Traceability and Discrimination among Differently Farmed Fish: A Case Study on Australian Murray Cod

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The development of traceability methods to distinguish between farmed and wild-caught fish and seafood is becoming increasingly important. However, very little is known about how to distinguish fish originating from different farms. The present study addresses this issue by attempting to discriminate among intensively farmed freshwater Murray cod originating from different farms (indoor recirculating, outdoor floating cage, and flow through systems) in different geographical areas, using a combination of morphological, chemical, and isotopic analyses. The results show that stable isotopes are the most informative variables. In particular, δ^{13}C and/or δ^{15}N clearly linked fish to a specific commercial diet, while δ^{18}O linked fish to a specific water source. Thus, the combination of these isotopes can distinguish among fish originating from different farms. On the contrary, fatty acid and tissue proximate compositions and morphological parameters, which are useful in distinguishing between farmed and wild fish, are less informative in discriminating among fish originating from different farms.

KEYWORDS: Aquaculture; chemiometric; discriminant function analysis; fatty acids; *Maccullochella peelii peelii*; stable isotopes; product tracing

INTRODUCTION

Recently, the trade of fish and seafood products has been increasingly influenced by food quality and safety norms (*1*). Thus, several directives have been introduced and will be progressively enhanced, aiming to establish safety standards and traceability/product tracing procedures into the fisheries and aquaculture market chains (*2*). Commonly, the different national and international norms developed, or under development, are based on the guidelines and principles provided by the Codex Alimentarius Commission compiled by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) (*1*). Traceability/product tracing have been defined by the Codex Alimentarius Commission as "the ability to follow the movement of a food through specified stage(s) of production, processing and distribution" (*2*), and traceability systems and associated regulations specifically developed for fish and seafood products have been, or will be soon, implemented worldwide (*1*).

Given the growing importance of aquaculture in Southeast Asian and Indian–Pacific nations, the traceability of fish and

seafood products and associated regulations are becoming increasingly important in Australia (*3*). In Australia and New Zealand, the statutory agency "Food Standards Australia New Zealand" has developed standard codes and is increasingly concerned with traceability and labeling issues of fish and seafood products. As a consequence of these codes, a variety of labeling schemes are being put in place for fish products with the broad aim of promoting product safety, distinction of quality, and resource sustainability (*1, 2*).

The future and economically sustainable development of the aquaculture sector will be progressively more market driven and, consequently, reliant on its capacity to meet consumers' expectations. A number of studies have recently pointed out increasing consumer concerns about safety and health issues (*1, 4*), with consumers also being aware of environmentally detrimental practices (*5*). Hence, environmental, organic, and ethical issues are growing in significance as factors affecting consumers' food choices (*6, 7*). Consequently, consumers expect to be able to easily access reliable information, through a labeling scheme, as to which aquaculture techniques and which types of feed or raw materials have been used in the feed formulation (*2*).

In light of the above issues, there has been much recent activity in developing analytical tools to distinguish between farmed and wild-caught fish (*8–11*). In contrast, only limited information is available for differentiating cultured fish farmed

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Table 1. General Farms Characteristics, Water Quality, and Details of the Commercial Diets Used in Four Different Murray Cod Farms

	farms			
	DU	GL	RU	DPI
location	Warrnambool (VIC)	Glenview (QLD)	Rutherglen (VIC)	Red Cliffs (VIC)
farm type	indoor	indoor	indoor	outdoor
latitude	38.38 °S	26.46 °S	36.06 °S	34.18 °S
elevation (m)	21	426	169	54
farming system	recirculating aquaculture system	recirculating aquaculture system	flow trough system	open-water cage farm in irrigation reservoir
stocking density (kg/m^3)	50−150	70−130	10−20	15−30
water source	bore water	mix of bore and earthen pond water	bore water	surface irrigation (Murray river)
water exchange (%/day)	15	10	300−500	
temperature (°C)	20−25	25−31	16−22	8.6−28.5
oxygen (mg/L)	7−15	8−12	6−8	3.5−16.5
ammonia (mg/L)	0.1−1.5	0.1−0.5	<1.0	0.0−2.73
nitrate (mg/L)	40−120	<30	<40	0.0−11.6
diet producer	Skretting	Ridley	Ridley	Skretting
diet name	Nova ME	45/20 Marine Sink	Aqua Native	Classic SS
pellet size (mm)	11	10	6	11

from different systems or different regions (*12*). However, it is accepted that the quality of farmed fish, which can vary greatly between farms, is mainly influenced by the quality of farming environment, the quality of the feed, the feed management, and the culture methods implemented (*13, 14*). Thus, there is a growing need to develop analytical methods for discriminating among farmed fish cultured in different systems and/or in different geographical locations.

The aim of the present study was to develop analytical methods to detect the origin of intensively cultured freshwater Murray cod (*Maccullochella peelii peelii*) in Australia, trying to distinguish between fish farmed in different locations, under different farming conditions, and fed with different commercial feeds. The rationale for choosing Murray cod is that it supports a new and emerging national aquaculture industry with export market potential, it is farmed from southeastern South Australia (SA), through Victoria (VIC) and New South Wales (NSW), to southeastern Queensland (QLD), and Murray cod are commonly reared in a variety of different culture systems; the latter including highly intensive recirculating systems, flow-through earthen raceway or pond systems, and more recently also in floating cages, raceways, and tanks in irrigation storage reser-

voirs as a component of integrated agri-aquaculture production systems (*15, 16*).

MATERIALS AND METHODS

Fish Sampling and Farms Description. Twelve commercial size Murray cod (∼750 g) were collected during March, 2007, from four different farms specialized in Murray cod production (DU, Deakin University Murray cod farm; GL, Glenview Murray cod farm; RU, Rutherglen Murray cod farm; and DPI, Department of Primary Industry farm; **Table 1** and **Figure 1**). They were culled using an ice slurry, bled, immediately shipped on ice to the Deakin University laboratories, and then stored at −20 °C until required. In the four farms, Murray cod were fed for the last part (>3 months) of the production cycle with different commercial extruded diets (**Table 1**).

Morphological Parameters. The morphological parameters measured included total length (TL), total weight (TW), somatic weight (SW; gutted carcass weight), total viscera weight (VW; including liver, gut, and perivisceral fat), and fillet weight (FW; with skin on). All weights were in grams, and lengths were in centimeters. Also estimated were the following morphologic parameters: Fulton's condition factor $K = TW \times TL^{-3} \times 100$; dress-out percentage (%) = $SW \times TW^{-1} \times 100$; and fillet yield (%) = $FW \times TW^{-1} \times 100$.

Chemical Analyses. Proximate compositions of commercial diets
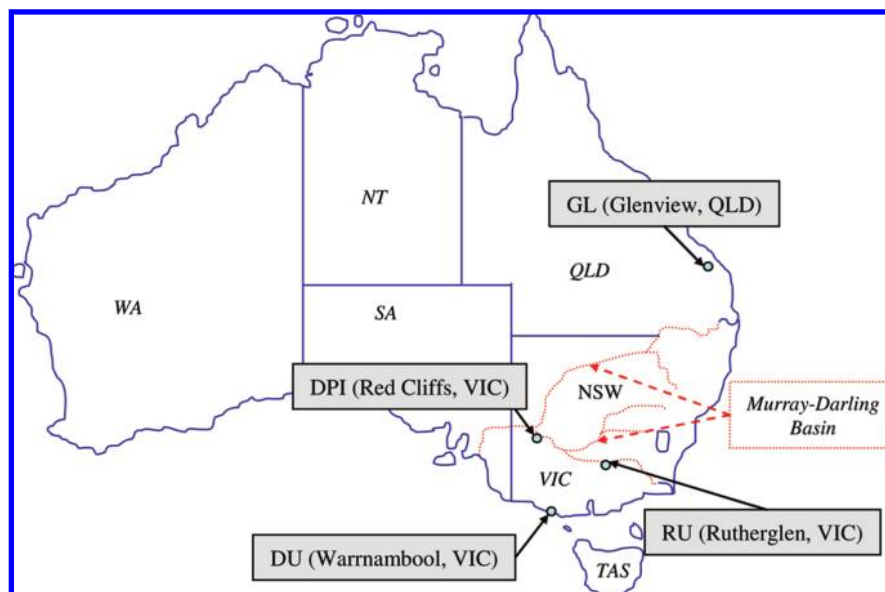
Figure 1. Schematic map of Australia with the location of the four Murray cod farms.

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and fish fillets were determined according to standard methods previously described (*17, 18*). Briefly, moisture was determined by drying samples in an oven at 80 °C to constant weight. The protein content was determined using an automated Kjeltech 2300 (Foss Tecator, Höganäs, Sweden). Lipid was determined by chloroform: methanol (2:1) extraction. The ash content was determined by incinerating samples in a muffle furnace (Wit, C & L Tetlow, Blackburn, Australia) at 550 °C for 18 h. Three subsamples of each commercial diet and the whole left fillet of each fish, denuded from the skin and finely minced and mixed, were used for the proximate analysis. All analyses were performed in triplicate.

Fatty acid analysis was performed on three subsamples of the commercial diets and on the individual right fillets, denuded from the skin and finely minced and mixed, from each farm. All analyses were carried out in triplicate as previously described (*17, 18*). Briefly, after lipid extraction, fatty acids were esterified into methyl esters using the acid-catalyzed methylation method, and fatty acid methyl esters were isolated and identified by gas chromatography. The resulting peak areas were corrected by theoretical relative FID (flame ionization detector) response factors and quantified relative to the internal standard. Fatty acids accounting for less than 10 mg per gram of lipid were omitted, and the final fatty acid composition was reported as a percentage (% w/w) of total fatty acids.

Stable Isotopes Analysis. Bulk isotope ratios $^{13}C/^{12}C$ and $^{15}N/^{14}N$ were determined in diets and fish fillets, and $^{18}O/^{16}O$ was determined in diets, fish fillets, and culture water by stable isotope ratio mass spectrometry (IRMS). The isotopic composition of a sample was reported accordingly to the standard δ notation. Subsamples of the commercial diets and fish fillets were freeze-dried and pulverized, while water samples were filtered (0.45 μm). The stable isotoped analysis was implemented at the Flinders Advanced Analytical Laboratory (Flinders University, Adelaide, SA) following standard procedures using a GV IsoPrime, stable isotope mass spectrometer (GV Instruments, Manchester, United Kingdom), and a EuroVector elemental analyzer (Milan, Italy). Standards certified by the International Atomic Energy Agency (IAEA, Vienna, Austria) were used, and the $\delta^{18}O$ on solid samples (diets and fillets) was performed as previously described by Stuart-Williams et al. (*19*).

Statistical and Chemiometric Analysis. Where appropriate, data are reported as means ± standard errors of means (SEM). After normality and homogeneity of variances were confirmed, one-way analysis of variance (ANOVA) was used to test for differences between means of the four farms for each variable. Differences were considered statistically significant at $P < 0.05$. Student–Newman–Keuls posthoc tests were used to separate groups after a significant ANOVA. Discriminant function analysis (DFA) was used to determine which variables were most effective at identifying the source of fish (i.e., among the four farms). As is typical of such data, the number of variables far exceeded the number of individual replicate fish, so DFA was not possible on the whole data set (*20*). The ratio of variables to replicate fish was reduced by (i) doing separate analyses for isotopes, morphological and proximate composition variables, and fatty acid variables and (ii) only including those variables that showed significant differences between the four farms in the one-way ANOVAs, to maximize the discriminatory ability of the analyses. As the main aim of the DFA was to identify those variables most important in distinguishing which farm the fish came from, it was assumed that those variables that were not significantly different between farms would not be good discriminators. All statistical analyses were performed using SPSS (SPSS Inc. Chicago, Illinois) v.14.0 for Windows.

RESULTS

Three out of the four commercial diets had similar proximate compositions with protein contents varying from 457 to 470 mg g^{-1} and lipid contents varying from 182 to 193 mg g^{-1} (**Table 2**). The RU diet was notably different because it was characterized by a very high protein content (532 mg g^{-1}) and very low lipid content (110 mg g^{-1}). DU and DPI diets had very similar fatty acid compositions with the three major classes

Table 2. Proximate (mg g^{-1}) and Fatty Acid (% w/w) Composition of the Commercial Diets Used in Four Different Murray Cod Farms

	farms[a]			
	DU	GL	RU	DPI
proximate composition (mg g^{-1})				
moisture	94.1	81.0	71.6	95.6
protein	470.0	464.9	532.4	457.9
lipid	182.2	193.9	110.7	191.0
ash	88.4	115.0	106.5	74.9
NFE[b]	165.3	145.2	178.9	180.7
fatty acid composition (% w/w)				
14:0	3.9	7.3	3.8	3.7
16:0	21.7	21.1	14.6	20.6
18:0	5.3	5.9	5.0	5.2
16:1n-7	7.1	9.3	4.8	5.7
18:1n-9	26.6	15.4	33.3	27.7
18:1n-7	3.4	3.5	4.0	3.3
20:1[c]	0.9	0.9	1.0	3.5
22:1[d]	0.6	0.8	0.3	2.3
16:2n-4	0.5	1.2	0.6	0.2
16:3n-4	0.7	1.5	0.6	0.4
18:2n-6	10.3	4.5	12.9	10.7
20:4n-6	0.7	1.0	0.6	0.6
18:3n-3	1.2	0.9	4.2	1.2
18:4n-3	1.3	1.7	0.9	1.0
20:5n-3	7.2	12.6	5.1	4.9
22:5n-3	0.9	1.8	1.1	0.9
22:6n-3	5.9	7.7	5.1	5.9
SFA	31.4	34.8	24.0	30.1
MUFA	38.6	29.9	43.5	42.6
PUFA	30.0	35.2	32.5	27.4
n-3 PUFA	16.8	25.4	16.9	14.5
n-4 PUFA	1.4	3.1	1.4	0.8
n-6 PUFA	11.8	6.7	14.2	12.1
HUFA	15.6	24.6	12.8	13.3
n-3 HUFA	14.4	22.8	11.7	12.3
n-6 HUFA	1.3	1.8	1.0	1.0

[a] See **Table 1** for farm descriptions. [b] NFE, nitrogen free extract calculated by difference. [c] Represents the sum of 20:1 isomers. [d] Represents the sum of 22:1 isomers.

of fatty acids, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA), and the principal individual fatty acids, oleic acid (OA; 18:1n-9), linoleic acid (LA; 18:2n-6), α-linolenic acid (ALA; 18:3n-3), arachidonic acid (AA; 20:4n-6), eicosapentaenoic acid (EPA; 20:5n-3), and docosahexaenoic acid (DHA; 22:6n-3), not showing noteworthy differences (**Table 2**). The GL diet was characterized by a relatively low content of OA and LA (typically derived from vegetable oils) and relatively high content of n-4 PUFA (namely, 16:2n-4, 16:3n-4, and 18:3n-4), EPA, and in general n-3 highly unsaturated fatty acids (HUFA; typically derived from marine fish oils). In contrast, the RU diet had a very high content of OA, LA, and MUFA and relatively low content of palmitic acid (PA; 16:0), EPA, and n-3 HUFA (**Table 2**).

No major variations in the stable isotope ratios of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ of the four diets were recorded (**Table 3**). However, DU and DPI diets (both produced by the same feed manufacturer) recorded $\delta^{13}C$ ratios of −22.9 and −22.1‰, respectively, while GL and RU diets (both produced by the other feed mill) recorded higher values of $\delta^{13}C$ ratios of −20.6 and −20.4‰, respectively. DU and DPI diets were characterized and found to have similar $\delta^{15}N$ ratios (7.3 and 7.7‰, respectively), while GL and RU diets differed from each other and recorded values of $\delta^{15}N$ ratios of 8.8 and 9.7‰, respectively. $\delta^{18}O$ ratios were almost constant across the four diets, varying from 23.5 to 24.2‰, while noteworthy differences were recorded in the water

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Table 3. Stable Isotope Ratios (δ^{13}C, δ^{15}N, and δ^{18}O) of Commercial Diets Fed to Murray Cod and the δ^{18}O Stable Isotope Ratios of Culture Water in Four Different Farms and the Stable Isotope Ratios (δ^{13}C, δ^{15}N, and δ^{18}O) in Muscle Flesh of Market Size Murray Cod Produced in the Four Different Farms

	farm[a]			
	DU	GL	RU	DPI
δ^{13}C ‰ (diet)	−22.9	−20.6	−20.4	−22.1
δ^{15}N ‰ (diet)	7.3	8.8	9.7	7.7
δ^{18}O ‰ (diet)	24.1	24.1	23.5	24.2
δ^{18}O ‰ (water)	−3.8	2.5	−4.8	−2.0

	fish[b]			
	DU	GL	RU	DPI
δ^{13}C ‰ (muscle)	−20.4 ± 0.08 c	−19.6 ± 0.07 b	−18.6 ± 0.12 a	−20.5 ± 0.31 c
δ^{15}N ‰ (muscle)	10.5 ± 0.19 a	12.6 ± 0.10 b	11.2 ± 0.07 a	10.5 ± 0.29 a
δ^{18}O ‰ (muscle)	15.4 ± 0.47 a	18.2 ± 0.13 c	15.0 ± 0.18 a	16.5 ± 0.15 b

[a] See **Table 1** for farm descriptions. [b] Values are reported as means ± pooled SEM ($N = 3$). Values with the same letter in each row are not significantly different ($P > 0.05$).

Table 4. Morphologic Data and Fillet Proximate Composition of Market Size Murray Cod Produced in Four Different Farms[a]

	farms[b]			
	DU	GL	RU	DPI
	morphologic data			
length (cm)	31.0 ± 0.58 a	38.7 ± 0.67 b	39.7 ± 0.97 b	34.2 ± 2.92 ab
TW (g)	636.0 ± 34.42	828.5 ± 59.91	792.0 ± 83.16	746.4 ± 250.47
SW (g)[c]	579.0 ± 32.58	755.7 ± 55.96	761.1 ± 79.81	653.0 ± 210.32
VW (g)	57.0 ± 1.98	72.8 ± 5.64	30.9 ± 3.47	93.5 ± 40.15
fillets weight (g)	322.2 ± 18.64	429.7 ± 33.07	392.9 ± 45.05	366.2 ± 132.04
K[d]	2.1 ± 0.10 c	1.4 ± 0.04 a	1.3 ± 0.04 a	1.7 ± 0.11 b
dress-out percentage[e]	91.0 ± 0.22 b	91.2 ± 0.53 b	96.1 ± 0.12 c	88.2 ± 1.08 a
fillet yield[f]	50.6 ± 0.44	51.8 ± 0.48	49.6 ± 2.17	48.3 ± 1.77
	fillet proximate composition (mg g^{-1})			
moisture	747.1 ± 5.07 a	753.1 ± 3.41 a	774.7 ± 7.21 b	742.4 ± 4.16 a
protein	199.8 ± 4.28	182.5 ± 1.61	189.7 ± 2.93	190.9 ± 8.01
lipid	42.2 ± 6.50 ab	53.2 ± 3.96 b	25.4 ± 7.90 a	55.4 ± 6.83 b
ash	10.9 ± 0.25	11.2 ± 1.45	10.2 ± 0.52	11.3 ± 0.17

[a] Values are reported as means ± pooled SEMs ($N = 3$). Values with the same letter in each row are not significantly different ($P > 0.05$). [b] See **Table 1** for farm descriptions. [c] SW = gutted carcass weight. [d] K = Fulton's condition factor = $100 \times$ TW $\times$ length^{-3}. [e] Dress-out percentage = $100 \times$ SW $\times$ TW^{-1}. [f] Fillet yield = $100 \times$ fillets weight $\times$ TW^{-1}.

sampling: −3.8, 2.5, −4.8, and −2.0‰ for DU, GL, RU, and DPI, respectively (**Table 3**).

The average fish weight varied from 636 to 828 g, but they were not statistically different between farms (**Table 4**). However, RU and GL fish were significantly longer than DU fish. No statistically significant differences were recorded for SW, VW, FW, and fillet yield. DU fish had the highest condition factor (K) (2.1), followed by DPI fish (1.7) and GL and RU fish (1.4 and 1.3, respectively). The dress-out percentage was significantly higher in RU fish (96.1%), followed by DU and GL fish, and the lowest value recorded (88.2%) was for DPI fish (**Table 4**). No statistically significant differences were recorded in the fillet protein and ash contents (**Table 3**). However, significantly lower lipid contents (25.4 mg g^{-1}) and significantly higher moisture contents (774.7 mg g^{-1}) were recorded in the fillet of RU fish as compared to the other farms.

δ^{13}C was significantly lower in RU fish (−18.6‰), followed by GL fish (−19.6 ‰) and by DU and DPI fish [−20.4 and −20.5‰, respectively (**Table 3**)]. GL fish recorded the highest δ^{15}N value (12.6‰), while δ^{18}O was significantly lower in DU and RU fish (15.4 and 15.0‰, respectively), followed by DPI fish (16.5‰) and GL fish (18.2‰) (**Table 3**).

Despite the fact that several statistically significant differences were recorded in the fatty acid composition of fish fillets from the different farms, the overall fatty acid makeup of different

fish fillets was relatively similar (**Table 5**). The 17 fatty acids that were present in the diets (accounting for more than 10 mg per g of lipid) were also identified in the muscle. The fatty acids found in highest amounts in Murray cod fillets, irrespective of the farm origin, were, in order, OA, PA, LA, DHA, and EPA. PA was significantly lower in GL and RU fish, while the fillets of DPI fish had a significantly higher level of OA as compared to the fillets of RU fish.

16:2n-4 was highest in GL fillet, and n-4 PUFA was significantly higher in GL and RU fish as compared to the others. No statistically significant differences were recorded in LA and MUFA contents. EPA, DHA, and AA were significantly lower in DPI fish, even though no statistically significant differences were recorded for the total n-3 HUFA and n-6 HUFA (**Table 5**).

For isotope and morphologic variables, the first discriminant function explained most of the variation in the data (**Table 6**) and best separated the four farms (isotopes: Pillai Trace $F_{9,24} = 7.3$, $P < 0.001$; morphological parameters: Pillai Trace $F_{15,18} = 2.5$, $P = 0.033$). δ^{18}O was the strongest discriminator among the isotopes, with δ^{13}C and δ^{15}N having smaller, but similar, correlations with the first discriminant function. Dress-out percentage and condition factor were the main variables contributing to the first discriminant function based on morphology, although correlations were low (<0.3).

Table 5. Fatty Acid Composition (% w/w) of Muscle Flesh of Market Size Murray Cod Produced in Four Different Farms[a]

	farms[b]			
	DU	GL	RU	DPI
14:0	3.6 ± 0.09 a	4.6 ± 0.08 b	3.6 ± 0.10 a	3.7 ± 0.25 a
16:0	20.4 ± 0.05 b	18.2 ± 0.23 a	18.5 ± 0.27 a	21.6 ± 0.76 b
18:0	4.7 ± 0.08 ab	4.4 ± 0.07 a	5.3 ± 0.29 b	4.7 ± 0.11 ab
16:1n-7	5.5 ± 1.18	6.7 ± 0.14	4.0 ± 1.13	5.9 ± 1.28
18:1n-9	26.8 ± 0.75 ab	27.3 ± 0.05 ab	25.1 ± 1.54 a	29.5 ± 0.74 b
18:1n-7	3.6 ± 0.27	4.0 ± 0.05	4.3 ± 0.12	4.2 ± 0.06
20:1[c]	1.0 ± 0.05 a	0.9 ± 0.06 a	1.2 ± 0.08 a	2.1 ± 0.35 b
22:1[d]	0.4 ± 0.05 a	0.3 ± 0.04 a	0.6 ± 0.13 ab	0.8 ± 0.12 b
16:2n-4	0.5 ± 0.06 ab	0.8 ± 0.02 c	0.6 ± 0.06 bc	0.3 ± 0.07 a
16:3n-4	0.5 ± 0.11	0.4 ± 0.06	0.6 ± 0.04	0.4 ± 0.01
18:2n-6	10.0 ± 0.32	9.0 ± 0.04	9.6 ± 0.53	10.4 ± 0.13
20:4n-6	1.2 ± 0.13 ab	1.0 ± 0.02 ab	1.4 ± 0.29 b	0.7 ± 0.12 a
18:3n-3	1.0 ± 0.04 a	2.5 ± 0.05 c	2.0 ± 0.17 b	1.1 ± 0.07 a
18:4n-3	0.9 ± 0.03	1.1 ± 0.02	1.0 ± 0.03	0.9 ± 0.08
20:5n-3	5.3 ± 0.18 b	5.1 ± 0.09 b	4.4 ± 0.49 ab	3.2 ± 0.70 a
22:5n-3	3.3 ± 0.18 b	3.2 ± 0.08 b	3.0 ± 0.22 b	2.1 ± 0.28 a
22:6n-3	8.2 ± 0.58 ab	7.5 ± 0.08 ab	11.1 ± 1.58 c	6.1 ± 1.23 a
SFA	29.3 ± 0.11 ab	27.6 ± 0.29 a	28.1 ± 0.62 ab	30.2 ± 0.89 b
MUFA	37.3 ± 1.26	39.2 ± 0.11	35.2 ± 2.59	42.5 ± 1.73
PUFA	33.4 ± 1.15 ab	33.2 ± 0.24 ab	36.7 ± 2.08 b	27.3 ± 2.13 a
n-3 PUFA	19.5 ± 0.97 ab	20.3 ± 0.11 ab	22.4 ± 2.13 b	14.1 ± 2.23 a
n-4 PUFA	1.2 ± 0.14 ab	1.6 ± 0.08 b	1.7 ± 0.15 b	0.9 ± 0.10 a
n-6 PUFA	12.7 ± 0.06 c	11.3 ± 0.10 a	12.7 ± 0.17 c	12.2 ± 0.09 b
HUFA	19.6 ± 1.12 ab	18.5 ± 0.06 ab	21.8 ± 2.60 b	13.5 ± 2.20 a
n-3 HUFA	17.5 ± 0.93	16.7 ± 0.05	19.4 ± 2.27	12.2 ± 2.17
n-6 HUFA	2.1 ± 0.25	1.8 ± 0.02	2.4 ± 0.39	1.4 ± 0.06

[a] Values are reported as means ± pooled SEMs ($N = 3$). Values with the same letter in each row are not significantly different ($P > 0.05$). [b] See **Table 1** for farm descriptions. [c] Represents the sum of 20:1 isomers. [d] Represents the sum of 22:1 isomers.

Table 6. Results of Discriminant Function Analyses (Based on 12 Fish, Three Replicate Fish from Each of Four Farms) for the Three Subsets of Variables, Only Including Those That Were Significantly Different among the Four Farms (ANOVA)[a]

	discriminant functions	
stable isotopes on fillets	1	2
% variation explained	**53.9**	**44.3**
$\delta^{18}O$ ‰	0.780	0.305
$\delta^{15}N$ ‰	0.436	0.817
$\delta^{13}C$ ‰	−0.450	0.729
morphologics and fillet proximate	1	2
% variation explained	**94.2**	**5.7**
dress-out percentage (%)	0.232	0.634
K (condition factor)	−0.240	0.357
fillet lipid (mg/g)	−0.068	−0.332
fillet moisture (mg/g)	0.133	0.218
fish length (cm)	0.126	−0.161
fillet fatty acids	1	2
% variation explained	**78.5**	**20.2**
16:0	0.142	0.057
20:1 isomers	0.067	0.165
22:5n-3	−0.054	−0.159
18:3n-3	−0.258	0.031
14:0	−0.063	−0.014
18:0	−0.020	0.035
22:1 isomers	0.046	0.140
16:2n-4	−0.118	−0.083

[a] Only the first two discriminant functions are presented, with loadings indicating correlations between each variable and each function.

Dress-out percentage also contributed strongly to the second but much less discriminating function (**Table 6**). The results for fatty acids need to be interpreted cautiously as the ratio of variables to replicate fish was still high and the analysis could not use all variables due to collinearity issues. Again, the first discriminant function explained most of the variability between the farms, although the second function was more important than for isotopes and morphological variables (**Table 6**). 18:3n-3 and 16:0 were most correlated with the first function and 20:1, 22:5n-3, and 22:1 in decreasing order for the second function. None of the correlations were strong (all < 0.3).

DISCUSSION

The potential effects of different farming methods on final product quality of farmed Murray cod involve modification of fish appearance (shape, morphological proportions, and coloration), the occurrence of off-flavor, and the modification of nutritional characteristics (*16, 17, 21*). As such, product differentiation has been identified as a fundamental issue for further development of the Murray cod industry (*16*). Murray cod are commonly sold into the market as "head-on, gilled and gutted" (HOGG) and/or whole/bled fish. External appearance is typically an indicator of product taste and overall quality and plays a very important role in consumers' purchasing behavior. The market size Murray cod from the four different farming systems were characterized by different morphological parameters, likely derived from different farming conditions (environmental and production) more than by different fish strains. Murray cod aquaculture is, indeed, a relatively new industry, and no genetically different fish strains have yet been developed, with almost all farmed fish being derived by a relatively limited captive progeny. However, it is also possible that, considering not all of the fish came from the same source or breeding pairs, different morphologies in the present trial could also be attributable to family inheritance. Previous work on similar size Murray cod reported Fulton's condition factor (K) values ranging from 1.4 to 1.8 (*21, 22*), and accordingly, GL, RU, and DPI fish recorded values within this range. However, fish produced at DU, which is a highly intensive/high stocking density recirculating aquaculture system, recorded a significantly higher

K value (up to 2.1). However, dress-out percentage and fillet yield for DU fish were similar to other sampled fish, and on the contrary, a significant lower dress-out percentage was recorded in DPI fish, which were farmed in relatively low stocking density flow-through system.

Fillet proximate composition can be affected by on-farm feeding management and feeding rate (*23*). The culture system can also influence fillet proximate composition although little information is available in this regard. Higher fillet lipid deposition was reported in tank-reared sharpsnout sea bream (*Diplodus puntazzo*) as compared to cage reared fish (*24*), and modification of fillet lipid deposition was also reported in rainbow trout (*Oncorhynchus mykiss*) farmed in various locations and differently managed intensive flow trough systems (*12*). In the present study, despite the high condition factor of DU recirculation aquaculture system (RAS) reared fish, their fillets were characterized by lower levels of lipid as compared to GL RAS and DPI cage fish, all of which were fed with diets of very similar nutritional composition. Thus, fillet lipid composition appears to be influenced by both feeding regime and culture system. In addition to feed management and farming conditions, diet composition is well-known to be the primary factor affecting fillet proximate composition (*14*). Accordingly, RU fish, which were fed with a diet formulated for Australian native freshwater species, with a high protein and low lipid content, had a significantly lower fat content in their fillets as compared to all other fish, which were fed with commercial diets formulated for barramundi (higher lipid and energy content). A significantly higher moisture content was also recorded, as lipid and moisture are inversely related (*14*). It is known that a modification of fat and moisture level in fish fillets can affect sensorial characteristics (*25*) and liquid-holding capacity (*26*). While this can be seen as a positive characteristic (leaner food) from a consumer point of view, it is important to emphasize that a consumer eating a portion of Murray cod fillet derived from the RU farm will also be receiving significantly less of the beneficial and health-promoting n-3 HUFA. However, by observing the fillet fatty acid composition expressed as percentage of total fatty acids, RU fish recorded the highest DHA level. A possible explanation for this might be that in the total lipid fraction of the fillet of RU fish, which were fed the leanest diet and, hence, the fillet contained significantly less fat, a relatively higher portion of membrane lipids was occurring. In fact, it is known that fish membrane lipids contain a higher proportion of phospholipids, as compared to storage lipids, which are richer in triacylglycerols, and phospholipids usually contain higher amount of DHA as compared to triacylglycerols (*27*).

It is now well-accepted that fatty acid composition of fish tissues is primarily affected by the dietary fatty acid composition (*25, 27*), and this has also been confirmed in the Australian native Murray cod (*17, 28, 29*). However, incorporation of fatty acids into fish tissue is also under various metabolic influences such as preferential incorporation, β-oxidation, lipogenic activity, or fatty acid elongation and desaturation processes (*18, 27*), all of which are likely influenced by growth stage, culture system, and general environmental conditions (*27*) and thus may somewhat confound dietary and fillet fatty acid correlations. The fatty acid composition of formulated commercial aquafeed is commonly very different from the natural available food sources in aquatic environments, and as such, the tissue fatty acid composition has been used widely to discriminate farmed and wild-caught fish (*2, 8, 9, 30*). In situations where diets contain markedly different fatty acid profiles, it has also been

possible to distinguish among fish cultured in different systems (intensive, semi-intensive, or extensive) (*12, 30–33*). However, the fatty acid composition of fish farmed in different systems and fed commercial diets with similar fatty acid profiles is likely to be an ineffective discrimination tool. In the present study, the fatty acid composition of Murray cod fillets was quite similar, and none of the individual fatty acids were strongly correlated with the discriminant functions separating farms. Thus, in common with studies on other species, the fillet fatty acid makeup does not appear to be a particularly good discriminator of the origin of farmed Murray cod but, rather, appears to be more reflective of diet than system.

DU and DPI diets were produced by the same feed mill company and had a very similar fatty acid composition. GL and RU diets, even though produced by the same feed mill company, were two differently formulated diets (one developed for barramundi and the other for Australian native freshwater species), and their fatty acid composition was slightly different. The fatty acid composition of aquafeed is affected by the lipid source utilized in the formulation and by the fat content of other raw materials (*27*). The RU diet had a relatively low fat content, and its fatty acid composition was likely largely affected by the vegetable materials used in the formulation as the contents of OA and LA (two typical fatty acids of vegetable products) were relatively high as compared to the other diets. On the other hand, the GL diet, because of the very limited content of LA and high content of EPA, likely contained high amounts of lipid source derived from marine origin raw materials. These relatively minor differences in dietary fatty acid composition were only partially reflected in the fatty acid makeup of fillets, not surprisingly given the other potential influences on fillet fatty acid fillet composition mentioned above. In addition, it is common practice to shift from one diet to another according to temporary availability, feed cost, and growth stage of fish, and such variable feeding history likely influences the overall fatty acid profile, thereby potentially further confounding inferences that can be made by analyzing only the final grow-out diet. Indeed, in the present study, although farmers confirmed that fish had been fed for at least the previous 3 months on the diet analyzed, the fillet fatty acid compositions recorded indicated that fish had been previously fed with different diets or different batches.

Interestingly, the GL diet had a relatively high content of n-4 PUFA. These fatty acids, and in particular 16:2n-4, are known to be readily synthesized by some species of marine photosynthetic algae (*34*) and hence can be transferred up the trophic chain to aquatic animals, which are used in the production of aquafeed. Thus, it might be that the GL diet was formulated with raw materials, particularly fish oil, of different origin as compared to other diets, which subsequently resulted in fillets containing a higher content of these fatty acids. Thus, individual fatty acids in fish flesh may provide some level of feed ingredient discrimination. However, RU fish also showed a relatively high content of n-4 PUFA, confirming that those fish were probably previously fed with a different diet or different feed batch.

Given the results of the DFA and the various potential influences on fillet fatty acid composition, it is evident that using fatty acid composition for product discrimination is problematic. Furthermore, the large number of fatty acid classes requires analysis of large numbers of replicate fillet samples, and this poses logistical and financial issues. Nevertheless, some specific fatty acids, particularly those that are only derived from the diet and undergo little/no in vivo bioconversion, such as the

n-4 fatty acids, can be a useful and powerful discriminator of dietary ingredient source, but a high number of samples must be analyzed.

Stable isotopes have been used in biological and ecological investigations for many years, such as in trophic food studies. Recently, they have also been successfully used to distinguish among cultured and wild fish, as the natural food and commercial diets are characterized by different isotopic ratios (*2, 8*). In particular, $\delta^{13}C$ and $\delta^{15}N$, being derived directly from the diet, are extremely valuable in linking a specified animal to a particular food source. In the present study, the $\delta^{13}C$ analysis of the four commercial diets revealed that Skretting Aquafeeds (fed to DU and DPI) were isotopically lighter than Ridley aquafeeds (fed to GL and RU), likely due to the utilization of similar raw materials within each feed mill company for the production of the different aquafeed batches. Accordingly, there were significant differences among Murray cod fillets fed different commercial diets, with Murray cod from DU and DPI farms being isotopically lighter than fish originating from GL and RU farms. There was a significant correlation between the $\delta^{13}C$ value of the diets and of the fish fillets ($\delta^{13}C$ fish = 0.6359 $\delta^{13}C$ diet −6.1022; R^2 = 0.75; p < 0.05). A different pattern was recorded for $\delta^{15}N$ ($\delta^{15}N$ fish = 0.5130 $\delta^{15}N$ diet +6.90.3; R^2 = 0.32; p > 0.05), with DU and DPI fish receiving lighter isotopic ratios showing a lighter isotopic ratio in the flesh, while GL fish recorded the heaviest $\delta^{15}N$ isotopic ratio as compared to RU fish, although the GL diet was lighter than RU diet.

In water, $\delta^{18}O$ is known to be affected by evaporation rate and precipitation and consequently is influenced also by temperature and therefore, in surface water, by season, latitude, and elevation (*35*). As such, water characterized by different $\delta^{18}O$ ratios, entering the food web, can affect the $\delta^{18}O$ of living organisms. No important differences were noted in $\delta^{18}O$ value of different commercial diets, while marked variability of $\delta^{18}O$ value was recorded in the water of the different farms. This variability can be easily explained by the fact that the four farms were located at different latitudes and longitudes but also were using water from different origins (bore, earthen ponds, or river). Moreover, the evaporation rate on farms was likely to be significantly different considering that DU and GL were indoor recirculating systems, RU was a flow-through system, and DPI was an outdoor cage farm located in a water reservoir. As a result of this significant variation in $\delta^{18}O$ in the water of different farms, significant differences were recorded in $\delta^{18}O$ of fish fillet. Of all of the variables measured in this experiment, $\delta^{18}O$ had the strongest correlation with a discriminant function separating fish from the four farms. Furthermore, an almost perfect linear relationship was observed between $\delta^{18}O$ value in fish fillet and in culture water ($\delta^{18}O$ fish = 0.4407 $\delta^{18}O$ water +17.168; R^2 = 0.99; p < 0.05). Both of these results suggest that $\delta^{18}O$ will be an extremely informative parameter for origin identification in farmed fish.

The DFA used to determine which variables were the best discriminators of fish from the four farms is a commonly implemented statistical approach previously used for the purposes of discriminating between farmed and wild fish (*2, 8, 11, 30*). While the results of our analyses were informative for isotope ratios and they suggested that none of the morphological or fatty acid variables were useful discriminators between the four farm sources of fish, the latter results need to be interpreted cautiously. DFA is based on correlations between variables that explain most of the variation between groups and therefore relies on the ratio of variables to replicate animals not being too high. For morphological and fatty acid characteristics, the number of

variables will nearly always exceed the number of fish that can be realistically harvested and analyzed. Future work on discriminating between different sources of farmed aquatic animals will need to consider alternative statistical methods, such as those based on dissimilarities between fish rather than on correlations between variables.

In summary, the present study showed that stable isotopes are informative variables that can be useful in distinguishing among fish produced in different farms. In particular, $\delta^{13}C$ and/ or $\delta^{15}N$ can clearly link a fish product to a specific commercial diet, while $\delta^{18}O$ is useful for linking a fish product to a specific water source, and their combination can therefore distinguish among fish originating from different farms. On the other hand, fatty acid composition and other variables such as morphological parameters and tissue proximate composition, which have been proven to be useful to distinguish farmed and wild fish, seem to be less informative in discriminating among farmed products.

ABBREVIATIONS USED

AA, arachidonic acid; ALA, α-linolenic acid; DFA, discriminant function analysis; DHA, docosahexaenoic acid; DPI, Department of Primary Industry farm; DU, Deakin University farm; EPA, eicosapentaenoic acid; GL, Glenview farm; HUFA, highly unsaturated fatty acids; LA, linoleic acid; MUFA, monounsaturated fatty acids; OA, oleic acid; PA, palmitic acid; PUFA, polyunsaturated fatty acids; RAS, recirculation aquaculture system; RU, Rutherglen farm; SFA, saturated fatty acids.

ACKNOWLEDGMENT

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