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Habitat related variations in fatty acids of catadromous *Galaxias maculatus*

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Abstract – The fatty acid profile of the galaxiid fish *Galaxias maculatus* (Salmoniformes) collected from an estuarine habitat (a river mouth), a freshwater creek and two land-locked freshwater lakes in Victoria, Australia, were investigated with a view to evaluating habitat influences on the fatty acid profile. Fish from the freshwater creek were unpigmented, fresh-run juveniles (referred to as whitebait), the others adult fish. The fatty acids that predominated in adult *G. maculatus* muscle were 16:0 and 22:6(n-3), and 16:0 and 18:1(n-9), respectively. In whitebait 22:6(n-3), 20:5(n-3) and 16:0 were predominant. Hopkins estuary fish had the highest amount of 18:2(n-6) but the lowest 18:3(n-3). In all galaxiid populations, polyunsaturated fatty acids (PUFA) were the main group of fatty acids in total lipid in muscle, and accounted for more than 40 %, followed by saturates and monoenes. In landlocked populations, the amount of monoenes was lower than in other galaxiid populations. *G. maculatus* whitebait had significantly lower amount of saturates and the highest amount of n-3 fatty acids. The ratio of n-3 to n-6 PUFA ranged from 4.6 (Hopkins estuary) to 7.6 (whitebait), and the former differed significantly from all the other populations. The main fatty acids predominating in the liver of galaxiids from landlocked (Lake Bullen Merri) and estuarine populations were 16:0, 22:6(n-3) and 18:1(n-9), and generally reflected the corresponding pattern in muscle. A principal component analysis of the fatty acid composition of muscle samples confirmed and highlighted the distinct fatty acid profile of the populations investigated, when three groupings could be discerned. The study suggests that in addition to the diet, other habitat related factors may influence the fatty acid profile in catadromous *G. maculatus*.
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Muscle / polyunsaturated fatty acids / euryhaline / freshwater / Galaxiid fish / Salmoniformes

Résumé – Variabilité des acides gras du poisson catadrome *Galaxias maculatus* en fonction de l'habitat. L'influence de l'habitat sur le profil en acides gras de poissons Galaxidés *Galaxias maculatus* (Salmoniformes) a été étudiée en réalisant des analyses sur des échantillons prélevés dans un estuaire (embouchure de fleuve), dans un ruisseau et dans deux lacs d'eau douce à Victoria (Australie). Les poissons provenant du ruisseau étaient des juvéniles non pigmentés, tandis que les autres poissons étudiés étaient des adultes. Les acides gras prédominant dans le muscle des adultes *G. maculatus* étaient 16:0 et 22:6 de la série n-3 et 16:0 et 18:1 de la série n-9. Chez les juvéniles, 22:6(n-3), 20:5(n-3) et 16:0 étaient majoritaires. Ce sont les poissons pêchés dans l'estuaire de Hopkins qui contenaient le plus de 18:2(n-6) et le moins de 18:3(n-3). Dans toutes les populations de Galaxidés, les acides gras polyinsaturés (PUFA) constituaient le groupe le plus important (plus de 40 %) parmi les lipides du muscle, suivi par les acides gras saturés et les mono-insaturés. La proportion d'acides gras mono-insaturés était plus faible chez les Galaxidés des lacs que chez les autres. Par rapport aux adultes, les juvéniles avaient des acides gras saturés en quantité significativement inférieure mais le taux le plus élevé de n-3. Le rapport n-3/n-6 variait de 4,6 (estuaire du Hopkins) à 7,6 (juvéniles), ce dernier étant significativement différent des autres populations. Les acides gras dominants dans le foie des poissons de lac (lac de Bullen Merri) et des populations estuariennes étaient 16:0, 22:6(n-3) et 18:1(n-9) et reflétaient généralement le profil en acides gras du muscle. Une analyse des composants principaux de la composition en acides gras du muscle a confirmé et mis en évidence le profil précis en acides gras des différentes populations étudiées où trois groupes peuvent être distingués. Cette étude suggère qu'en plus du régime alimentaire, d'autres facteurs liés à l'habitat peuvent influencer le profil en acides gras chez le poisson catadrome *G. maculatus*.
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Muscle / acides gras polyinsaturés / poissons euryhalins / eau douce / Galaxidés / Salmoniformes

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1. INTRODUCTION

Fatty acids, in particular polyunsaturated (PUFA) ones, are a major component of membrane phospholipids and also act as precursors of biologically active eicasanoids [3, 4]. Fish are incapable of synthesising PUFA de novo, and are therefore dependent on their diet for these nutrients [14, 24]. The fatty acid profiles, and the dietary essential groups of fatty acids, of freshwater fish differ from those of marine fish. The differences are considered to reflect the diet [27]. This may result in different desaturation and chain elongation capabilities in the two groups of fish [28]. For example, the ratio of n-3 to n-6 fatty acids in marine fish typically ranges from 4.7 to 14.4, and from only 0.5 to 3.8 in freshwater fish, and within each group, the lowest values occur in tropical species [14].

There have been limited studies on diadromous fish, particularly with reference to the changes in fatty acids associated with the transition from a marine and/or estuarine habitat to a freshwater habitat or vice versa. Sheridan et al. [28] documented the changes associated with the parr-smolt transformation in steelhead trout *Oncorhynchus mykiss* (Walbaum). The changes in the fatty acid profiles of the Australian shortfin eel *Anguilla australis* Richardson in relation to development were evaluated by De Silva et al. [11]. Both these studies suggested that a certain degree of pre-adaptiveness in the fatty acid profiles may be involved in the transformations associated with their migratory behaviour. In addition, changes in the fatty acid profiles of skeletal tissues during spawning migration of Pacific pink salmon, *Oncorhynchus gorbuscha* have also been reported [22]. According to these authors, pink salmon during spawning migration preferentially depleted PUFA, mostly 20:5(n-3) and 22:6(n-3).

Borlongan and Benitez [5] compared the fatty acid profiles of sea- and freshwater grown milkfish *Chanos chanos* Forsskål. Changes in fatty acids of fed and starved hybrid red tilapia, raised in seawater and freshwater have also been examined [10]. These studies showed that there were major differences in the fatty acid profiles of freshwater and seawater raised fish [5, 10], and during starvation, different fatty acids tended to be conserved in the two groups of fish [10].

Members of the family Galaxiidae (order: Salmoniformes) are a widely distributed, typically freshwater group of fish [21]. The species *Galaxias maculatus* (Jenyns) is an exception amongst galaxiids in that it is a normally catadromous, stream dwelling species but it also has a number of viable, landlocked populations [7, 23]. The diet of landlocked populations of *G. maculatus* typically consists of crustaceans (amphipods, copepods and cladocerans) and insect larvae. Given that different populations of *G. maculatus* inhabit different environments, with widely different diets and a catadromous habit, this species lends itself well to a comparative study of fatty acids. As such, they are a suitable species to test the hypothesis of whether changes associated with the fatty acid profiles are pre-adaptive or are linked to dietary changes. The present study therefore, was instigated on whitebait (fresh-run, unpigmented juvenile fish [19]) and adult *G. maculatus* with a view to evaluate changes in the fatty acids of different populations from different habitats. It is expected that the findings will also provide further support for the aforementioned hypotheses.

2. MATERIALS AND METHODS

2.1. *Galaxias maculatus* samples

Galaxias maculatus samples were obtained from different environments: an estuarine habitat (Hopkins river estuary); a freshwater creek (Curdies creek); and two land-locked, freshwater lakes (Lake Colongulac and Lake Bullen Merri) in southeast Victoria, Australia (142°20'-143°15' E; 38°15'-38°30' S). The fish were caught with a scoop net of approximately 30-cm diameter opening, and transported live to the laboratory. The samples from Curdies creek were whitebait, whereas all the other samples were adolescent or mature fish with stage IV or V gonads [13]. Random samples of fish, a minimum of eight individuals from each population, were killed in anaesthetic (1:8 000 benzocaine). Total length (to the nearest mm) and body weight (to the nearest 0.1 g) of individual fish were determined and viscera removed. From each fish, a portion of the body muscle, devoid of skin, scales and bone, and the liver (from two populations only) were taken. The muscle and/or liver samples from individual fish, from each population, were pooled and divided in to two subsamples, and frozen at –70 °C until further analysis.

2.2. Fatty acid analysis

The methods used for lipid and fatty acid analyses were the same as those used in our previous studies on fatty acids [10, 11]. Briefly, muscle and liver subsamples from each population were homogenised in chloroform-methanol (2:1, v/v) using an Ika-Labortechnik Ultra-Turrax T8 homogeniser and total lipid was extracted and estimated gravimetrically [12]. The fatty acids in the total lipid were esterified into methyl esters by saponification with 0.5 N methanolic NaOH and transesterified with 14 % BF_3 (w/v) in methanol [1]. Three aliquots of each esterified sample (fatty acid methyl esters) were analysed in a Carlo Erba GC 600 equipped with a Omegawax 250 capillary column (30 mL × 0.32 mm internal diameter), a FID detector and a split-splitless injection system. The carrier gas was helium and both injector port and detector temperatures were 260 °C. The temperature programme was 200 °C for 5 min, 200–240 °C at 4 °C/min, and held at 240 °C for 35 min. Fatty acids were identified relative to known external standards and the resulting peaks

were quantified using C17:0 as an internal standard (Sigma, USA).

2.3. Statistical analysis

The differences in individual fatty acids (expressed as percent of total identified fatty acids in total lipid), and the gross changes in the fatty acid profiles (percentage of saturated, monounsaturated and polyunsaturated fatty acids or percent saturates, monoenes and PUFA, respectively) were compared using ANOVA. As in our previous studies [10, 11, 27] and that of Lie et al. [16], a principal component analysis (PCA) was carried out on arc sine transformed data [33]. This summarised the relative differences amongst different populations in relation to their overall fatty acid profiles and determined the contribution of individual fatty acids to these differences.

3. RESULTS

3.1. *G. maculatus* populations

The mean length and weight of *G. maculatus* from the different populations sampled are given in *table I*. The mean length and weight data, and the pigmentation pattern suggest that the Curdies creek fish were fresh-run juveniles or whitebait [18]. All other fish sampled were adult fish and in all these populations, both mature females and males were found. In adult fish, the amount of total lipid in muscle was higher ($P < 0.05$) than in whitebait *G. maculatus* (*table II*).

3.2. Fatty acids in muscle of *G. maculatus*

A total of 18 fatty acids were identified in muscle and liver samples of different populations of *G. maculatus* (*table II*). In all galaxiid populations investigated, polyunsaturated fatty acids (PUFA) was the main group of fatty acids in total lipids in muscle, and accounted for more than 45 %, except in fish from the Hopkins estuary, followed by saturates and monoenes (*table II*). In landlocked populations (Lake Colongulac and Lake Bullen Merri) and whitebait, the amount of monoenes was lower ($P < 0.05$) than in the estuarine population. Estuarine *G. maculatus* also had the lowest proportion of PUFA ($P < 0.05$) but the highest proportion of monoenes (*table II*). *G. maculatus* whitebait, on the other hand, had a significantly lower amount of saturates than the other populations. In the Hopkin estuary population, the amount of n-3 PUFA in muscle was significantly lower than in whitebait and in landlocked populations (*table II*), primarily due to a lower amount of 22:6(n-3). The ratio of n-3 to n-6 PUFA ranged from 4.6 (Hopkins estuary) to 7.6 (whitebait), and the former differed significantly from the other populations.

The fatty acids that predominated in adult *G. maculatus* muscle from landlocked and estuarine populations were palmitic acid (16:0) and docosahexaenoic acid [DHA; 22:6(n-3)], and 16:0, 22:(6n-3) and oleic acid [18:1(n-9)], respectively. In whitebait 22:6(n-3), eicosapentaenoic acid [EPA; 20:5(n-3)] and 16:0 were the predominant fatty acids (*table II*). The amount of 16:0 in whitebait were significantly lower than in other populations, but the reverse was true of stearic acid (18:0) and 20:5(n-3). The amount of linolenic acid [18:3(n-3)] in fish from the estuary was higher ($P < 0.05$) than in other populations, and the reverse was true of linoleic acid [18:2(n-6)].

3.2.1. PCA analysis

A principal component analysis (PCA) of the fatty acid composition of muscle samples confirms the preceding results and highlights the distinctiveness of the Curdies creek whitebait. The first and second axes of the analysis summarise (*figure 1*) efficiently the variation in fatty acid levels amongst samples accounting for 49.5 and 29.2 % of the total variation within the data set, respectively. The variation in sample scores on axis 1 contrasts the fatty acid profiles of small whitebait (CC) with high positive scores against larger landlocked fish (LBM and LC) with negative scores. The intermediate sized fish from the Hopkins estuary

Table I. The mean total length (TL) and weight (± SE) of *G. maculatus* samples used in the present study, the range in salinity of the habitats and the likely main food items of each population inferred from published data (see text for details). The codes used for each population in the text and other tables are given in parentheses.

Population	TL (mm)	Body wt. (g)	Salinity (‰)	Diet*
Landlocked				
Lake Colongulac (LC)	70 ± 2	2.11 ± 0.21	< 1	am; oc; inl
Lake Bullen Merri (LBM)	72 ± 4	1.66 ± 0.21	< 1	am; oc; inl
Freshwater streams/creek				
Curdies creek (CC)	44 ± 1	0.24 ± 0.01	0–8	cp; am
Estuarine				
Hopkins estuary (HE)	58 ± 2	1.04 ± 0.10	0–30	cp; mol

* Information summarised from Pollard [23]; am, amhipods; cp, copepods; oc, ostracods; inl, insect larvae; mol, molluscs.

Table II. Percentage lipid (by wet weight), and the amount of individual fatty acids expressed as a percentage (± SE) of total identified fatty acids in total lipid of muscle samples of *G. maculatus* from different habitats. Each value is based on two subsamples of muscle, each analysed in duplicate. Values in the same row, with different superscripts are significantly different from each other ($P < 0.05$).

Lipid/fatty acid	Adult landlocked		Estuarine	Whitebait freshwater
	LC	LBM	HE	CC
Lipid (%)	4.7 ± 0.9^{c}	2.4 ± 0.1^{b}	2.8 ± 0.2^{b}	1.3 ± 0.4^{a}
14:0	3.3 ± 0.2^{b}	0.5 ± 0.3^{a}	3.1 ± 0.2^{b}	3.0 ± 0.3^{b}
16:0	30.2 ± 1.2^{b}	29.7 ± 1.7^{b}	28.1 ± 0.3^{b}	13.5 ± 0.8^{a}
18:0	3.9 ± 0.5^{ab}	3.4 ± 0.1^{a}	4.9 ± 0.3^{b}	6.8 ± 0.2^{c}
Σ saturates	37.5 ± 0.9^{c}	33.6 ± 1.4^{b}	36.1 ± 0.6^{bc}	23.4 ± 0.8^{a}
16:1(n-7)	6.3 ± 0.8^{b}	2.0 ± 0.1^{a}	9.9 ± 0.2^{c}	8.7 ± 0.2^{c}
18:1(n-9)	6.2 ± 0.7^{a}	8.7 ± 1.0^{ab}	14.8 ± 3.6^{c}	9.2 ± 0.5^{ab}
20:1(n-7)	tr	tr	tr	tr
22:1(n-7)	0.9 ± 0.2	1.2 ± 0.2	0.6 ± 0.0	tr
Σ monoenes	13.5 ± 1.8^{a}	11.9 ± 0.6^{a}	25.4 ± 3.6^{b}	18.0 ± 0.3^{a}
18:2(n-6)	1.5 ± 0.3^{a}	3.3 ± 0.3^{ab}	4.7 ± 0.9^{b}	2.8 ± 0.2^{a}
18:3(n-3)	3.7 ± 0.4^{b}	3.4 ± 0.4^{b}	1.9 ± 0.4^{a}	3.8 ± 0.2^{b}
18:4(n-3)	nd	nd	nd	1.8 ± 0.2
20:2(n-6)	tr	tr	tr	0.1 ± 0.0
20:3(n-6)	tr	tr	tr	0.1 ± 0.0
20:4(n-6)	6.4 ± 0.3^{c}	4.0 ± 0.2^{b}	2.1 ± 0.4^{a}	3.6 ± 0.2^{b}
20:3(n-3)	tr	tr	tr	0.3 ± 0.0
20:5(n-3)	5.9 ± 0.6^{a}	6.2 ± 0.4^{a}	8.9 ± 0.9^{b}	15.9 ± 0.3^{c}
22:4(n-6)	tr	tr	tr	tr
22:5(n-3)	3.4 ± 0.2^{ab}	1.5 ± 0.0^{a}	3.9 ± 0.6^{c}	2.9 ± 0.2^{bc}
22:6(n-3)	28.9 ± 1.6^{b}	35.9 ± 1.2^{c}	16.7 ± 2.8^{a}	27.2 ± 0.2^{b}
Σ n-3	40.9 ± 1.0^{b}	47.0 ± 2.1^{bc}	31.5 ± 4.7^{a}	51.2 ± 0.7^{c}
Σ n-6	7.9 ± 0.1	7.3 ± 0.4	6.8± 0.5	6.7 ± 0.4
Σ PUFA	48.8 ± 1.1^{b}	54.4 ± 1.9^{bc}	38.3 ± 4.2^{a}	58.5 ± 1.1^{c}

tr, The amount present is less than 0.1 %; nd, not detected. Landlocked, LC - Lake Colongulac, LBM - Lake Bullen Merri; estuarine, HE - Hopkins estuary; freshwater: CC - Curdies creek.

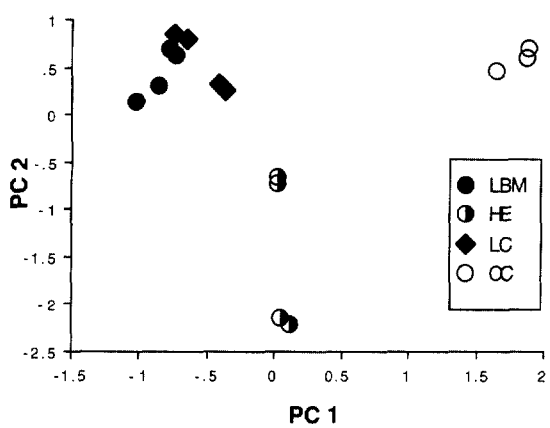

Figure 1. Principal coordinates analysis of relationships of the fatty acids of muscle samples of four populations of *G. maculatus*. The first and second axes account for 49.5 and 29.2 % of the total variation, respectively. LBM, Landlocked Lake Bullen Merri; LC, landlocked Lake Colongulac; HE, estuarine Hopkins estuary; CC, freshwater Curdies creek.

(HE) fall into an essentially intermediate position on this first axis between landlocked fish and whitebait, but are differentiated from all other populations by negative scores on axis 2. From the component loadings (*table III*), it can be seen that the majority of fatty acids contribute to the differences amongst populations on axis 1. Whitebait has elevated levels of 18:0, 16:1(n-7), 18:1(n-9), 18:4(n-3), 20:2(n-6), 20:3(n-6)

Table III. Factor loading for individual fatty acids from a principal component analysis of muscle samples from four populations of *G. maculatus*.

Fatty acid	Axis 1	Axis 2
14:0	0.459	–0.202
16:0	–0.949	–0.213
16:1(n-7)	0.640	–0.500
18:0	0.900	–0.236
18:1(n-9)	0.178	–0.884
18:2(n-6)	0.123	–0.811
18:3(n-3)	0.124	0.873
18:4(n-3)	0.929	0.303
20:1(n-7)	0.920	0.300
20:2(n-6)	0.932	0.302
20:3(n-6)	0.931	0.301
20:4(n-6)	–0.294	0.839
20:5(n-3)	0.940	0.067
22:1(n-7)	–0.894	–0.121
22:5(n-3)	0.471	–0.422
22:6(n-3)	–0.302	0.901

and 22:5(n-3), and lower levels of 16:0 and 22:1(n-7) compared to landlocked fish. The distinctiveness of the Hopkins estuary galaxiids on axis 2 is due to elevated levels of 16:1(n-7), 18:1(n-9), 18:2(n-6) and 22:5(n-3), and reduced levels of 18:3(n-3) and 20:4(n-6).

3.3. Fatty acids of the liver

In the liver of *G. maculatus* from Lake Bullen Merri and Hopkins estuary, the proportion of saturates in fatty acids in total lipids were higher, and the proportion of PUFA lower, than the corresponding amounts in muscle. As in muscle, the total PUFA and total n-3 in the liver of galaxiids from the landlocked population was significantly higher than in the Hopkins estuary population (*table IV*). The main fatty acids predominating in the liver of these fish were 16:0, 22:6(n-3) and 18:1(n-9), and generally reflected the corresponding pattern in muscle (*table IV*). These predominant fatty acids accounted for nearly 50 % of the fatty acids in total lipid in the liver of both populations investigated.

Table IV. Percentage lipid (by wet weight), and the amount of individual fatty acids expressed as a percentage (± SE) of total identified fatty acids in total lipid of liver samples of *G. maculatus* from three different habitats. Each value is based on two subsamples of liver, each analysed in duplicate. Values in the same row, with different superscripts are significantly different from each other ($P < 0.05$). LBM and HE refer to habitats as defined in *tables I* and *II*.

Lipid/fatty acid	LBM lake	HE estuary
Lipid (%)	13.0 ± 1.1^{c}	9.2 ± 0.7^{b}
14:0	1.9 ± 0.8^{a}	5.1 ± 0.0^{b}
16:0	31.4 ± 0.1	32.1 ± 4.0
18:0	3.6 ± 0.1^{a}	5.8 ± 0.4^{b}
Σ saturates	37.0 ± 1.1	43.0 ± 3.6
16:1(n-7)	2.6 ± 2.0^{a}	8.8 ± 0.5^{b}
18:1(n-9)	14.5 ± 1.2^{b}	10.6 ± 0.7^{a}
20:1(n-7)	nd	0.2 ± 0.0
22:1(n-7)	0.6 ± 0.0	0.4 ± 0.0
Σ monoenes	17.8 ± 0.8	20.1 ± 1.3
18:2(n-6)	4.5 ± 0.2^{b}	2.8 ± 0.1^{a}
18:3(n-3)	3.6 ± 0.4^{b}	1.5 ± 0.0^{a}
18:4(n-3)	0.3 ± 0.0	0.2 ± 0.0
20:2(n-6)	nd	0.3 ± 0.0
20:3(n-6)	nd	0.3 ± 0.0
20:4(n-6)	3.5 ± 0.1^{a}	4.4 ± 0.3^{b}
20:3(n-3)	tr	tr
20:5(n-3)	3.5 ± 0.1^{a}	7.5 ± 0.5^{b}
22:4(n-6)	1.2 ± 0.3	nd
22:5(n-3)	1.7 ± 0.2^{a}	3.5 ± 0.2^{b}
22:6(n-3)	26.5 ± 1.9^{b}	16.0 ± 1.1^{a}
Σ n-3	35.8 ± 2.1	28.7 ± 1.9
Σ n-6	9.3 ± 0.1	7.8 ± 0.3
Σ PUFA	45.1 ± 1.9	36.6 ± 2.3

nd, Not detected.

4. DISCUSSION

In animal tissues, the common fatty acids vary in chain length from C14 to C22 and on occasions from C2 to C36 or even more [9]. The most commonly reported fatty acids in fish range from C14 to C22. The galaxiid populations studied presently originated from three different habitats and were also of at least two different life stages: whitebait from Curdies creek and adult populations from other habitats. The results indicate major differences in the fatty acid profiles of the galaxiid populations.

Differences in individual fatty acids of these populations were clearly reflected in the PCA, where three distinct groupings (the Curdies creek whitebait; the landlocked populations of Lake Colongulac and Lake Bullen Merri, and Hopkins estuary populations) were discernible. The three groupings correspond to three habitats and life history stages, including size. Differences in fatty acid profiles in relation to development [15, 29], food habits [16, 20, 26], starvation/food availability [10, 31, 32], temperature [31], salinity [5, 10] and migratory habits [10, 22, 28] have been reported in different fish species. However, differences in fatty acid profile in relation to body size per se have not been demonstrated hitherto, but possible differences between sexes have been alluded to [10]. At this stage, the reasons for the observed differences in the fatty acid profiles of *G. maculatus* in relation to size have to be speculative. It is unlikely that such differences are caused by differences in the diet, as the food habits of juvenile and adult fish are reported to be similar [6, 8, 21]. With increasing size, the proportion of different tissues in the body changes and, in most fish, this is accompanied by a concomitant increase in muscle and liver lipid content [17]. These changes can be expected to result in an increase in neutral lipid relative to polar lipids, which are primarily structural [24, 25], and such differences are also manifest in the fatty acid profile. Unfortunately, in the present study, the profiles of different lipid fractions were not discerned, but the results indicate that this area warrants further investigation.

Differences in fatty acid profiles should not just be considered with respect to species habitat (freshwater and/or marine), but should also be based on a consideration of its natural diet, in particular whether a species is herbivorous, omnivorous or carnivorous [26]. The dietary composition of galaxiid fish in Australian and New Zealand waters is well documented [6, 18, 19], and that of *G. maculatus* in particular has been studied in detail [8, 18, 23]. Generally, juvenile and adult galaxiids, irrespective of habitat, are known to be selective feeders. Pollard [23] concluded that the diet of *G. maculatus* consisted primarily of crustaceans (amphipods, cladocerans and copepods), significant amounts of insect larvae in landlocked populations, insect larvae and molluscs in stream dwelling and freshwater reaches, and mostly of crustaceans (amphipods, copepods and cladocerans) in coastal river and

upper estuarine populations. The food of fresh-run *G. maculatus* whitebait is mostly copepods [18].

It is well accepted that marine and freshwater fish differ in their fatty acid requirements, and they have evolved different capabilities of chain elongation and desaturation of basic acids, 18:3(n-3) and 18:2(n-6) [14, 25]. The likely reason(s) for such differences, from an evolutionary viewpoint, have been linked to the fatty acid content of the common food resources in the habitats [25]. Within this general framework, the current food sources are thought to influence the overall fatty acid profile of a species, or in a stage of its life-cycle. On the other hand, it has been suggested that certain pre-adaptiveness may be evident in the fatty acid profile of fish, particularly those which are diadromous [11, 27]. The influence of salinity on fatty acid profile [5, 10] lends indirect support to the previous hypothesis.

In all the galaxiid populations, the predominant group of fatty acids was PUFA, and of these the n-3 fatty acids series. This dominance was also reflected in the relatively high range of the n-3 to n-6 ratio that is typical of marine species [14]. Based on the diet of different galaxiid populations and the dominance of crustacean types in all of them, one would expect the fatty acid profiles to be dominated by those of the n-3 series, and in particular 22:6(n-3), the common PUFA in the lipids of crustaceans [24]. Our observations support this. It is therefore plausible that even in landlocked galaxiids, the very high degree of selectivity for amphipods and large ostracods [23] in their diet influences the fatty acid profile that is typical of a marine species.

It is however, important to note that whitebait and Hopkins estuary fish had significantly higher amounts of monoenes than landlocked fish, brought about by elevated levels of 16:1(n-7) and 18:1(n-9). Relatively high levels of these two fatty acids were also reported in a preliminary study on galaxiid whitebait from New Zealand [30]. These fatty acids are primarily energy substrates. It may be that elevated levels of monoenes in these populations is a reflection of their migratory habit, which in contrast will not occur in landlocked populations.

In general, it is apparent in galaxiids that change of habitat from an estuarine to freshwater is not reflected in the fatty acid profile of a 'marine' to 'freshwater' type or vice versa in their n-3 to n-6 ratios [14]. This trend, however, is different from that reported for some of the anadromous salmonids such as steelhead trout [28], Atlantic salmon (*Salmo salar*) [14] but closer to that reported for chinook salmon (*Oncorhynchus tshawytscha*) eggs [2]. It is interesting to note that in spite of the 'secondary' invasion of the estuarine habitat by *G. maculatus* [18], its fatty acid profile bears little resemblance to that which is typical of freshwater fish. Perhaps this is indicative of the predominant influence dietary habits impose on fatty acid profiles [24]. On the other hand, within this overall pattern, three groupings could be discerned based on the fatty acid profile. Such differences may reflect habitat differences, suggesting that diet may not be the only factor that influences the nature of the fatty profile in fish.

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