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Effects of Starvation and Water Quality on the Purging Process of Farmed Murray Cod (*Maccullochella peelii peelii*)

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The aim of this study was to determine the effects of starvation and water quality during the purging process on the biometric parameters, fatty acids, and flavor volatiles of Murray cod farmed in a recirculation system. Market size Murray cod, at the end of the grow-out stage, were divided into eight treatments. The treatments were either fed/starved (F or S) and kept in clean water (CW: CWF2, CWS2, CWF4, and CWS4) or fed/starved and kept in recycled water (RW: RWF2, RWS2, RWF4, and RWS4) for either 2 or 4 weeks. Fish were sampled at 0, 2, and 4 week intervals. Food deprivation was responsible for a significant ($P < 0.05$) weight loss compared to that of fed treatments. The same was observed for the condition factor (K), hepatosomatic index (HSI), and dress-out percentage (DP). No significant changes were, however, observed in the visceral fat index (VFI). Saturated fatty acids (SFA) were highest in RWF4 and lowest in CWS4 ($P < 0.05$), while monounsaturated fatty acids (MUFA) were lowest in CWF4 ($P < 0.05$). Starvation did not affect the flavor volatile compounds, which were mainly affected by changes in water quality. Specifically, total aldehyde (% w/w) content was significantly ($P < 0.05$) affected by water quality, but the time of purging was not responsible for any noteworthy differences. This study was able to separate the effects of starvation and water quality, in the purging process, on the final eating quality of farmed market size Murray cod. It is concluded that because of the inevitable weight loss during starvation, Murray cod should be fed during the purging stage but kept in clean water and deprived of food only for the time necessary to empty the gastro-intestinal tract.

KEYWORDS: Aquaculture; fatty acids; fish; off-flavor; SPME; GCMS; volatile compounds

INTRODUCTION

Purging is a common practice in aquaculture to ensure that the product reaches the market with an empty gastro-intestinal tract and without off-flavors (*1*).

The duration of purging is dependent on many factors, mainly the species cultured, the nature of the off-flavor contaminants, intensity of the off-flavor, culture method, and environmental conditions (*2*). Usually, fish intensively reared at high stocking density require more time to be purged compared to fish kept at low stocking densities in semi-intensive or extensive environments. This is mainly due to the fact that under intensive rearing conditions degraded water quality due to over accumulation of feed and nutrients from fecal matter, can play a major role in contributing unpleasant taints to the flesh (*3*). However, it is also possible that in extensive pond conditions, environmental contaminants, namely, geosmin and 2-methylisoborneol (MIB), can seriously affect the marketability of the product (*4*).

The purging process commonly involves moving market sized fish to clean water and starving them from a few days to many weeks (*5*) before they are processed and packaged, or transported live. In more recent years, a longer term purging up to 60 days and associated longer starvation has also been adopted to stabilize and improve the flesh quality (*6, 7*). In fact, starvation can also be considered a conditioning technique as it enhances the biochemical and microbial storage stability of the carcass. By reducing the amount of feces in the intestine, spoilage is delayed, and digestive enzyme activity is reduced. If further processing steps are considered, e.g., filleting and freezing, an interruption in the feeding before slaughter may be a determinant factor of product shelf life (*8*). Furthermore, purging can also

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improve the overall final nutritional qualities of farmed fish by reducing excessive fat deposition and increase in the percent content of the health-promoting long chain omega-3 polyunsaturated fatty acids (*7, 9*).

A drawback of purging procedures is that, due to feed deprivation, there is an inevitable weight loss (*1, 9*), and under some circumstances, potential increased aggressive behavior can lead to external damage (such as fin and skin abrasions, which can be responsible for depreciation at the market) or even cannibalism and death.

Murray cod, *Maccullochella peelii peelii* (Mitchell), is the largest Australian native, freshwater, carnivorous, warm water fish. Currently, Murray cod supports a small but growing aquaculture industry within Australia (*10*), with a production of 87.2 tons valued at just over $1.4 million in 2005/2006 (*11*). Most of this production is from indoor, controlled-environment, recirculating aquaculture systems (RAS) and is sold domestically.

Palmeri et al. (*9*) found that Murray cod, on average, lose between 4% and 9% body weight after 2 and 4 weeks of purging, respectively. It was also found that, following a consumer test, Murray cod purged for 2 weeks were preferred to unpurged fish and not dissimilar to fish purged for 4 weeks. During this study, protein and hepatic reserves, not fat, either body or perivisceral, were affected by the purging procedures.

As described previously, commonly implemented and studied purging procedures involve a combination of starvation and transfer of fish into clean water. No studies, to date, have investigated the individual and combined effects of feed restriction and water quality on the purging process. It is possible that by limiting starvation time to 2−3 days while maintaining good water quality, acceptable edible qualities are maintained without major weight losses. This study was therefore designed to determine if Murray cod can be purged in clean water without undergoing a prolonged regime of feed restriction past the time necessary to empty the gut.

MATERIALS AND METHODS

Purging Facility. Two recirculating facilities were used for the purpose of this trial. The first one was the commercial production facility in which fish have been previously grown, designed to produce 20 tons of Murray cod per year. The system is fitted with microbead biofiltration, UV sterilization, and a 40 μm drum filter for collection and waste disposal. All fish were housed in a 2500 L tank during the grow-out phase and then transferred, within the same system, into 6 circular 600 L tanks for the experimental purging period.

The second system was a RAS purging system designed to hold 1 ton of fish at any one time. The purging set up consists of 6 circular 600 L tanks, part of a 15 tank recirculating system with a total volume of approximately 15, 000 L fitted with trickling biofiltration and sand filter for waste collection and removal. The water (dechlorinated town water) was exchanged at a rate of >2,000 L day^{-1}. Salinity was kept between 2 and 4 g L^{-1} and temperature at ∼17 °C. All the other water quality parameters for both systems were adequate to the culture of this species (*12*) and are reported in **Table 1**.

Experimental Fish and Sampling Procedures. Average market size fish (534.1 ± 16.3 g) were sourced from the stock of the intensive RAS facility located at Deakin University, Warrnambool, Australia. During the final stages of the grow-out period, the fish were fed a commercially extruded diet (Classic, Skretting, Tasmania, Australia; moisture = 86.7 ± 0.3 mg g^{-1}; crude protein = 419.7 ± 0.1 mg g^{-1}; total lipid = 187.3 ± 0.0 mg g^{-1}; ash = 76.7 ± 0.3 mg g^{-1}; energy = 20.5 kJ/g).

Individual weight and length of harvested fish were determined to the nearest g and cm, respectively, and 40 fish per tank were allocated to 12 purging tanks (three tanks per treatment).

At the beginning of the trial fish were divided into two groups and randomly allocated to 12 tanks, 6 in the commercial facility and 6 in the purging facility and treatments designed as follows. Purging facility: CWF2/CWF4 = clean water and fed for 2 weeks/4 weeks (N = 3); CWS2/CWS4 = clean water and starved for 2 weeks/4 weeks (N = 3). Commercial facility: RWF2/RWF4 = recycled water and fed for 2 weeks/4 weeks (N = 3); RWS2/RWS4 = recycled water and starved for 2/4 weeks (N = 3).

Table 1. Water Quality Parameters for Systems Using Recycled and Clean Water

water quality parameter	recycled water	clean water
temperature °C	22.6	17.1
pH	7.8	6.9
DO[a] (mg L^{-1})	7.7	8.8
NH_3-N (mg L^{-1})	0.5	0.5
NO_2-N (mg L^{-1})	2.5	1
NO_3-N (mg L^{-1})	50	30
alkalinity (mg L^{-1})	430	86
BOD[a] (mg L^{-1})	10	0
salinity (gL^{-1})	0	3
total P (mg L^{-1})	3	0.05
turbidity (NTU)	18	0
TSS[a] (mg L^{-1})	17.2	0
TDS[a] (mg L^{-1})	1400	2750

[a] See Abbreviations Used.

Treatments that were not undergoing starvation were fed (2% b/w day^{-1}) a Skretting Classic diet by means of 12 h belt feeders. Fish were sampled at the beginning of the trial (time zero) and at weeks 2 and 4. Fish sampled at time zero were not starved, and they were regularly fed the day before culling. Three fish per replicate tank were harvested, transferred to a drum containing ice slurry, and subsequently culled by cutting the main arterial vessel in the throat and left in the ice slurry until no movement was observed. They were then removed from the ice, dried with a paper towel, gutted, gilled, filleted, and frozen at −20 °C until needed for analysis. All procedures used were approved by the Deakin University Animal Welfare Committee.

Biometric and Growth Parameters. The main biometric parameters including total weight (TW), total length (TL), somatic weight (SW), liver weight (LW), fillet weight (FW), and perivisceral fat weight (PFW) were recorded (all weights were in g and length in cm).

The following parameters were also calculated: Fulton's condition factor, K = (TW ÷ L^3) × 100; hepatosomatic index, HSI (%) = (LW ÷ TW) × 100; visceral fat index, VFI (%) = (PFW ÷ TW) × 100; dress-out percentage, DP (%) = (SW ÷ TW) × 100; and fillet yield, FY (%) = (FW ÷ TW) × 100.

Growth or weight decrease during the experimental period was measured with the computation of the following parameters: food conversion ratio, FCR, dry food fed (g) ÷ increase in wet biomass (g); weight gain percent, (final weight − initial weight) ÷ (initial weight) × 100; and specific growth rate, SGR, $(\ln_{w2} - \ln_{w1}) \div (t_2 - t_1) \times 100$, where w_2 and w_1 were the weight in grams at time t_2 (end of trial) and t_1 (start of trial), respectively.

Water Quality Parameters. The principal water quality parameters such as temperature, pH, dissolved oxygen (DO), ammonia-N, nitrite-N, nitrate-N, alkalinity, biological oxygen demand (BOD), salinity, total phosphorus, turbidity, total suspended solids (TSS), and total dissolved solids (TDS) were assessed on the water of both systems and analyzed by the Deakin University Water Quality Laboratory (NATA accredited) using standardized methodology routinely implemented in the laboratory.

Fatty Acid Analysis. The quantification of fatty acids was conducted as previously reported in our laboratory (*9, 13*). Briefly, after extraction with chloroform/methanol (2:1v/v) (*14*) as modified by Ways and Hanahan (*15*), fatty acids were esterified into methyl esters using the acid catalyzed methylation method (*16*) and followed by the methods previously used in the laboratory. The internal standard used was 23:0 (Sigma-Aldrich, Inc., St. Louis, MO, USA), and fatty acid methyl esters were isolated and identified using a Shimadzu GC 17A (Shimadzu, Chiyoda-ku, Tokyo, Japan) equipped with an Omegawax 250 capillary column (30 m × 0.25 mm internal diameter, 0.25 μm film thickness, Supelco, Bellefonte, PA, USA), a flame ionization detector (FID), a

Shimadzu AOC-20i auto injector, and a split injection system (split ratio 50: 1). The temperature program was 150 to 180 at 3 °C min^{-1}, then from 180 to 250 at 2.5 °C min^{-1}, and held at 250 °C for 10 min. The carrier gas was helium at 1.0 mL min^{-1}, at a constant flow. Each of the fatty acids was identified relative to known external standards. The resulting peaks were then corrected by the theoretical relative FID response factors (*17*) and quantified relative to the internal standard.

Flavour Volatile Compounds and Off-Flavor Analysis. A 50 g sample of thoroughly homogenized Murray cod fillet was placed in a 100 mL modified Pyrex bottle fitted with a Shimadzu predrilled rubber septum. The bottle was placed in a water bath and heated to 70 °C. When the temperature was reached, a preconditioned 100 μm polydimethylsiloxane (PDMS) SPME fiber (Supelco) was manually inserted into the Pyrex vial and exposed to the headspace for 60 min. This solid-phase microextraction procedure has been chosen after modification of previously reported conditions (*18*) and several optimizations trials implemented within the laboratory.

The fiber was then withdrawn from the sample and immediately desorbed at 270 °C in the injection port of an HP6890 gas chromatograph equipped with a 5973 mass selective detector (Agilent Technologies, Palo Alto, CA). The fiber was left inside the injection port during the entire run to allow removal of all possible residues before the next analysis. The injection port was operated in splitless mode. The head pressure was set to 30 psi of helium for 1 min and then to a constant velocity of 33 cm s^{-1} for the remainder of the GC run. The initial oven temperature was set at 40 °C for 3 min, then ramped to 200 at 5 °C min^{-1}, and finally ramped to 250 at 50 °C min^{-1} for a total run time of 40 min. A BPX5 capillary column (30 m × 0.25 mm i.d., 0.25 μm thickness) was used in this study. Each sample was extracted and injected twice, in triplicate, for flavor volatile compounds and for specific geosmin and MIB quantification. The GC was operated in full scan mode for flavor volatile compounds and in selective ion monitoring (SIM) mode for detection and identification of geosmin and 2-methyisoborneol (MIB). Ions at *m/z* 112, 126, and 182 were monitored for geosmin, and *m/z* 95, 135, and 168 were monitored for MIB. Identification of compounds was based on mass spectra from library databases (NIST 98, WILEY 275) and known external standards. Data were recorded and analyzed with the Agilent Chemstation Software. The data were calculated as percentage of the total volatile compounds. Because of the different kinetics and partitioning coefficients of different analytes onto the SPME fiber, these analyses were mainly aimed to determine differences between samples rather than comparing different analytes within the same sample.

Statistical Analysis. Data are reported as the mean ± SEM (n = 3). After normality and homogeneity of variance were confirmed, one way analysis of variance (ANOVA) was used to determine differences between means and the multivariate general linear model (GLM) to separate the effects of feeding regime, water, and interaction of the two for data relative to growth, fatty acid, and volatile compounds analysis. Differences were considered statistically significant at $P < 0.05$. Data were subject to Duncan's post hoc test where differences were detected for homogeneous subsets. All statistical analyses were performed using SPSS (SPSS Inc. Chicago, Illinois) v.15 for Windows.

RESULTS

Water Quality Parameters. Nitrite, nitrate, alkalinity, BOD, total phosphorus, turbidity, and TSS were higher in the recycled water (**Table 1**). The high TDS value in the clean water was due to the salt used for primary prophylaxis, while dissolved solids in the recycled water entirely originated from leached uneaten food and feces.

Biometric and Growth Parameters. During the experimental purging, starved Murray cod lost weight. All starved treatments had a significant ($P < 0.05$) reduction in body weight compared to the start of the trial, while fed fish, apart from RWF2, significantly gained weight (**Table 2**).

The same trend ($P < 0.05$) was observed for the condition factor (K), the hepatosomatic index (HSI), and dress-out percentage (DP). The fed treatments showed no differences

Table 2. Biometry and Growth Data (Mean ± SEM) Relative to the Purging Process in Commercial Size Murray Cod[a]

	0 W Purging	2 W purging[b]				4 W purging[b]			
biometry	initial	CWS2	CWF2	RWS2	RWF2	CWS4	CWF4	RWS4	RWF4
initial weight (g)	561.5 ± 17.2	557.0 ± 3.0	557.9 ± 5.0	561.1 ± 10.5	570.4 ± 7.2	557.0 ± 3.0	557.9 ± 5.0	561.1 ± 10.5	570.4 ± 7.2
final weight (g)	561.5 ± 17.2	498.6 ± 3.8*	581.0 ± 4.0*	518.2 ± 11.2*	594.1 ± 7.6	482.6 ± 6.9*	604.0 ± 1.5*	485.7 ± 9.3*	601.7 ± 8.7*
somatic weight (g)	475.9 ± 15.9 abc	473.5 ± 31.5 abc	504.9 ± 30.9 abc	478.4 ± 22.8 abc	527.9 ± 28.6 bc	441.1 ± 30.0 ab	509.7 ± 32.5 bc	404.7 ± 20.5 a	566.7 ± 33.4 c
liver weight (g)	8.0 ± 0.9 bc	4.9 ± 0.5 a	7.4 ± 1.0 b	4.0 ± 0.3 a	10.6 ± 1.1d	3.5 ± 0.1 a	9.9 ± 0.7 cd	3.4 ± 0.2 a	11.3 ± 0.7d
VFI[c]	3.2 ± 0.5	3.8 ± 0.4	3.7 ± 0.8	3.6 ± 0.6	3.8 ± 0.5	3.0 ± 0.4	3.2 ± 0.4	3.0 ± 0.3	3.7 ± 0.4
K[c]	1.6 ± 0.0d	1.4 ± 0.0 bc	1.5 ± 0.0 cd	1.4 ± 0.0 bc	1.5 ± 0.0 cd	1.3 ± 0.0 a	1.5 ± 0.1 cd	1.3 ± 0.0 ab	1.6 ± 0.0d
HSI[c]	1.5 ± 0.1 bc	1.0 ± 0.1 a	1.3 ± 0.2 b	0.8 ± 0.0 a	1.8 ± 0.1 c	0.8 ± 0.1 a	1.8 ± 0.2 c	0.8 ± 0.0 a	1.8 ± 0.1 c
FY (%)[c]	38.8 ± 26.5	38.5 ± 0.7	38.2 ± 1.2	38.7 ± 0.5	38.0 ± 0.5	38.9 ± 0.6	36.9 ± 2.8	38.8 ± 0.7	40.1 ± 0.4
DP (%)[c]	90.2 ± 0.1 a	92.1 ± 0.4 bc	91.5 ± 0.6 abc	92.5 ± 0.7 c	90.1 ± 0.9 a	92.6 ± 0.4 c	90.4 ± 0.4 ab	93.0 ± 0.4 c	90.4 ± 0.5 ab
				Growth					
FCR[c]			0.80 ± 0.0 a		0.8 ± 0.0 a		1.2 ± 0.0 c		1.0 ± 0.1 b
weight gain (%)[c]		−10.5 ± 0.6 b	4.1 ± 0.3 d	−7.6 ± 0.3 c	4.2 ± 0.5 d	−13.4 ± 0.8 a	8.3 ± 0.7 e	−13.4 ± 0.9 a	5.5 ± 0.2 d
SGR[c]			0.3 ± 0.0 b		0.3 ± 0.0 b		0.3 ± 0.0 b		0.2 ± 0.0 a

[a] Values with the same lower case letter in each row are not significantly different ($P > 0.05$). An asterisk (*) after the final weight indicates a statistically significant difference ($P < 0.05$) compared to the initial weight of the fish. [b] Treatments abbreviations: CWS2, clean water starved for 2 weeks; CWS4, clean water starved for 4 weeks; RWS2, recycled water starved for 2 weeks; RWS4, recycled water starved for 4 weeks; CWF2, clean water fed for 2 weeks; CWF4, clean water fed for 4 weeks; RWF2, recycled water fed for 2 weeks; RWF4, recycled water fed for 4 weeks. [c] Refer to Abbreviations Used.

Table 3. Effects of Feeding Regime, Water Quality, and Time on the Growth, Body Composition, and Recovery Indices of Murray Cod[a]

	feeding regime		water		time		feeding regime × water		feeding regime × time		water × time		feeding regime × water × time	
	F value	*P*	*F* value	*P*	*F* value	*P*	*F* value	*P*	*F* value	*P*	*F* value	*P*	*F* value	*P*
weight	16.206	***	0.391	ns	1.504	ns	1.993	ns	3.260	ns	0.036	ns	0.579	ns
somatic weight	13.238	**	0.321	ns	1.536	ns	1.689	ns	3.057	ns	0.007	ns	0.775	ns
liver	150.816	***	3.531	ns	4.450	*	8.874	*	7.685	ns	0.193	ns	1.741	ns
VFI	0.628	ns	0.059	ns	1.582	ns	0.330	ns	0.507	ns	0.247	ns	0.037	ns
fillet	10.576	**	0.872	ns	0.731	ns	2.747	ns	2.710	ns	0.068	ns	1.278	ns
HSI	137.501	***	1.1582	ns	2.328	ns	5.114	*	5.367	*	0.301	ns	4.322	*
K	32.564	***	2.770	ns	0.932	ns	0.104	ns	7.434	**	0.463	ns	0.009	ns
DP	22.980	***	0.137	ns	0.003	ns	1.751	ns	1.288	ns	0.808	ns	0.616	ns
FY	0.302	ns	1.027	ns	0.360	ns	0.874	ns	0.001	ns	0.941	ns	1.354	ns

[a] Asterisks denote the level of significance: *P* *** <0.001, ** <0.01, and * <0.05; ns, not significant.

Table 4. Total Lipid (% of Wet Weight) and Fatty Acid Composition (% on Total Fatty Acids) (Mean ± SEM) of Starved and Fed Murray Cod during the Trial[a]

	0 W purging	2 W purging				4 W purging			
	initial	CWS2	CWF2	RWS2	RWF2	CWS4	CWF4	RWS4	RWF4
total lipid %	4.5 ± 0.0 a	4.4 ± 0.0 a	4.5 ± 0.1 a	4.3 ± 0.0 a	5.3 ± 0. 1 bc	4.8 ± 0.4 ab	4.5 ± 0.2 a	5.9 ± 0.0d	5.7 ± 0.0 cd
					Fatty Acid %				
14:0	4.5 ± 0.0d	4.1 ± 0.0 abc	3.9 ± 0.0 a	4.2 ± 0.0 bc	4.1 ± 0.0 abc	4.0 ± 0.1 ab	4.2 ± 0.2 abc	4.3 ± 0.1 c	4.2 ± 0.0 bc
16:0	19.9 ± 0.1	19.7 ± 0.2	19.9 ± 0.0	20.0 ± 0.0	19.8 ± 0.1	19.0 ± 0.5	20.1 ± 1.0	19.5 ± 0.0	20.3 ± 0.0
16:1 *n*-7	6.9 ± 0.0	6.5 ± 0.0	6.4 ± 0.0	6.5 ± 0.0	6.4 ± 0.0	6.6 ± 0.0	6.4 ± 0.0	6.6 ± 0.1	6.7 ± 0.1
18:0	4.7 ± 0.1 c	4.6 ± 0.1 bc	4.6 ± 0.0 bc	4.6 ± 0.0 bc	4.7 ± 0.0 c	4.2 ± 0.1 a	4.6 ± 0.1 bc	4.4 ± 0.2 ab	4.6 ± 0.0 bc
18:1 *n*-9	22.8 ± 0.5 a	24.1 ± 0.1 ab	25.4 ± 0.1 b	24.3 ± 0.1 ab	24.7 ± 0.2 b	24.9 ± 0.0 b	25.0 ± 1.4 b	24.9 ± 0.0 b	25.0 ± 0.1 b
18:1 *n*-7	3.6 ± 0.1 ab	3.4 ± 0.1 a	3.4 ± 0.0 a	3.5 ± 0.1 ab	3.4 ± 0.1 a	3.7 ± 0.1 b	3.6 ± 0.1 ab	3.5 ± 0.2 ab	3.4 ± 0.0 a
18:2 *n*-6	8.0 ± 0.1 a	9.1 ± 0.1 b	9.6 ± 0.0 bc	9.3 ± 0.0 bc	9.4 ± 0.0 bc	9.5 ± 0.0 bc	9.7 ± 0.4 c	9.6 ± 0.0 bc	9.3 ± 0.1 bc
18:3 *n*-3	1.0 ± 0.0 a	1.2 ± 0.0 b	1.0 ± 0.0 a	1.0 ± 0.0 a	1.0 ± 0.0 a	1.0 ± 0.0 a	1.3 ± 0.1 c	1.1 ± 0.2 a	1.1 ± 0.0 a
18:4 *n*-3	1.0 ± 0.3	1.3 ± 0.1	1.0 ± 0.0	1.1 ± 0.1	1.1 ± 0.0	1.1 ± 0.0	1.4 ± 0.2	1.3 ± 0.0	1.1 ± 0.1
20:1*	0.7 ± 0.0 a	1.2 ± 0.1 bcd	1.3 ± 0.0 cde	1.3 ± 0.1 cde	1.4 ± 0.0 de	1.3 ± 0.1 cde	1.1 ± 0.0 b	1.2 ± 0.4 bc	1.5 ± 0.1e
20:4 *n*-6	1.2 ± 0.1 b	1.2 ± 0.2 ab	1.0 ± 0.0 ab	1.1 ± 0.0 ab	0.9 ± 0.1 ab	0.9 ± 0.0 ab	1.0 ± 0.1 ab	0.9 ± 0.0 ab	0.9 ± 0.0 a
20:5 *n*-3	5.0 ± 0.2 bc	4.9 ± 0.1 bc	4.7 ± 0.0 ab	4.9 ± 0. bc	4.7 ± 0.1 ab	5.1 ± 0.2 c	5.2 ± 0.1 c	5.1 ± 0.0 c	4.7 ± 0.0 a
22:5 *n*-3	3.9 ± 0.1 c	2.9 ± 0.1 ab	2.8 ± 0.0 ab	2.8 ± 0.0 ab	2.9 ± 0.0 ab	2.9 ± 0.0 ab	3.1 ± 0.2 b	2.8 ± 0.0 ab	2.8 ± 0.0 a
22:6 *n*-3	9.2 ± 0.3 b	9.0 ± 0.2 b	9.0 ± 0.0 ab	9.0 ± 0.0 ab	8.8 ± 0.0 ab	8.9 ± 0.1 ab	9.0 ± 0.6 b	8.7 ± 0.0 ab	8.3 ± 0.0 a
SFA	29.1 ± 0.0 b	28.5 ± 0.3 ab	28.4 ± 0.0 ab	28.8 ± 0.0 ab	28.6 ± 0.0 ab	27.3 ± 0.7 a	28.8 ± 1.3 ab	28.2 ± 0.0 ab	29.1 ± 0.0 b
MUFA	34.0 ± 0.5 ab	35.2 ± 0.4 bc	36.5 ± 0.1 c	35.7 ± 0.1 bc	35.9 ± 0.1 bc	36.6 ± 0.0 c	33.0 ± 1.6 a	36.1 ± 0.0 c	36.6 ± 0.0 c
PUFA	29.4 ± 0.2 abc	29.6 ± 0.0 bc	29.1 ± 0.1 ab	29.3 ± 0.1 abc	29.0 ± 0.2 ab	29.4 ± 0.1 abc	30.7 ± 1.2 c	29.4 ± 0.0 abc	28.2 ± 0.2 a
HUFA	19.5 ± 0.6 c	18.1 ± 0.1 b	17.4 ± 0.0 ab	17.9 ± 0.1 b	17.5 ± 0.2 ab	17.8 ± 0.1 b	18.3 ± 0.9 b	17.5 ± 0.0 ab	16.6 ± 0.1 a
n-3	20.1 ± 0.2d	19.4 ± 0.1 cd	18.5 ± 0.1 ab	18.9 ± 0.1 bc	18.7 ± 0.2 abc	19.0 ± 0.19 bc	20.0 ± 0.6d	18.9 ± 0.1 bc	17.9 ± 0.1 a
n-6	9.3 ± 0.0 a	10.2 ± 0.1 b	10.6 ± 0.0 b	10.4 ± 0.1 b	10.3 ± 0.0 b	10.4 ± 0.0 b	10.7 ± 0.6 b	10.5 ± 0.0 b	10.2 ± 0.1 b
n-3 HUFA	18.9 ± 0.6 c	16.9 ± 0.1 b	16.4 ± 0.0 ab	16.8 ± 0.1 b	16.5 ± 0.1 ab	16.8 ± 0.1 b	17.3 ± 0.8 b	16.6 ± 0.0 ab	15.7 ± 0.0 a
n-6 HUFA	1.2 ± 0.1 b	1.2 ± 0.2 ab	1.0 ± 0.0 ab	1.1 ± 0.0 ab	0.9 ± 0.1 ab	0.9 ± 0.0 ab	1.0 ± 0. ab	0.9 ± 0.0 ab	0.9 ± 0.0 ab
n-3/*n*-6	2.2 ± 0.0e	1.9 ± 0.0d	1.7 ± 0.0 a	1.8 ± 0.0 bcd	1.8 ± 0.0 abc	1.8 ± 0.0 bcd	1.9 ± 0.0 cd	1.8 ± 0.0 abc	1.8 ± 0.0 ab

[a] Values in the same row with the same lowercase letter are not significantly different (*P* > 0.05). Only principal fatty acids have been reported. *20:1 represents the sum of 20:1 isomers (20:1*n*-9 and 20:1*n*-11).

compared to the start of the trial. The somatic weight, fillet yield (FY), and visceral fat index (VFI) were not affected by starvation ($P > 0.05$).

Food conversion ratio (FCR) was highest ($P < 0.05$) in CWF4 (1.21 ± 0.0), while the lowest was recorded by CWF2 (0.8 ± 0.0). RWF4 had the lowest ($P < 0.05$) specific growth rate (SGR) (0.23 ± 0.0%), while the other treatments were not significantly different between each other (CWF2, 0.3 ± 0.0%; CWF4, 0.3 ± 0.0%; and RWF2, 0.3 ± 0.0%). The highest growth rate ($P < 0.05$) was recorded in CWF4 (8.3 ± 0.7%) followed by SWF4 (5.5 ± 0.2%). Among the starved treatments, CSW4 and RWS4 had the highest weight loss (−13.4 ± 0.8% and −13.4 ± 0.9%, respectively).

Feeding regime was the factor affecting the most changes in body weight and body composition as shown in **Table 3**, with significant effects on all parameters apart from VFI and FY. Only minimal ($P < 0.05$) interactions between feeding regime and water, feeding regime and time and among feeding regime, water, and time on liver weight and HSI were recorded; no interactions between water and time have been shown (**Table 3**).

Lipid Content and Fatty Acid Composition. Total lipid content of the fish fillet did not show any significant ($P > 0.05$) change in fish at time zero and in fish reared in clean water (CWS2, CWF2, CWS4, and CWF4) and in recycled water for two weeks (RWS2). All other treatments recorded a significantly higher lipid content compared to the start of the trial as shown in **Table 4**, with the highest values recorded for fish fed and starved in recycled water for 4 weeks (RWS4 and RWF4).

Fatty acid composition of fillet of fish (expressed as percent of all fatty acids) was significantly modified by the purging strategy implemented (**Table 4**). Saturated fatty acids (SFA) were significantly higher in RWF4 fish (29.1 ± 0.0%) compared to CWS4 (27.3 ± 0.7%), (the two most extreme treatments), while monounsaturated fatty acids (MUFA) were lowest for CWF4 (33.0 ± 1.6%), compared to all other treatments. Also, CWF4 recorded the highest polyunsaturated fatty acid (PUFA) content (30.7 ± 1.2%), while the lowest value was recorded in RWF4 (28.2 ± 0.2%). RWF4 also recorded the lowest value for the highly unsaturated fatty acids (HUFA; the polyunsaturated fatty acids with three or more double bonds and a chain length of 20 or more carbons).

Table 5. Effect of Feeding Regime, Water Quality and Time on the Fatty Acid Composition of Farmed Murray Cod[a]

	feeding regime		water		time		feeding regime × water		feeding regime × time		water × time		feeding regime × water × time	
	F value		*F* value	*P*	*F* value	*P*	*F* value	*P*	*F* value	*P*	*F* value	*P*	*F* value	P
14:0	11.661	**	1.278	ns	13.091	**	1.278	ns	2.161	ns	0.013	ns	3.273	ns
16:0	0.177	ns	2.987	ns	0.572	ns	0.281	ns	3.629	ns	0.271	ns	0.039	ns
16:1 *n*-7	0.412	ns	1.353	ns	1.384	ns	1.392	ns	0.969	ns	1.253	ns	1.432	ns
18:0	5.578	*	10.177	*	1.424	ns	0.029	ns	6.790	*	0.171	ns	3.336	ns
18:1 *n*-9	5.655	*	1.662	ns	0.193	ns	0.452	ns	1.002	ns	0.075	ns	0.528	ns
18:1 *n*-7	4.988	*	2.372	ns	2.076	ns	0.062	ns	0.484	ns	4.779	ns	0.142	ns
18:2 *n*-6	25.183	***	1.735	ns	0.304	ns	3.045	ns	2.045	ns	0.257	ns	0.001	ns
18:3 *n*-3	5.742	*	3.962	ns	24.397	***	1.571	ns	30.571	***	0.005	ns	32.223	***
18:4 *n*-3	0.956	ns	2.454	ns	0.435	ns	0.275	ns	1.188	ns	0.022	ns	3.202	ns
20:1	50.846	***	2.397	ns	5.674	ns	8.170	*	0.227	ns	0.014	ns	11.929	**
20:4 *n*-6	5.907	*	1.099	ns	1.757	ns	0.536	ns	1.757	ns	0.010	ns	0.113	ns
20:5 *n*-3	12.478	**	16.608	**	2.673	ns	2.673	ns	1.673	ns	11.506	**	10.220	*
22:5 *n*-3	70.871	***	0.108	ns	2.074	ns	0.001	ns	0.338	ns	2.866	ns	6.258	*
22:6 *n*-3	4.025	ns	0.590	ns	3.444	ns	1.440	ns	0.009	ns	1.557	ns	0.473	ns
SFA	0.322	ns	2.666	ns	1.471	ns	0.326	ns	4.065	ns	0.199	ns	0.133	ns
MUFA	4.966	*	0.964	ns	3.150	ns	3.345	ns	7.684	*	3.965	ns	9.699	*
PUFA	1.795	ns	0.534	ns	6.712	*	4.204	ns	0.534	ns	3.313	ns	5.825	*
HUFA	19.169	***	2.745	ns	5.231	*	1.919	ns	0.554	ns	3.803	ns	3.272	ns
n-3	21.466	***	3.232	ns	13.558	**	4.662	ns	3.121	ns	8.668	*	16.990	**
n-6	8.990	**	0.353	ns	1.091	ns	2.926	ns	0.312	ns	0.105	ns	0.026	ns
n-3 HUFA	108.782	***	7.506	*	4.389	ns	0.234	ns	6.649	*	5.091	ns	14.960	**
n-6 HUFA	31.158	***	2.373	ns	4.800	ns	1.806	ns	0.169	ns	4.963	ns	3.785	ns
n-3/*n*-6	5.907	*	1.099	ns	1.757	ns	0.536	ns	1.757	ns	0.010	ns	0.113	ns

[a] Asterisks denote the level of significance: *** <0.001, ** <0.01, and * <0.05; ns, not significant.

In all tested treatments, linolenic acid (LA; 18:2*n*-6) and total *n*-6 PUFA showed significant increase ($P < 0.05$) compared to that at the start of the trial, with the highest values recorded in fish fed for 4 weeks in clean water, CWF4 (9.7 ± 0.4% and 10.7 ± 0.6%, respectively). Arachidonic acid (AA; 20:4*n*-6) did not show any difference ($P > 0.05$) among treatments; however, an overall reduction across all treatments compared to the initial fish was observed. The total *n*-3 PUFA content was markedly affected by the purging strategy with the lower value recorded in RWF4 (17.9 ± 0.1%). Accordingly, the levels of eicosapentaenoic acid (EPA; 20:5*n*-3) and docosahexaenoic acid (DHA; 22:6*n*-3) in RWF4 were significantly lower ($P < 0.05$) (4.7 ± 0.0% and 8.3 ± 0.0%, respectively) compared to those in the other treatments throughout the whole duration of the trial. Overall, *n*-3 HUFA and the *n*-3/*n*-6 ratio were all significantly higher in fish at the start of the trial ($P < 0.05$).

The data indicate that the changes in the majority of fatty acid were not water or time dependent but starvation dependent (**Table 5**). Only stearic acid (18:0), EPA, and *n*-3 HUFA were affected by the water, while myristic (14:0), α-linoleic (18:3*n*-3), PUFA, HUFA, and *n*-3 were affected by time. All but palmitic (16:0), palmitoleic (16:1*n*-7), stearidonic (18:4*n*-3), DHA, SFA, and PUFA were affected by the starvation process.

Flavour Volatile Compounds and Off-Flavor Analysis. During this study, a total of 20 volatile compounds were identified. Aldehyde was the chemical class with the most compounds, 11, while hydrocarbons accounted for 6, alcohols for 2, and butylated hydroxytoluene (BHT) was the only phenol compound isolated and identified (**Table 6**).

Hexadecanal, the aldehyde found in the highest concentration, was significantly lower in all fish purged for 4 weeks and in RWS2, while nonanal was significantly higher ($P < 0.05$) at the start of the trial. Hexanal, however, was generally higher in fish purged for 4 weeks compared to that in fish purged for 2 weeks only, with the lowest concentration in CWF2 ($P < 0.05$). Octanal and 2-nonenal were lowest ($P < 0.05$) in fish purged for two weeks, 2−4 decadienal was lowest in fish kept in clean water ($P < 0.05$), while CWS2 showed the lowest level of 2 decenal ($P < 0.05$). Hexadecanal and heptanal were significantly different ($P < 0.05$) in all treatments with the exception of RWS2 and RWS2. Among alcohols, 1-nonanol did not show any significant difference throughout the whole duration of the trial, while 1-octanol was significantly higher ($P < 0.05$) in CWS4 and CWF4 (0.3 ± 0.0%) compared to that in CWF2 (0.2 ± 0.0%).

Total hydrocarbons were higher ($P < 0.05$) in all purged fish compared to that in the initial sample. Pristane, the hydrocarbon found in highest concentrations, although not significantly ($P > 0.05$), was lower in fish kept in recycled water. Among other hydrocarbons, D-limonene was found at very low concentrations at the start of the trial and in all RW treatments, but was not found in CW treatments. BHT was also found at low concentrations in all treatments and ranged between 0.3 ± 0.1% and 0.7 ± 0.1%.

Geosmin and MIB were not detected in either purged or unpurged fish. Samples were also distilled (sample was placed in a heated round flask and purged with N_2 and the condensed distillate collected in a chilled receiving vessel) and the distillate analyzed, but also in this instance no tainting compounds were detected. To confirm that nondetection of geosmin and MIB was actually due to the absence of these compounds in samples, spiked fish samples (2.5 ppb of 100 μg mL^{-1} of geosmin and MIB in methanol, Supelco) were analyzed. Both geosmin and MIB were then detected in the spiked sample; hence, it was confirmed that both compounds were absent (limit <2.5 ppb of 100 μg mL^{-1}) in the analyzed samples.

It is evident that water was the main factor responsible for differences between treatments (**Table 7**), significantly affecting the content of 2-decenal, 2-nonenal, 2-pentenal, heptanal, hexadecanal, nonanal, octadecanal, octanal, 8-heptadecene, D-limonene, heptadecane, octane, pentadecane, and pristane, while the feeding regime did not produce any significant effects. Time significantly affected the concentration of heptanal, limonene, and pristane, while only minimal ($P < 0.05$) interactions among the three variables were recorded with heptanal, hexadecanal, and nonanal affected by water and time interaction.

Table 6. Volatile Compounds (% Total Volatile Compounds; 100 μm PDMS-SPME Fiber) Isolated and Identified during This Trial[a]

		0 W purging	2 W purging				4 W purging				
compound	RI	initial	CWS2	CWF2	RWS2	RWF2	CWS4	CWF4	RWS4	RWF4	description
2,4-decadienal	1204	2.2 ± 0.3 a	2.0 ± 0.7 a	1.9 ± 0.4 a	1.9 ± 0.7 a	1.4 ± 0.3 a	4.5 ± 1.1 b	4.5 ± 1.1 b	3.5 ± 0.7 ab	2.9 ± 0.3 ab	fried, cod oil
2,4-heptadienal	1002	3.2 ± 0.2	3.3 ± 0.8	3.8 ± 0.2	3.1 ± 0.7	2.9 ± 0.7	4.8 ± 1.3	4.8 ± 1.2	3.8 ± 0.7	4.5 ± 1.0	green, cucumber
2-decenal	1127	0.9 ± 0.3 a	0.5 ± 0.5 a	0.9 ± 0.5 ab	0.5 ± 0.5 a	0.5 ± 0.5 a	2.9 ± 1.0 b	2.9 ± 1.0 b	2.3 ± 0.7 ab	1.9 ± 0.4 ab	fatty, fried
2-nonenal	1141	0.7 ± 0.2 a	0.8 ± 0.2 a	0.7 ± 0.2 a	0.8 ± 0.2 a	0.7 ± 0.2 a	2.0 ± 0.3 b	2.0 ± 0.3 b	1.6 ± 0.1 b	1.9 ± 0.2 b	cucumber, floral,
2-pentenal	768	2.1 ± 0.3 ab	0.8 ± 0.2 a	0.8 ± 0.1 a	0.8 ± 0.2 a	0.8 ± 0.1 a	3.7 ± 1.0 c	3.7 ± 1.0 c	2.9 ± 0.7 bc	2.2 ± 0.2 abc	green, grass
heptanal	904	2.0 ± 0.1 ab	1.5 ± 0.1 a	1.5 ± 0.1 a	2.7 ± 0.1 bc	2.2 ± 0.5 bc	2.8 ± 0.1 cd	2.8 ± 0.1 cd	2.7 ± 0.1 cd	3.2 ± 0.1d	oily, fatty, rancid
hexadecanal	1321	25.9 ± 1.6 b	28.5 ± 1.3 b	26.5 ± 1.1 b	18.5 ± 2.3 a	27.1 ± 0.4 b	12.4 ± 4.5 a	12.4 ± 4.4 a	18.3 ± 0.7 a	18.1 ± 1.3 a	cardboard
hexanal	804	4.9 ± 0.5 b	3.3 ± 0.7 ab	2.6 ± 0.2 a	3.9 ± 0.5 ab	3.7 ± 0.4 ab	4.4 ± 0.2 b	3.4 ± 0.2 ab	3.8 ± 0.0 ab	4.2 ± 0.3 b	oxidised
nonanal	1103	9.1 ± 0.4d	2.9 ± 0.3 a	2.4 ± 0.2 a	5.3 ± 0.7 b	2.8 ± 0. 2 a	6.5 ± 0.2 c	6.5 ± 0.3 c	3.6 ± 0.5 a	4.9 ± 0.4 b	floral, citrus
octadecanal	1348	2.8 ± 0.5e	2.5 ± 0.3 de	2.2 ± 0.3 cd	2.3 ± 0.3d	2.2 ± 0.3 bcd	1.3 ± 0.3 abc	1.3 ± 0.3 abc	1.0 ± 0.3 a	1.2 ± 0.3 ab	-
octanal	1009	1.5 ± 0.4 ab	0.2 ± 0.2 a	0.1 ± 0.1 a	0.1 ± 0.2 a	0.2 ± 0.1 a	1.9 ± 0.4 c	1.9 ± 0.3 c	1.5 ± 0.2 bc	1.8 ± 0.3 c	tallowy, citrus
Σ aldehydes		***55.1 ± 0.4 c***	***46.1 ± 0.4 b***	***43.5 ± 0.3 ab***	***39.8 ± 0.6 a***	***44.6 ± 0.2 ab***	***47.3 ± 1.3 b***	***46.1 ± 1.2 b***	***44.9 ± 0.3 ab***	***46.8 ± 0.4 b***	**low threshold**
1 octanol	1066	0.2 ± 0.0 ab	0.2 ± 0.0 ab	0.2 ± 0.0 a	0.2 ± 0.0 ab	0.3 ± 0.1 b	0.3 ± 0.0 b	0.3 ± 0.0 b	0.2 ± 0.0 ab	0.3 ± 0.1 ab	
1 nonanol	1665	0.4 ± 0.1	0.5 ± 0.2	0.4 ± 0.1	0.3 ± 0.0	0.4 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.5 ± 0.1	
Σ alcohols		***0.6 ± 0.0***	0.7 ± 0.1	***0.5 ± 0.0***	0.5 ± 0.0	0.7 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.8 ± 0.1	**high threshold**
8-heptadecene	1298	0.2 ± 0.1	0.5 ± 0.3	0.6 ± 0.1	0.5 ± 0.2	0.6 ± 0.1	0.3 ± 0.3	0.3 ± 0.3	0.2 ± 0.2	0.5 ± 0.3	-
D-limonene	1031	0.6 ± 0.2 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.5 ± 0.2 b	0.1 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.5 ± 0.1 b	0.1 ± 0.0 a	lemon, orange
heptadecane	1303	2.1 ± 0.1 a	2.8 ± 0.2 b	2.6 ± 0.2 b	2.7 ± 0.1 b	2.5 ± 0.1 b	2.4 ± 0.2 ab	2.4 ± 0.2 ab	2.0 ± 0.2 a	2.3 ± 0.3 ab	alkane
octane	800	2.4 ± 0.5 b	0.6 ± 0.1 a	0.6 ± 0.1 a	0.6 ± 0.1 a	0.6 ± 0.1 a	1.1 ± 0.1 a	1.1 ± 0.1a	0.9 ± 0.2 a	1.0 ± 0.1 a	alkane
pentadecane	1264	10.9 ± 0.1 ab	15.7 ± 0.2 b	14.5 ± 0.2 ab	11.9 ± 0.4 ab	14.1 ± 0.1 ab	11.8 ± 2.4 ab	11.8 ± 2.6 ab	9.8 ± 2.5 a	14.4 ± 0.5 ab	alkane
pristane	1304	27.5 ± 0.8 a	33.0 ± 2.1 b	37.2 ± 1.6 bc	43.0 ± 2.2d	36.3 ± 0.7 bc	35.9 ± 2.1 bc	37.1 ± 3.2 bc	40.9 ± 0.5 cd	33.7 ± 0.9 b	sweet, crayfish
Σ hydrocarbons		***43.6 ± 0.3 a***	***52.7 ± 0.8 b***	***55.5 ± 0.6 bc***	***59.2 ± 0.8 c***	***54.2 ± 0.3 bc***	***51.4 ± 1.1 b***	***52.7 ± 1.4 b***	***54.3 ± 1.0 bc***	***52.1 ± 0.3 b***	**high threshold**
BHT		0.6 ± 0.1 ab	0.4 ± 0.1 ab	0.5 ± 0.1 ab	0.4 ± 0.1 ab	0.5 ± 0.2 ab	0.7 ± 0.1 b	0.6 ± 0.1 ab	0.3 ± 0.1 a	0.4 ± 0.1 ab	
Σ others		**0.6 ± 0.1^{ab}**	**0.4 ± 0.1^{ab}**	**0.5 ± 0.1^{ab}**	**0.4 ± 0.1^{ab}**	**0.5 ± 0.2^{ab}**	**0.7 ± 0.1^{b}**	**0.6 ± 0.1^{ab}**	**0.3 ± 0.1^{a}**	**0.4 ± 0.1^{ab}**	

[a] Values are the mean ± SEM. Values in the same row with the same lowercase letter are not significantly different ($P > 0.05$).

Table 7. Effect of Feeding Regime, Water Quality, and Time on the Volatile Compound Composition of Farmed Murray Cod[a]

	feeding regime		water		time		feeding regime × water		feeding regime × time		water × time		feeding regime × water × time	
	F value	*P*	*F* value	*P*	*F* value	*P*	*F* value	*P*	*F* value	*P*	*F* value	*P*	*F* value	*P*
2,4-decadienal	0.366	ns	9.153	ns	2.641	ns	0.271	ns	0.001	ns	0.991	ns	0.004	ns
2,4-heptadienal	0.191	ns	2.494	ns	1.000	ns	0.006	ns	0.027	ns	0.015	ns	0.358	ns
2-decenal	0.001	ns	9.478	***	1.457	ns	0.205	ns	0.196	ns	0.471	ns	0.003	ns
2-nonenal	0.109	ns	28.094	***	0.863	ns	0.263	ns	0.398	ns	0.493	ns	0.246	ns
2-pentenal	0.139	ns	16.829	***	2.162	ns	0.213	ns	0.291	ns	1.973	ns	0.250	ns
heptanal	0.022	ns	29.706	***	17.926	***	0.011	ns	3.650	ns	11.378	**	3.217	ns
hexadecanal	0.901	ns	18.957	ns	0.102	ns	2.355	ns	1.018	ns	9.405	**	2.575	ns
hexanal	1.798	ns	2.634	***	2.591	ns	3.225	ns	0.050	ns	1.859	ns	0.806	ns
nonanal	2.156	ns	101.893	***	2.720	ns	0.440	ns	15.699	***	45.348	***	9.979	**
octadecanal	0.056	ns	21.650	***	0.261	ns	0.088	ns	0.322	ns	0.022	ns	0.008	ns
octanal	0.221	ns	39.368	***	0.343	ns	0.274	ns	0.094	ns	0.608	ns	0.097	ns
Σ aldehydes	0.345	ns	15.161	ns	2.043	ns	4.591	*	0.099	ns	0.528	ns	0.808	ns
1 octanol	0.372	ns	0.443	ns	0.124	ns	3.757	ns	0.052	ns	8.197	ns	1.208	ns
1 nonanol	0.449	ns	0.220	ns	0.038	ns	2.592	ns	1.436	ns	1.861	ns	0.069	ns
Σ alcohols	0.780	ns	0.068	ns	0.001	ns	5.401	*	1.469	ns	0.002	ns	0.051	ns
8-heptadecene	0.713	ns	1.945	**	0.020	ns	0.194	ns	0.013	ns	0.160	ns	0.188	ns
D-limonene	7.826	ns	6.388	***	24.554	*	7.826	ns	0.037	ns	0.005	ns	0.037	ns
heptadecane	0.009	ns	7.446	***	2.329	ns	0.967	ns	2.525	ns	0.577	ns	0.227	ns
octane	0.009	ns	24.662	*	0.283	ns	0.117	ns	0.230	ns	0.163	ns	0.058	ns
pentadecane	1.812	ns	3.758	**	0.821	ns	3.618	ns	0.799	ns	1.339	ns	0.078	ns
pristane	2.883	ns	6.979	***	4.601	*	14.522	ns	0.485	ns	2.237	ns	0.227	ns
Σ hydrocarbons	0.436	ns	15.505	ns	2.377	ns	5.373	*	0.077	ns	0.418	ns	0.782	ns
BHT	0.139	ns	0.551	ns	3.920	ns	0.342	ns	0.300	ns	2.742	ns	0.386	ns
Σ others	0.139	ns	0.551	ns	3.920	ns	0.342	ns	0.300	ns	2.742	ns	0.386	ns

[a] Asterisks denote the level of significance: *** <0.001, ** <0.01, and * <0.05; ns, not significant.

DISCUSSION

It is common practice in aquaculture to purge fish to be made ready for the market in clean water without feeding (*19, 20*). This routine process is aimed primarily to eliminate undigested food from the stomach and feces from the intestine (*21*) and also to remove possible off-flavors that may have accumulated during growth (*1, 22*).

During this study, it was evident that starvation negatively affected fish weight, significantly reducing the overall harvestable product and consequently reducing the profit for the producer. Also the hepatosomatic index and dress-out percentage were negatively affected by starvation, while VFI and FY did not show any significant change, all in conformity with previous findings (*9*). Furthermore, no reduction of total lipids in the fillet was recorded during starvation, suggesting that Murray cod preferentially utilize protein and hepatic reserves instead of fillet or perivisceral lipid storages (*9*).

The starvation period did not have a major impact on the fatty acid profile of the fish. Overall, the nutritional qualities of fillets of Murray cod under the different purging treatments were characterized by the presence of abundant health promoting fatty acids (*9*).

A major finding of the present study was that the feeding restriction during purging was not necessary to ameliorate the organoleptic properties of Murray cod. In fact, volatile compound composition was not affected by starvation. However, changes in water quality were effective in changing the volatile compounds profiles of the flesh. It is at this point important to emphasize that, in the present study, volatile compounds have been analyzed with a 100 μm polydimethylsiloxane (PDMS) SPME fiber, and it is well accepted that specific SPME selectivity should be taken into account to avoid overstatement or misinterpretation.

Freshwater fish are usually characterized by a sweet and delicate aroma (*23*), which is a result of the interaction of volatile aldehydes and alcohols derived from the oxidative deterioration of *n*-3 and *n*-6 PUFA (*23*−*25*). The odor threshold of aldehydes is generally lower than those of other volatile compounds (*26*); thus, they contribute more to total flavor. In this study, aldehydes accounted for more than 40% of all volatile compounds, and hence, they likely abundantly contributed to the overall aroma of fish.

In the present study, it has been shown that fatty acid composition of Murray cod fillet is only partially affected by purging and that in particular the feeding regime (starvation) is the primary cause of modification, while water quality is almost unimportant. However, despite the known direct links between fatty acid composition and flavor volatile compound formation (*27*), the overall modifications of fillet flavor volatile compound composition were fundamentally affected by water quality and in no instance significantly affected by feeding regime.

The results of the multivariate general linear model clearly demonstrated that water quality is the most influential parameter in determining the flavor volatile compounds composition. Within the analyzed aldehydes, some are characterized by unpleasant aroma such as 2-decenal (fatty, fried), heptanal (oily, fatty, rancid), hexanal (oxidized) and others by more pleasant descriptions such as 2- nonenal (cucumber, floral), 2-pentenal (green, grass), nonanal (floral, citrus), and octanal (tallowy, citrus), and they were all significantly modified by water quality but not feeding regime during the purging process.

In particular, the compound 2-nonenal, which is characterized by a pleasant cucumber-like, floral smell and which is generated by the oxidative degradation of *n*-6 unsaturated fatty acids (*28*), such as LA and AA, was significantly higher in fish purged for 4 weeks. Similarly also concentrations of 2,4-decadienal, which on the contrary is commonly associated with unpleasant fired oil smell and is derived by *n*-6 PUFA oxidation (*29*), were higher in fish purged for 4 weeks. However, no significant modification in *n*-6 PUFA content of the fillet of fish purged

for 4 weeks was recorded. Hexanal, which is also derived by the oxidation of fatty acids (PUFA) and in particular the oxidation of LA (*29*), and is usually considered an off-flavor in seafood and an indicator of food degradation (*30*), was found in higher concentrations in unpurged fish and was significantly affected by water quality, confirming the importance of clean water in the purging process.

In accordance with earlier findings of Palmeri et al. (*6*), the concentration of pristane, a compound which gives a sweet, pleasant aroma, was higher in fish purged for either 2 or 4 weeks, and was particularly high in fish starved and kept in clean water. Also, D-limonene, a common terpene contaminant, was found in fish kept in recycled water. The fish that were found to show traces of D-limonene came from the same system and were all kept in recycled water, and therefore, the presence of this compound must be attributed to some contamination in the system. D-Limonene is indeed a very common aromatic scenting agent used in many detergents, and water contamination is a very common occurrence (*31*).

Butylated hydroxytoluene (BHT) was also found in very small concentrations. BHT is a synthetic antioxidant, commonly used in fish meal and fish oil production, and in the preparation of commercial feeds, and therefore, its presence is most likely to be diet derived.

Reports of geosmin and MIB, two of the most common volatile compounds responsible for the typical muddy off-flavor of intensively farmed fish, in fish produced in closed recirculation systems are relatively sporadic (*18*), but worth investigating. Accordingly, in the present study, no traces of geosmin or MIB were identified, indicating that the RAS farming procedures of Murray cod were ideally suited to avoid this occurrence.

Palmeri et al. (*9*) conducted a consumer test (triangle test) on similar size farmed Murray cod purged for 0, 2, and 4 weeks in clean water. During this study, consumers were able to distinguish between unpurged (0 weeks) and purged fish (2 and 4 weeks), with purged fish being preferred to unpurged fish, but unable to distinguish between fish purged for 2 and 4 weeks. Therefore, it was concluded that intensively farmed Murray cod should be purged for no longer than 2 weeks to minimize weight loss due to prolonged starvation. Chemical analyses conducted on the same fish corroborated the outcome of the consumer test. Aldehydes, in fact, were significantly higher in unpurged fish, whereas hydrocarbons were significantly lower. Also in that instance, no geosmin or MIB were detected (*9*).

In the current study, the same general trend was observed, although some differences were evident. Aldehydes in fish purged for two weeks were much higher in the current trial. Also during this trial, new compounds were detected, namely, D-limonene, butylated hydroxytoluene, 1-octanol, and 1-nonanol, while others reported in the previous study were not found. Such differences, however, may also be due to the different extraction techniques adopted: SPME versus SDE.

This study was able to demonstrate the separation of the effect of starvation and water quality, and, in regard to previously reported drawbacks of the implementation of period of starvation, this is an important finding that needs to be considered for optimizing purging strategies. The presence of tainting compounds in the suspended solids and especially in the dissolved solids affected the volatile make up of fish by being likely absorbed through the gills and gut epithelium (*2*). Admittedly, with the present experimental design, it was not possible to attribute such effects to a specific water parameter. However, it is possible to speculate that the combination of increased BOD, TSS, phosphorus, nitrite, and nitrate can play a negative role on the development of taints that can affect the overall fish flavor. However, it is also possible that salt added to the purging water, which is a common practice in Murray cod farming, can have a positive role in the depletion of the tainting compounds. Further studies, specifically focusing on the relationship between fish flavor and water quality parameters, are then needed to increase our comprehension of such phenomena.

This study also suggested that starvation is not a crucial part of the purging strategy, while water quality seemed to be the main factor affecting the final volatile compound composition. As starvation causes substantial weight loss if protracted past the necessary time to empty the gut and intestine, it is recommended that intensively farmed Murray cod are to be purged in clean water for at least 2 weeks and starved for a minimal number of days. The potential of off-flavor presence is removed by implementing this procedure, the main eating qualities are maintained, and weight loss minimized.

ABBREVIATIONS USED

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; HUFA, highly unsaturated fatty acids; MIB, methylisoborneol; SPME, solid phase microextraction; PDMS, polydimethylsiloxane; HSI, hepatosomatic index; DP, dress out percentage; VFI, visceral fat index; FY, fillet yield; *K*, condition factor; FCR, feed conversion ratio; SGR, specific growth rate; BHT, butylated hydroxytoluene; RAS, recirculating aquaculture system; DO, dissolved oxygen; TSS, total suspended solids; TDS, total dissolved solids; NTU, nephelometric turbidity units; BOD, biological oxygen demand.

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