egg FA composition even differed between the wild fish stocks that were from the same area, whereas there were no differences in egg proximate composition or FA composition among the three feeding groups even though the compositions of the feeds fed to these fish differed significantly. The present study suggested that the proportions of essential FAs supplied in the dry feeds fed to the captive broodstocks were inappropriate for this species, and feeds must be adapted further to meet the nutritional requirements of this species to improve the quality of farmed whitefish eggs.

Keywords: broodstock nutrition, dry feed, fatty acid analysis, highly unsaturated fatty acids, mussel meat, offspring improvement, *Coregonus maraena*

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Introduction

Maraena whitefish (*Coregonus maraena*, Bloch 1779) is a relatively new species in intensive aquaculture (Baer et al. 2021). For production in re-circulating aquaculture systems (RAS), fishes adapted to husbandry conditions are of immense advantage. Regardless of outdoor conditions, fishes can be kept in indoor RAS and reproduced independently of season. While optimal chemical and physical environmental conditions are important, above all,

fishes need adequate diets that meet their nutritional requirements (Adams 1999). These needs change with age and reproductive season, and different commercially available feeds are used at fish farms since there is no standardization for this fish species. During female gonad development, all necessary substances are provided to each developing egg, and this provides optimal developmental conditions for embryos as they mature, which gives the offspring the best chances of survival (Adams 1999, Araújo et al. 2012, Callan et al. 2012). In contrast to natural conditions, where fishes must actively search for food, under controlled conditions in RAS, fish farmers can provide the appropriate nutrients in the feed.

It is well documented that broodstock diets strongly influence the fat content and especially the fatty acid profiles not only of the body tissues (Soivio et al. 1989, Wang 1990), but especially of eggs (Kaitaranta and Linko 1984, Harel et al. 1994, Fernández-Palácios et al. 1995, Rodríguez et al. 1998, Almansa et al. 1999, Cejas et al. 2003, Mejri et al. 2017). Inadequate nutrition of broodstocks leads to problems with eggs especially for final egg development and can lead to reduced hatching and poor growth rates in embryos and larvae. Additionally, broodstock malnutrition can lead to increased deformations or poor larval development (Divanach et al. 1993, Cejas et al. 2003, Callan et al. 2012). Positive changes in egg quality through improved broodstock nutrition have been documented in a variety of fish species (Izquierdo et al. 2001).

Essential fatty acids (EFAs) are exceptionally important in human nutrition and also in fish reproduction (Soivio et al. 1989, Rainuzzo et al. 1997, Adams 1999, Glencross 2009). Requirements for EFAs vary with habitat (marine, brackish, or freshwater) since freshwater fishes can synthesize long-chain polyunsaturated fatty acids (LC-PUFA) from the 18-C fatty acids linolenic acid (C18:3 n-3, αLNA; C18:3 n-6, γLNA) and linoleic acid (C18:2 n-6, LOA). Marine species cannot do this and require more 20-C and 22-C EFAs (Glencross 2009). Watanabe et al. (1989) demonstrated that, for coregonids, the n-3 FA class and LC-PUFA are enormously important but that enriching diets with LNA and LOA had no positive effects. This implies that coregonids, which are usually classified as freshwater fishes, should be counted among brackish or marine species (Glencross 2009) in terms of FA metabolism.

C. maraena is described by Kottelat and Freyhof (2007) as an anadromous fish species that occurs throughout the Baltic Sea in coastal feeding grounds (Czerniejewski and Rybczyk 2010). The fish migrate to spawn in shallow freshwater or brackish areas and lower segments of rivers. This species feeds on benthic animals, especially crabs, mussels, and insect larvae. Currently, there is little evidence of what is suitable feed for maraena whitefish in RAS since as no nutritional studies have been conducted on them to date. Therefore, fish farmers use different feeds for *Coregonus* in aquaculture systems. Maraena whitefish is fed commercial feeds developed for other species like rainbow trout (*Oncorhynchus mykiss* (Walbaum)), seabass (*Dicentrarchus labrax* (L.)), or sturgeon (*Acipenser* sp.). Developing suitable feeds for new species in aquaculture with low production volumes is rather unattractive for feed manufacturers. While alternative feeds can be sufficient for fattening commercial fishes, they are often unsuitable for broodstocks since the nutritional requirements of fishes that are maturing and spawning are much higher. What constitutes adequate nutrition for broodstocks is currently poorly understood and remains a challenge (Izquierdo et al. 2001, Glencross 2009).

The FA profiles of eggs can differ depending on the diets of benthic animals in wild fish and dry feed in farmed fish. To gain an understanding of what constitutes appropriate nutrition for spawning maraena whitefish, wild and farmed fish are compared as in shortfin corvine (*Cynoscion parvipinnis* Ayres) (González-Félix et al. 2017). Successful reproduction and quality eggs can only be guaranteed by a sufficient supply of FAs (Almansa et al. 1999, Izquierdo et al. 2001).

The aim of this study was to improve knowledge of the nutritional requirements of maraena whitefish spawners since current problems with the reproduction of this species and rearing its larvae indicate that the feeds fed to farmed fish are of an inadequate

composition. This study analyzed three commercially available feeds used by fish farmers in Mecklenburg-Vorpommern and compared the influence of them on egg quality. Our hypothesis was that feeding commercial dry diets with the addition of mussel meat would improve egg FA profiles and the resulting ratios of important indices by making the diets more similar to that that wild fish consume.

Materials and Methods

Fish and rearing conditions

Wild population

Whitefish eggs were collected during the spawning season from two fishing areas in the southern Baltic Sea (Germany) in 2019. These areas are known as potential spawning grounds. The fish were caught by commercial fishers with gillnets in the Oder Estuary (Oder Lagoon, Szczecin Lagoon) (53.79732, 14.06473) and in Peenestrom (54.00567, 13.91573). Each sampling location was assumed to comprise one stock. Eggs were collected by stripping them manually from a total of 19 females (0.87–1.95 kg). The eggs were frozen at -80°C for further analyses.

Flow-through system

The material for this study were whitefish aged 2.5 years that were obtained from a commercial fish farm with a flow-through system. These fish were obtained as larvae from the aquaculture research station at the Institute of Fisheries of the State Research Centre for Agriculture and Fishery Mecklenburg-Vorpommern in Born, Germany, and were transferred as fingerlings to the fish farm to grow out in 2018. According to the seasonal environmental conditions, the gonads of these fish developed during the natural spawning season in fall. The experiment started at an early stage of gametogenesis in fall as water temperatures decreased. Adult whitefish were divided into two experimental groups in two different tanks in September 2020. The fish were fed commercial dry feed from the beginning of October. One experimental group received 4.5 mm Aller Arctic Support (Aller Aqua, Germany), while the other group was fed with 4.5 mm Aller Ivory ex (Aller Aqua, Germany). The fish were fed daily according to the manufacturer's instructions for fish size and water temperature. Eggs from four mature females per tank were collected by stripping them manually on December 16 and 18, 2020. Egg samples from eight females per experimental group were frozen separately for further analyses.

Indoor recirculating aquaculture system

The F2 aquaculture generation maraena whitefish hatched in 2018 were held in a climate room for out-of-season reproduction under the same conditions as described above from July 2019 (400–500 g body weight). The fish were fed 3 mm Supreme 10 (Alltech Coppens, Germany). After a two-month phase of artificial summer (temperature 20.5°C), the fish were gradually cooled starting in September 2020. The photoperiod (light during the day) was also shortened according to natural conditions. The lowest water temperatures of 5.5°C were reached at the end of December 2020. Supplementing feeding with frozen mussel meat (AquaHobby Fischfutterhandel, Germany) started at the end of October 2020 (body weight 700–800 g) at a daily feeding rate of 0.2% of body weight. Water quality was ensured by continuous purification and disinfection (filter mat, moving bed bio-filter, and UV light). The water parameters of NH_4^+, NO_2^-, and NO_3^- concentrations were measured weekly, while pH, temperature, and oxygen saturation were recorded continuously. Dissolved oxygen was maintained > 8 mg l^{-1} and all physical water parameters were maintained in the optimal range. Eggs from four mature females per tank (0.82–0.98 kg body weight) were collected by stripping the fish manually between January 5–10, 2021. Samples were frozen individually for further analyses.

Fatty acid analysis

All the samples were shipped to an accredited analytical laboratory for analyses. These were performed

Table 1
Proximate composition of commercial feeds (% wet weight)

Proximate composition	Ivory	Support	Coppens Supreme 10	Mussel
Dry matter	91.3±0.0[a]	92.7±0.0[a]	90.9±0.01[a]	27.3±1.27[b]
Water content	8.7±0.0[a]	7.3±0.0[a]	9.1±0.01[a]	72.7±1.27[b]
Crude protein	53.6±0.07[a]	45.7±0.03[b]	49.13±0.06[c]	16.2±0.42[d]
Crude fat	19.9±0.14[a]	22.5±0.24[b]	12.99±0.31[c]	2.3±0.01[d]
Ash	9.9±0.0[a]	7.4±0.0[b]	7.4±0.10[b]	2.9±0.28[c]

Means with different superscript letters differ significantly among the fish groups (one-way ANOVA, $P < 0.05$).

according to the standardized protocols for feed analysis of the VDLUFA as follows: water content and dry matter (VDLUFA Bd. III 3.1. (1975-11)), ash (VDLUFA 8.1/GAFTA method 12:0 (1975-11/2003-01)), crude fat (VDLUFA Bd. III 5.1.1 (1988)), crude protein (VDLUFA Bd. III 4.1.2 (2004), FA profile/concentrations (VDLUFA Bd. III 5.6.1.). Technical replications was performed for all samples.

Statistics

The results of all the measurements were analyzed statistically with SPSS 22.0 (IBM Corp., USA) software and Graphpad Prims 9.5.0. All data were tested for Gaussian distribution using the Shapiro-Wilk test, and data analysis was conducted with one-way ANOVA with Tukey's post-hoc test for multiple comparisons of several groups. Student's t-test was used for two-group comparisons. The results are presented as means ± SEM and differences are significant at $P < 0.05$. The graphs were created with Graphpad Prism 9.5.0 (Graphpad Software, USA). Graphic representations of multifactor analysis using radar charts were illustrated with Microsoft Excel.

Ethics Statement

All fish were kept under the most effective commercial and economic aquaculture conditions (with respect to animal welfare) that met current aquaculture standards in Germany. No fish were killed for this study.

Results

Analysis of feed composition

The experimental diets used showed significant differences in proximate composition (Table 1). Because of the high water content of the frozen mussel meat, values for dry matter, crude protein, crude fat, and ash were lower compared to those of the dry feeds used. Crude protein and crude fat also differed significantly among the three dry feeds.

Saturated fatty acid (SFA) content was significantly elevated in the mussels at almost twice that of the dry feeds. Palmitic acid (C16:0) was the most important FA in this group. Additionally, the proportions of fatty acids C14:0, C15:0, and C23:0 were nearly twice as high compared to those in the dry feeds. Almost no differences were detected among the Ivory, Support, or Coppens Supreme 10 dry feeds (Table 2).

The proportion of monounsaturated fatty acids (MUFA) in the mussels was about half of that in the dry feeds. The main fatty acids were oleic acid (C18:1, OLA) in the dry feeds and palmitoleic acid (C16:1) in the mussels (Table 2). C20:1 and C22:1 differed significantly among all the experimental diets.

The values of polyunsaturated fatty acids (PUFA) were low in the dry feeds at 33.7% and were highest in mussels at 40.86%. Significant differences to varying degrees were found in almost all the fatty acids of this group, and the greatest differences were in the essential fatty acids (EFA). In the dry feeds, this was LOA, which was hardly found in mussels. In contrast, eicosapentaenoic acid (EPA) was the most

Table 2
Fatty acid composition (% of total fatty acids) of experimental feeds in mean ± standard deviation.

Fatty acid composition (%)	Ivory	Support	Coppens Supreme 10	Mussel
C 4:0	ND	0.19±0.01	ND	ND
C 10:0	ND	0.05±0.00	ND	ND
C 12:0	0.05±0.00^{a}	0.15±0.00^{b}	0.25±0.01^{c}	ND
C 14:0	2.84±0.01^{a}	1.48±0.00^{b}	3.53±0.03^{a}	4.96±0.49^{c}
C 15:0	0.27±0.00^{a,b}	0.15±0.00^{a}	0.25±0.00^{a}	0.58±0.05^{b}
C 16:0	11.49±0.01^{a}	10.56±0.01^{a}	14.94±0.06^{a}	25.30±3.87^{b}
C 18:0	2.89±0.00^{a}	3.67±0.30^{a,b}	3.42±0.02^{a,b}	5.12±1.00^{b}
C 20:0	0.52±0.23	0.62±0.01	0.38±0.01	0.44±0.37
C 21:0	0.08±0.01	0.07±0.00	ND	ND
C 22:0	0.23±0.02	0.34±0.08	0.24±0.00	0.22±0.00
C 23:0	0.32±0.01^{a}	0.20±0.01^{a}	ND	1.29±0.31^{b}
C 24:0	0.10±0.00	0.14±0.00	ND	0.22±0.00
Σ SFA	18.78±0.20^{a}	17.59±0.21^{a}	23.11±0.13^{a}	37.89±5.42^{b}
C 14:1	ND	0.05±0.00	ND	0.12±0.00
C 16:1	3.32±0.01^{a}	2.23±0.00^{a}	4.62±0.02^{a}	13.44±1.66^{b}
C 18:1 (OLA)	37.69±0.07^{a}	48.53±0.11^{b}	34.37±0.09^{c}	3.03±0.94^{d}
C 20:1	4.91±0.01^{a}	2.45±0.01^{b}	2.15±0.05^{c}	3.71±0.09^{d}
C 22:1	0.76±0.02^{a}	0.42±0.00^{b}	0.32±0.01^{c}	0.07±0.01^{d}
C 24:1	0.60±0.08	0.30±0.01	ND	ND
Σ MUFA	47.51±0.13^{a}	54.14±0.10^{b}	41.90±0.14^{c}	21.27±2.26^{d}
C 18:2 n6 (LOA)	15.67±0.01^{a}	17.93±0.00^{b}	18.70±0.07^{b}	1.77±0.40^{c}
C 18:3 n3 (αLNA)	6.45±0.08^{a}	6.31±0.01^{a}	4.47±0.00^{b}	1.10±0.08^{c}
C 18:3 n6 (γLNA)	0.12±0.01	0.06±0.00	0.11±0.00	0.09±0.03
C 20:2 n6	1.36±0.06^{a}	0.41±0.18^{b}	0.58±0.00^{b}	1.52±0.16^{a}
C 20:3 n3	0.53±0.00^{a}	0.06±0.01^{b}	0.29±0.00^{c}	0.08±0.02^{b}
C 20:3 n6	0.23±0.00^{a}	0.07±0.00^{b}	0.18±0.00^{c}	0.28±0.01^{d}
C 20:4 n6 (ARA)	0.02±0.00^{a}	0.02±0.01^{a}	0.60±0.00^{b}	ND
C 20:5 n3 (EPA)	3.47±0.06^{a,b}	1.53±0.03^{a}	5.90±0.02^{b}	27.37±1.80^{c}
C 22:6 n3 (DHA)	5.85±0.21^{a}	1.89±0.03^{b}	4.20±0.04^{c}	8.66±0.75^{d}
Σ PUFA	33.70±0.31^{a,b}	28.27±0.13^{a}	35.01±0.01^{b,c}	40.86±3.19^{c}
Σ n3	16.30±0.24^{a}	9.79±0.05^{b}	14.85±0.06^{a,b}	37.20±1.85^{c}
Σ n6	17.40±0.02^{a}	18.49±0.13^{a}	20.16±0.07^{b}	3.66±0.40^{c}
n3/n6	0.94±0.02^{a}	0.53±0.01^{a}	0.74±0.01^{a}	10.25±0.63^{b}
DHA/EPA	1.69±0.02^{a}	1.24±0.00^{b}	0.71±0.00^{c}	0.32±0.00^{d}

Means with different superscript letters differ significantly among the feeds ($p<0.05$) (one-way ANOVA, $p < 0.05$). ND – not detected. OLA – oleic acid, LOA – linoleic acid, DHA – docosahexaenoic acid, EPA – eicosapentaenoic acid, αLNA – α-linolenic acid, γLNA – γ-linolenic acid, ARA – arachidonic acid, SFA – saturated fatty acid, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids.

abundant FA and was several times higher in mussels than in the dry feeds. Arachidonic acid (C20:4, ARA) was not detected in the mussels. This resulted in significant differences in the proportions of PUFA and highly unsaturated fatty acids (LC-PUFA: ≥ 20 carbons (C) and ≥ 3 double bonds; also referred to as HUFA) between mussel meat and the three dry feeds (Table 2). In combination with the different main

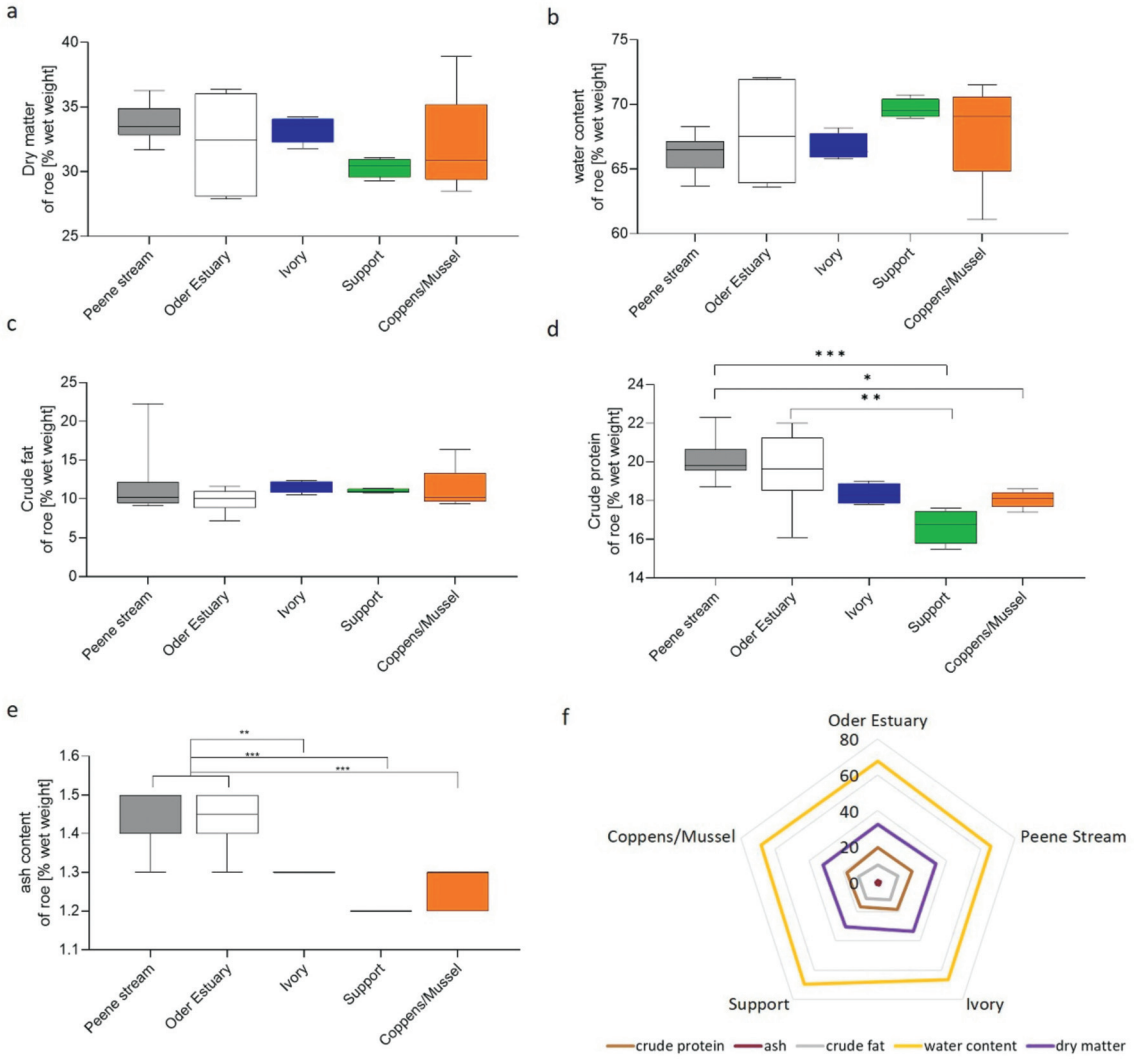

Figure 1. Proximate composition of roe (% wet weight). Analysis of dry matter (a), water content (b), crude fat (c), crude protein (d) and ash content (e). Control groups are of wild whitefish from two locations Peene Stream (grey bars) and Oder Estuary (white bars) were compared with whitefish from aquaculture fed with Ivory (blue), Support (green) and Coppens/Mussel (orange) Significant differences *P<0.05, ** P<0.01, ***P<0.001 (one Way Anova; Tukeys Multiple Comparison; Graphpad Prism 9.5.0). (f) Radar chart of the means from the proximate composition factors in all five animal groups (Excel); crude protein (brown), ash (dark red) crude fat (grey), water content (yellow) and dry matter (violet).

fatty acids (LOA and EPA), the amounts of n-3 and n-6 fatty acids determined differed significantly between the mussels and dry feeds, as did their ratio (n-3/n-6) and the ratio of docosahexaenoic acid (DHA) to EPA (Table 2).

Analysis of eggs

The wild fish used as control groups came from two different catch areas (the Oder Estuary and Peenestrom). There were no significant differences in the proximate composition of the eggs from fish from the two locations. Compared to the eggs of the fish from the three feeding groups, there were no differences in the contents of dry matter, water, or crude fat (Figs. 1a –c, f). The crude protein in the eggs of the wild fish was around 20%, whereas the values of eggs from the fish from the feeding groups were significantly lower (Ivory: 18.3 ± 0.47%; Support: 16.7 ± 0.76%; Coppens Supreme 10/mussel:

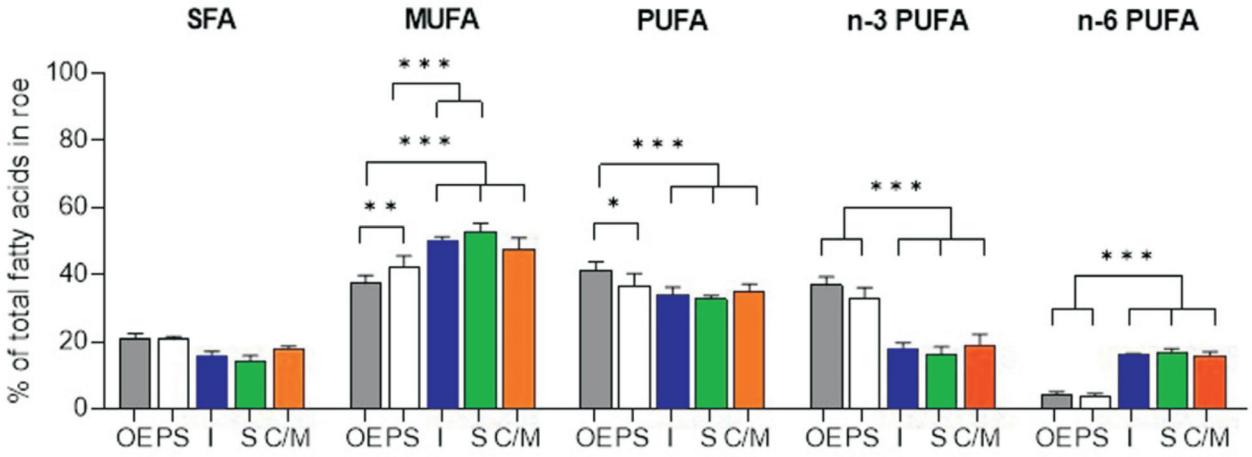

Figure 2. Total fatty acid concentration in roe. Analysis of sum fatty acid from saturated fatty acid (SFA), monounsaturated FA (MUFA), polyunsaturated FA (PUFA) of roe from animals of the Oder Estuary (OE – grey bars), Pene Stream (PS-white bars), fed with Ivory (I – blue bars), Support (S – green bars) and Coppens with mussel (C/M – orange bars) Significant differences *P<0.05, ** P<0.01, ***P<0.001 (one Way Anova; Tukeys Multiple Comparison).

18.1 ± 0.39%; Figs. 1d, f). The ash content was also significantly lower in the feeding groups compared to both wild fish groups (Figs. 1e, f).

Analysis of FA composition

Significant differences in the content of MUFA and PUFA in the sum FA concentrations (sum SFA, sum MUFA, sum PUFA, sum n-3 PUFA, sum n-6 PUFA) were noted in both control groups (Oder Estuary and Peenestrom). The fish from the Oder Estuary had higher percentages of MUFA, whereas those from Peenestrom had higher PUFA percentages (Fig. 2). In contrast to these wild fish, the fish from the three feeding groups had significantly higher MUFA and n-6 PUFA concentrations, whereas the concentrations of total PUFA and n-3 PUFAs were significantly lower compared to those of the wild fish. No differences were detected in SFA concentrations in any of the groups of fish.

Analysis of the FA content of the eggs from the two wild fish stocks revealed significant differences between the Oder Estuary and Peenestrom fishing grounds. The fish eggs from the Oder Estuary had higher values of C22:0, C14:1, C18:4, and EPA, while those from Peenestrom had significantly higher values of MUFA and OLA. However, the main fatty acids in the two wild fish groups were OLA, followed by DHA and C16:0 (Table 3).

The FA profiles of wild fish eggs differed significantly from those of the experimental group eggs. The C20:0, C21:0, and C23:0 SFAs were not detected in the eggs of either wild fish group. In contrast, C17:0 was not detected in the eggs from any of the feeding groups (Table 3). Of SFA, C16:0 dominated at 11.2 to 13.5% in the eggs of the farmed fish groups, while in those from the wild fish groups ranged from 15.7 to 16.3%. The most common MUFA was OLA in all groups, although in wild fish eggs this value was almost half of that of farmed whitefish eggs. The same observation was noted for C20:1. Here, too, the value was significantly lower in wild fish eggs. In contrast, significantly higher proportions of C16:1 were noted in the eggs of both wild fish groups, and C18:1 n-7 was only detected in these two groups. There were also clear differences in PUFA. DHA was the most abundant PUFA in all experimental trials, and its value was significantly higher in both wild fish groups. This was also true of EPA, the values of which were even three times higher. ARA, stearidonic acid (SDA, C18:4), and C22:5 n-3 were only noted in wild fish eggs (Table 3),

Table 3
Fatty acid composition (% of total fatty acids) of eggs of wild whitefish from two locations and experimental fish. Farmed whitefish were fed Ivory, Support, or Coppens/mussel and compared to wild *Coregonus* from the Oder Estuary and Peenestrom

Fatty acid composition (%)	Oder Estuary	Peenestrom	Ivory	Support	Coppens Supreme 10
C 14:0	1.49±0.24^{a,b}	1.30±0.17	1.14±0.14^{a}	0.99±0.09^{b}	1.25±0.11
C 15:0	0.50±0.00^{a,c,e}	0.43±0.00^{b,d,f}	0.19±0.01a^{,b}	0.15±0.02^{c,d}	0.21±0.04^{e,f}
C 16:0	15.76±0.01^{a,c,e}	16.27±0.01^{b,d,f}	12.34±1.21^{a,b}	11.22±1.25^{c,d,g}	13.51±0.70^{e,f,g}
C 17:0	0.42±0.07	0.38±0.09	ND	ND	ND
C 18:0	2.18±0.30^{a,c}	1.99±0.00^{b,d}	1.49±0.12^{a,b}	1.47±0.11^{c,d}	1.75±0.38
C 20:0	ND	ND	0.12±0.04	0.14±0.01	0.20±0.08
C 21:0	ND	ND	0.07±0.01	0.07±0.00	0.07±0.02
C 22:0	0.82±0.08^{a}	0.34±0.02	ND	ND	ND
C 23:0	ND	ND	0.49±0.10^{a}	0.37±0.02^{b}	0.64±0.08^{a,b}
C 14:1	0.27±0.13^{a}	0.12±0.08^{a}	ND	ND	ND
C 16:1	9.10±1.06^{a,c,e}	9.33±0.94^{b,d,f}	3.85±0.19^{a,b}	4.32±0.43^{c,d}	5.59±0.58^{e,f}
C 18:1 n7	4.55±0.35	4.92±0.52	ND	ND	ND
C 18:1 n9 (OLA)	22.91±1.80^{a,c,e,h}	27.18±2.50^{b,d,f,h}	44.19±1.28^{a,b}	46.76±2.50^{c,d,g}	40.09±3.15^{e,f,g}
C 20:1	0.54±0.20^{a,c,e,g}	0.87±0.21^{b,d,f,g}	1.63±0.23^{a,b}	1.48±0.16^{c,d}	1.48±0.20^{e,f}
C 22:1	ND	ND	0.14±0.02	0.14±0.01	0.12±0.01
C 18:2 n6 (LOA)	1.71±0.48^{a,c,e}	1.21±0.27^{b,d,f}	12.92±0.36^{a,b}	13.25±0.70^{c,d}	12.48±1.46^{e,f}
C 18:3 n3 (αLNA)	1.94±1.58	0.88±0.23^{a,b,c}	3.50±0.31^{a}	3.69±0.29^{b}	2.74±0.85^{c}
C 18:3 n6 (γLNA)	0.39±0.16^{a,c,e}	0.30±0.08^{b,d,f}	1.59±0.18^{a,b}	1.50±0.46^{c,d}	1.50±0.20^{e,f}
C 18:4 n3 (SDA)	2.18±1.19^{a}	0.42±0.16^{a}	ND	ND	ND
C 20:2 n6	0.31±0.07^{a,e,g}	0.34±0.08^{b,f,h}	0.88±0.07^{a,b,c,d}	1.18±0.11^{c,e,f}	1.15±0.23^{d,g,h}
C 20:3 n3	0.29±0.07^{a}	0.28±0.06	0.26±0.05	0.22±0.04	0.18±0.02^{a}
C 20:3 n6	0.22±0.07^{a,c,e}	0.11±0.07^{b,d,f}	0.92±0.21^{a,b}	0.72±0.14^{c,d}	0.77±0.19^{e,f}
C 20:4 n6 (ARA)	1.91±0.25	1.92±0.50	ND	ND	ND
C 20:5 n3 (EPA)	10.47±0.70^{a,c,f,h}	8.78±0.76^{b,d,g,h}	2.42±0.29^{a,b}	2.04±0.32^{c,d,e}	3.72±1.27^{e,f,g}
C 22:5 n3	2.96±0.23	2.96±0.33	ND	ND	ND
C 22:6 n3 (DHA)	19.04±3.93^{a,c,e}	19.60±2.63^{b,d,f}	11.64±1.16^{a,b}	10.24±1.87^{c,d}	12.21±2.64^{e,f}

Mean ± standard deviation. Means with different superscript letters differ significantly between the fish groups (one-way ANOVA and Student's t-test; $p < 0.05$). ND – not detected. OLA – oleic acid, LOA – linoleic acid, DHA – docosahexaenoic acid, EPA – eicosapentaenoic acid, αLNA – α-linolenic acid, γLNA – γ-linolenic acid, ARA – arachidonic acid.

which resulted in significantly higher PUFA n-3 values in both wild fish groups. Other FAs, such as LNA and C20:2 were significantly lower in wild fish eggs, and, in the case of LOA, it was substantially lower. Therefore, the farmed fish exhibited higher n-6 values compared to the wild fish groups.

Overall, even with some significant differences in concentrations of a few FAs, the three experimental farmed whitefish groups did not differ in sum FAs (SFA, MUFA, and PUFA or in PUFA n-3 and PUFA n-6; Fig. 2). In contrast, highly significant differences were noted in the eggs from the two wild fish groups (Fig. 2).

Influence of feed on eggs

The analysis of the influence of the feeds on whitefish egg quality indicated that the eggs from all the groups had high water content regardless of that in the feeds, which was very low in the Ivory (Fig. 3a), Support (Fig. 3b), and Coppens/mussel (Fig. 3c) feeds.

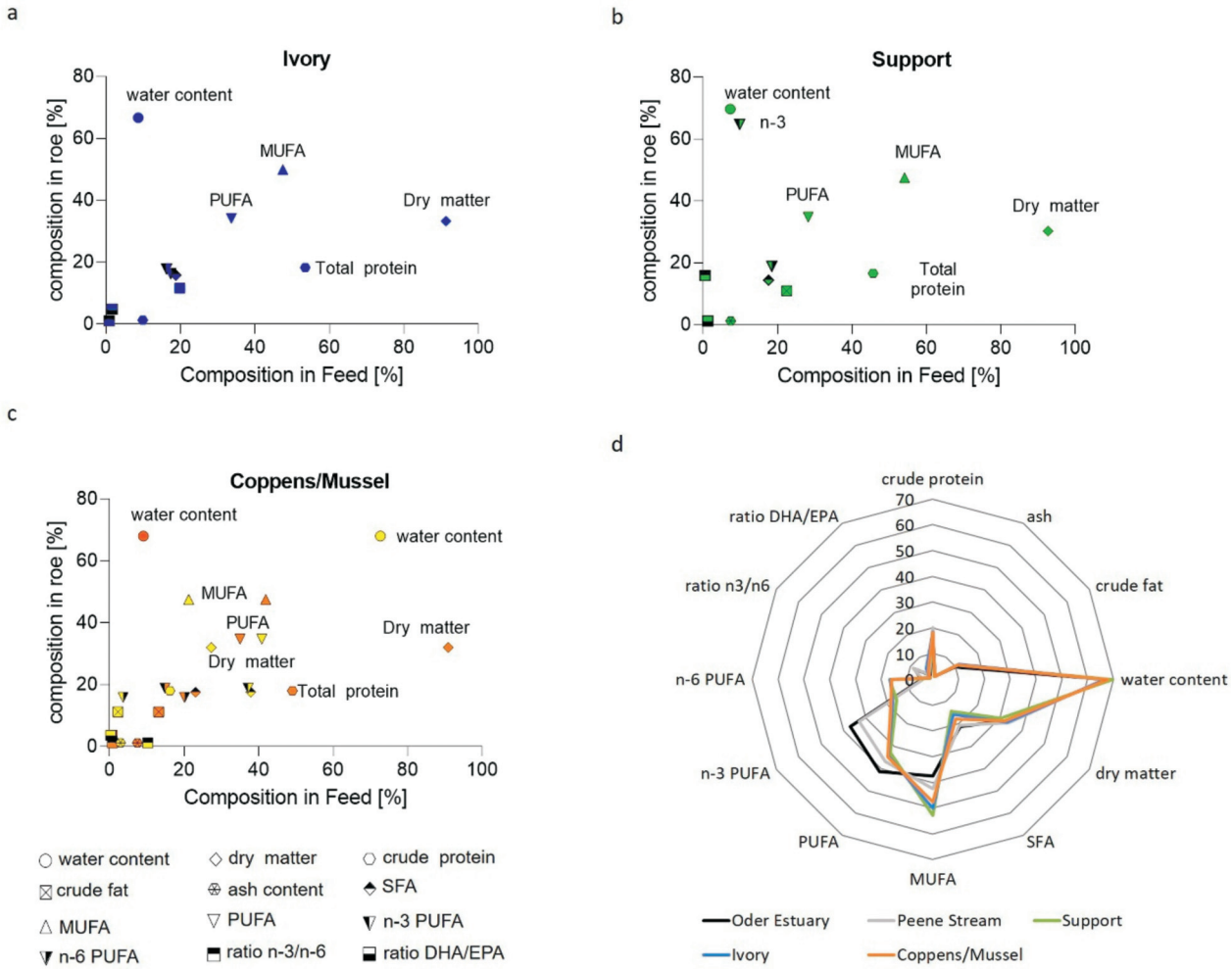

Figure 3. Summarised illustration of the relationship between feed composistion and roe quality. (a) Ivory feeding group (blue), (B) Support feeding group (green) and (c) experimental group fed with Coppens (orange) and Mussels (yellow). Illustration of water content, dry matter, crude protein; crude fat, saturated fatty acid (SFA), monounsaturated FS (MUFA), polyunsaturated FS (PUFA) as well as n-3 PUFA, n-6 PUFA, ratio of n-3/n-6 and the ratio of DHA/EPA; Graphpad Prism 9.5.0. (d) Spread of variables of roe quality of two wild fish groups (Oder Estuary – black and Peene Stream – grey) as well as the feeding groups (Support – green, Ivory – blue and Coppens/Mussel – orange); Excel.

Additionally, the crude fat content of the feeds also did not influence the eggs since the Ivory and Support feeds had crude fat contents of around 20%, whereas Coppens feed had only 13%, and the mussel had only around 2%. The crude fat content of the eggs from all three feeding groups did not differ from that of the eggs from the wild fish groups (Fig. 3d). As a rule, the water content in fish tissue depends directly on its fat content (Shadieva et al. 2020), and the higher the fat content, the lower the water content. Overall, the radar web indicated that the FAs in the feeds influenced egg quality (Fig. 3d). The MUFA content of the Support feed was around 54% compared to around 47% in Ivory and 41.9% in Coppens compared to 21.27±2.26 in mussel (Table 2). Even if no significant differences were noted among the three feeding groups, the whitefish eggs of the Support group had the highest values. Additionally, the MUFA values of the eggs in all three groups were significantly higher compared to those of the wild fish groups (Fig. 3d).

In contrast to MUFAs, PUFA concentrations were significantly higher in the eggs of both wild whitefish groups (Fig. 3d). The reason was the

significantly higher values of the n-3 groups. However, even for PUFAs, the diets had no significant effect on egg quality (Fig. 3). Thus, increased PUFA concentrations were present in the Coppens/mussel feed group, but the eggs did not have significantly increased PUFA values compared to the eggs from the other two feeding groups.

Discussion

This study determined the FA composition of eggs from wild whitefish, *C. maraena*. Samples from two spawning grounds were collected and analyzed. These FA profiles can be used as a natural model for assessing the quality of feed under aquaculture conditions (González-Félix et al. 2017). For the comparison of eggs from wild and farmed fish, first the influence of two commercially available dry feeds was tested. Second, eggs from maraena whitefish fed dry feed supplemented with frozen mussel meat were analyzed. Local fish farmers in Mecklenburg-Vorpommern (Germany) currently use these three different feeds since there are no standardized protocols for the husbandry and feeding of whitefish (especially during the spawning season). The total lipid values (9.8–11.8% wet weight [ww]; 30.4–36.2% dry weight [dw]) did not differ between the eggs of the *C. maraena* wild fish stocks and those of the fish fed experimental diets in this study. Furthermore, the crude fat contents analyzed were in the same range as that reported for other coregonids and salmonids (rainbow trout 9.2% ww; Kaitaranta and Ackmann 1981). Vuorela et al. (1979) and Kaitaranta (1980) reported total lipid contents of 9.8–9.9% ww in vendace, *Coregonus albula* (L.) eggs, while Lahti and Muje (1991) reported slightly higher values of 10.4–13.1% ww. Muir et al. (2014) reported values of 33.4–38.6% dw for eight spawning stocks of lake whitefish, *Coregonus clupeaformis* (Mitchill). Mueller et al. (2017) reported 29.5% dw for fertilized eggs in this species.

In the current study, FA profiles of the eggs from whitefish from the two catch areas differed significantly in MUFA and PUFA content and individual FAs such as OLA, SDA, and EPA. This suggests that these might be two different spawning populations that consumed different diets in different feeing grounds during gonad maturation. Muir et al. (2014) found even greater differences in the levels of certain FA classes, EFA and relevant indices in the eggs of mature lake whitefish when examining eight different spawning populations.

In the present study, PUFA was only detected at approximately 41% (Oder Estuary) compared to 37% (Peenestrom) of the total lipids of the eggs. Surprisingly, it was the opposite in MUFA with 42% in eggs of the fish from Peenestrom and 37% in the Oder Estuary group. These values differed from the results reported for other coregonids. Muir et al. (2014) reported higher proportions of PUFAs at 53–59% in the eggs of eight spawning populations of *C. clupeaformis*. Kaitaranta (1980) reported a PUFA content of 50% in *C. albula* eggs, and Lahti and Muje (1991) reported values of up to 60% PUFA for this species. Soivio et al. (1989) noted 42% PUFA in *Coregonus muksun* (Pallas) eggs, which was comparable to the wild fish eggs from the Oder Estuary. Soivio et al. (1989) and Kaitaranta (1980) reported that MUFAs and SFAs were present in almost equal proportions in the eggs of *C. albula* and *C. muksun*. In contrast, Lahti and Muje (1991) measured higher proportions of SFA than MUFA content in the eggs of *C. albula*, while Muir et al. (2014) reported the opposite proportions in *C. clupeaformis* eggs.

In whitefish, the FA composition of the meat and eggs are very similar regarding total lipid content, neutral lipid, and polar lipid fractions (Kaitaranta 1980). Özogul et al. (2007) reported that the muscle fat composition in eight marine and six freshwater fishes were basically comparable. What they all had in common was that MUFAs made up the smallest proportion of the total FAs. However, the PUFA content did not exceed 50% in any of the fish species analyzed. Very high MUFA levels of 58% were found in the gonads of farmed Chinese sturgeon that were fed a largely dry diet (Zhou et al. 2016). These authors considered improving the FA profile to be necessary for the adequate nutrition of the broodstock. In this

respect, certain variability among fish species is to be expected, and the FA profiles in the body and the eggs is species-specific and depends on the feed. However, the distribution of fats among the individual FA classes in the wild fish eggs analyzed in the present study, with approximately equal proportions of MUFAs and PUFAs, was difficult to classify.

The analysis of the feed compositions revealed that OLA was the main FA in the dry feed at proportions of 34–48% (Table 2), whereas mussel meat contained only 3%. In contrast, mussel meat had almost double proportions of C16:0 and also a significantly higher content of C16:1 compared to the Ivory, Support, and Coppens dry feeds. These had little influence on the quality of eggs with slightly higher values of C16:0 and C16:1 in the fish fed Coppens/mussel; however, these values were still low compared to the eggs of wild fish from both spawning grounds. Additionally, the very high levels of oleic acid in the feeds appeared to be of nutritional significance since this FA in the farmed fish eggs was nearly double that in eggs from the wild fish stocks.

In addition to differences in MUFAs, great differences in PUFAs were also noted between the eggs of wild and cultured fish, especially in LC-PUFAs. In the wild fish eggs, DHA and EPA dominated at a ratio of 2:1. Other long-chain FAs, such as C22:5, ARA, and SDA, were absent from the feeds, and, therefore, they were also absent from the cultured fish eggs. In contrast, LOA was found very abundantly in the cultured fish, and both LNAs were also in higher proportions, which could have also stemmed from feed composition. Nevertheless, no significant differences were determined in the values of the two LNAs among the three diets. The proportions of EFAs in the wild fish eggs analyzed in this study were comparable to those of lake whitefish, vendace, and *C. muksun* (Soivio et al. 1989, Lahti and Muje 1991, Muir et al. 2014). Of particular importance was DHA, which, occurred together with EPA at a ratio of about 1.5–3:1. The proportions of ARA were significant, while those of both LNAs were the lowest contents in wild fish eggs. High DHA and EPA levels > 60% of PUFAs are typical for marine fish (Soivio et al. 1989).

An insufficient supply of suitable FAs from class n-3 was also clearly evident in the feeding groups. Wild fish eggs consisted mainly of n-3 FA, while n-6 was hardly represented, resulting in an n-3/n-6 ratio of approximately 10, whereas in farmed fish it was less than 1. Muir et al. (2014) reported n-3/n-6 ratios of 5–8 in *C. clupeaformis*. However, more advantageous ratios were also reported, such as 1.2 in seabass (Tan et al. 2014), 2.1–3.2 in seabream (Cejas et al. 2003, Farhoudi et al. 2012) and approximately 2 in goldfish (Wiegand 1996). For maraena whitefish, the n-3 FAs seem to be of great importance based on the results of the present study. Soivio et al. (1989) concluded that marine fish generally have a higher proportion of n-3 FAs, and the authors concluded this was linked to the marine food chain from phytoplankton via zooplankton to fish. The spawning ground sampling locations of the current study are strongly influenced by the Baltic Sea, and marine species such as herring (*Clupea harengus* L.) also use these waters as spawning sites (Polte et al. 2021). Therefore, it can be assumed that *C. maranea* of the southern Baltic Sea also seem to feed intensively on zooplankton in their feeding grounds.

Comparisons of FA profiles in wild and farmed fish eggs is important for improving broodstock nutrition and has been highlighted in previous studies (Harrell and Woods 1995, Pickova et al. 1999, Cejas et al. 2003, Ben Khemis et al. 2014, González-Félix et al. 2017). Problems and deficits that occur in the early life stages of larvae and juveniles are directly related to the quality and quantity of broodstock nutrition (Izquierdo et al. 2001). Our results indicated that all three feeds used by fish farmers in Mecklenburg-Vorpommern (Ivory, Support, and Coppens supplemented with mussel meat), although different in composition, seem to result in same quality of farmed whitefish eggs. Our hypothesis that supplementing feed with mussel meat would improve egg quality was not proved to be correct. Rather, the diet in particular must be modified to allow the farmed whitefish to increase n-3 PUFA concentrations that match those of wild fish.

Further efforts are needed to improve the success in the propagation and breeding of maraena

whitefish. This fish species is new to aquaculture and is not at all genetically adapted to captive husbandry conditions, and there is a lack of basic knowledge about adequate broodstock nutrition. Adapting diets will lead to economic success for fish farmers. Future studies must also aim to shed light on further rearing. Fertilization, hatching, and survival, and deformation rates of eggs and larval and juvenile are but a few aspects that can be optimized by fully meeting the nutritional requirements of spawners.

Acknowledgments. The authors wish to express thanks to the staff––Rica Tielebier, Andreas Tielebier, Steffan Herper, and Daniel Genz––of the Research Station Aquaculture (LFA, Born) for technical assistance and fish husbandry. The authors are also grateful to Steffen Schulz (LFA), Lutz Krenkel (LFA), Dr. Jens Frankowski (LFA), Peter Möller, and Bernd Plötz (FIUM GmbH and Co.KG).

Author contributions. R.B. designed the study; R.B. and B.G. managed the aquaculture experiment; D.B. performed field sampling; R.B. and D.B. processed samples; B.G. supervised the F.A. analyses; R.B., B.G., and D.B. performed data entry; R.B. and B.G. did the statistical analyses; R.B., B.G., and D.B. analyzed the data. The manuscript was mainly written by R.B. with editorial contributions by B.G. and D.B.

Data Availability Statement. The data used to support the findings of this study are included in the article.

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