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Early ontogeny-related changes of the fatty acid composition in the Percichthyid fishes trout cod, *Maccullochella macquariensis* and Murray cod, *M. peelii peelii*

Rasanthi M. Gunasekera [a], Sena S. De Silva [a*], Brett A. Ingram [b]

[a] *School of Ecology and Environment, Deakin University, P.O. Box 423, Warrnambool, Victoria 3280, Australia.*

[b] *Marine and Freshwater Resources Institute, Snob's Creek, Private Bag 20, Alexandra, Victoria 3714, Australia.*

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Abstract — Changes in the fatty acid profiles of the Percichthyid fish trout cod, *Maccullochella macquariensis* (Cuvier), and Murray cod, *M. peelii peelii* (Mitchell), two Australian native freshwater fish species, were investigated during early development from egg to yolk-sac-resorbed larval stage. In the two Percichthyid fishes polyunsaturated fatty acids (PUFA) accounted for more than 50 % of the 19 quantified fatty acids in total lipid. The fatty acids that occurred in the highest abundance in both trout cod and Murray cod, in all developmental stages, in order, were docosahexaenoic acid [DHA 22:6(n-3)], arachidonic acid [AA 20:4(n-6)], oleic acid [18:1(n-9)] and palmitic acid (16:0), all of which exceeded 100 µg per mg total lipid in most instances. The ratio of 22:6(n-3) to eicosapentaenoic acid-20:5(n-3) in eggs of trout cod and Murray cod was 5.4:1 and 7.3:1, respectively, and remained almost unchanged through development, and was considerably higher than the 2:1 ratio generally reported for fish eggs. In trout cod, 11 of the 19 fatty acids in total lipid decreased during the transformation from egg to yolk-sac-resorbed larva. In Murray cod, only 16:1(n-7) showed a significant decrease whilst 20:4(n-6) increased significantly with development. Overall, there was a tendency in both species to conserve n-3 and n-6 series highly unsaturated fatty acids (HUFA), suggesting their essentiality in first feeding larvae. These observations are discussed in relation to the feeding habits of trout cod and Murray cod, which are top order, freshwater carnivores. © Ifremer/Cnrs/Inra/Ird/Cemagref/Elsevier, Paris

Fatty acids / eggs / larvae / yolk-sac resorption / freshwater fish

Résumé — Modification de la composition en acides gras durant le développement ontogénique (œufs et larves) chez les poissons Percichthyidés, *Maccullochella macquariensis* et *Maccullochella peelii peelii*. Les modifications des profils en acides gras ont été étudiées chez des poissons Percichthyidés *Maccullochella macquariensis* (Cuvier) et *Maccullochella peelii peelii* (Mitchell), deux poissons d'eau douce originaires d'Australie, durant leur développement du stade de l'œuf au stade larvaire, à la fin de la résorption de la vésicule vitelline. Chez ces deux poissons, les acides gras polyinsaturés (PAGPI) comptent pour plus de 50 % des 19 acides gras des lipides totaux. Les acides gras les plus abondants chez les deux espèces à tous les stades de leur développement sont, dans l'ordre, l'acide docosahexénoïque [DHA 22:6 n-3], l'acide arachidonique [(AA 20:4(n-6)], l'acide oléique [18:1(n-9)] l'acide palmitique (16:0), tous excèdent 100 µg par mg de lipides totaux dans la plupart des cas. Les rapports du 22:6(n-3) sur l'acide écosapenténoïque 20:5(n-3) dans les œufs de ces deux espèces sont de 5,4 et de 7,3 respectivement et restent presque inchangés tout au long du développement, alors que le rapport généralement rapporté pour les œufs de poissons est beaucoup plus faible : 2. Chez *M. macquariensis,* 11 des acides gras des lipides totaux diminuent lors de la transformation de l'œuf en larve. Chez *M. peelii peelii,* seul 16:1(n-7) montre une baisse significative tandis que 20:4(n-6) augmente de façon significative au cours du développement. Dans l'ensemble, il y a une tendance chez les deux espèces à conserver les acides gras hautement insaturés n-3 et n-6, ce qui laisse prévoir leur importance lors de la première alimentation des larves. Les observations sont discutées en relation avec le fait que ces poissons appartiennent à l'échelon le plus élevé des prédateurs dulçaquicoles. © Ifremer/Cnrs/Inra/Ird/Cemagref/Elsevier, Paris

Acides gras / œufs / larves / poissons d'eau douce

* Corresponding author, e-mail: sena@deakin.edu.au

1. INTRODUCTION

Fatty acid composition in fish has been studied from a number of viewpoints primarily because of its structural and physiological importance [6, 37]. For example, the fatty acid composition in fish has been evaluated in relation to feeding habits [14, 45], nutritive value [1, 50], starvation [10, 52, 60], osmoregulatory changes [7, 10] and migratory habits [11, 39, 46] amongst others. Apart from the above studies, there have been numerous others on essential fatty acid requirements of cultured fish species for growth and reproduction [53] and the importance of highly unsaturated fatty acids (HUFA) in larval development [56].

Since early work on red sea bream, *Pagrus major* [57, 58], the inter-relationships between broodstock diets, egg and larval quality, particularly in relation to the quality and quantity of highly unsaturated fatty acids (HUFA) in such diets, are well documented for a number of species [43]. Research on broodstock was complemented by that on enrichment of live larval food organisms with HUFA [48, 56]. These lines of research enabled significant advances in the development of larval culture, particularly of marine species.

The fatty acid composition of eggs, wild caught and domesticated, has been investigated in a number of species. For example, in striped bass *Morone saxatilis* [17, 27], Atlantic halibut *Hippoglossus hippoglossus* [19], Atlantic cod *Gadus morhua* [54], six species of Australian fish [2] and turbot *Scophthalmus maximus* [47]. Changes in relation to early development from egg to first feeding larvae, however, have been investigated in few instances. Tocher et al. [53] studied the fatty acid composition of phospholipids and neutral lipids during embryonic and early larval development in Atlantic herring *Clupea harengus*, and Soivio et al. [49] investigated the changes in fatty acid composition during incubation, hatching and feeding in whitefish *Coregonus muskun*. More recently, the biochemical composition and fatty acid content of fertilised eggs, yolk-sac and first feeding larvae of Senegal sole *Solea senegalensis* [37, 55], and of striped bass *Morone saxatilis* [9] and pike *Esox lucius* [14] eggs and larvae during early development have been studied.

In general, there is a paucity of studies on changes in fatty acid composition in relation to early development in freshwater fish [15, 59]. The present study was instigated to determine the changes in fatty acid composition during early ontogeny in two Australian Percichthyid fish, trout cod *Maccullochella macquariensis* (Cuvier) and Murray cod *M. peelii peelii* (Mitchell). It is expected that the present study will add to our knowledge on fatty acid changes and their physiological role in relation to development in fish. Moreover, it will help us fill a void in our relatively limited knowledge on these aspects in freshwater fish. The information may also be useful in bringing about improvements in artificial propagation, via the manipulation of broodstock and/or larval diets, of these two species which are of important conservational value [32] and of aquaculture potential.

2. MATERIALS AND METHODS

2.1. Procurement of egg and larval samples

The broodstocks of trout cod and Murray cod were maintained in earthen ponds at the Fisheries Station, of the Marine and Freshwater Resources Institute, Alexandra, Victoria [25, 31]. Both trout cod and Murray cod broodstock were dependent on the natural food available in the ponds, supplemented with yabbies, an Australian freshwater crayfish species (approximately 5 kg per pond per month), depending on availability. In August and September, prior to the spawning season, chopped ox liver was also provided to both broodstock. Trout cod are propagated by hypophysation of pond reared [16, 31] broodstock. Murray cod spawns naturally in ponds, in response to increasing water temperature and day length during mid-late spring and early summer. The eggs are laid in nesting boxes, described previously in detail [25, 41].

The procurement of egg and larval samples for the present study was similar to that described previously [13, 26]. Briefly, in the 1997 spawning session (southern spring; September–October) samples of fertilised eggs (approximately 24 h after fertilisation) from ten female trout cod, which ovulated within 50–60 h of hypophysation, were obtained. The spawns, after mixing with a pool of milt from two or three males from the stock, were washed and incubated (20 ± 1.5°C) and reared separately ([16, 31] for details). The fertilisation and hatching success was estimated for each spawn. Eggs were considered fertilised if they remained translucent 24 h after mixing with sperm, as opposed to being opaque (non-fertilised). From each spawn, samples of newly hatched larvae and yolk-sac-resorbed larvae (about 150–200 per spawn) were collected. However, yolk-sac-resorbed larvae could not be obtained from certain females owing to mortality of hatched larvae before yolk-sac resorption. Accordingly, the study is based on eight and four spawns of trout cod and Murray cod, respectively.

2.2. Sample analysis

From each spawn of both species, 250–300 each of eggs, newly hatched and yolk-sac-resorbed larvae, but prior to onset of feeding, were taken and frozen at –70 °C until further analysis. At the time of taking the egg and larval samples for fatty acid analysis, samples were also taken for proximate and amino acid analysis [26]. The egg/larval samples from each spawn were analysed separately for fatty acids. Eggs and/or larval samples from individual spawns were divided into two sub-samples and were treated identically, and each sub-sample was analysed in duplicate.

The methods used for fatty acid analyses were the same as those used in our previous studies on fatty acids [10, 11, 12, 25]. Briefly, sub-samples from each developmental stage were homogenised in chloroform-methanol (2:1, v/v) using a Ika-Labortechnik Ultra-Turrax T8 homogeniser and total lipid was extracted and estimated gravimetrically according to Folch et al. [20]. 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 [3]. Two aliquots of each esterified sample (fatty acid methyl esters) were analysed in a Carlo Erba GC 6000 vega series 2 (Carlo Erba Instruments, Rodano, Italy) equipped with a Omegawax 250 capillary column (30 mL × 0.25 mm internal diameter; Supelco Incorporation, USA), a FID detector and a split-splitless injection system. The injection solvent was n-hexane (99+ % capillary GC; Sigma Chemicals, USA). 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^{-1}, 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).

All data were subjected to ANOVA followed by Duncan's multiple range test for comparison of the means among different developmental stages studied in each species. Analyses were conducted using the SPSS PC+ software package.

3. RESULTS

3.1. Spawning

The length and weight of trout cod and Murray cod females of which spawns were used in this study ranged from 48.5 to 65.5 cm and 1.62 to 4.76 kg, and from 59.0 to 82.0 cm and 3.0 to 8.32 kg, respectively. The percentage of fertilisation and hatching exceeded 75 and 60 %, respectively, for all the spawns used in the present study. The average number of days taken from fertilisation to hatching, and hatching to yolk-sac resorption were 10–11 and 6–7 d (at 20 ± 1.0 °C), and 8–9 and 7–8 d (at 20 ± 1.5 °C) in trout cod and Murray cod, respectively. The mean diameter of fertilised eggs of trout cod and Murray cod were 3.45 ± 0.07 and 3.52 ± 0.09 mm, respectively.

3.2. Gross changes

Gross changes in proximate composition have been dealt with earlier [26]. It suffices to point out that in both species, the moisture content increased and protein content decreased ($P < 0.05$) with development from egg to yolk-sac-resorbed larvae. The total lipid (mg per egg/or larva) in fertilised eggs, newly hatched larvae and yolk-sac-resorbed larvae of trout cod and Murray cod were 1.1, 1.1 and 0.7, and 1.0, 1.1 and 0.8, respectively. In both species, the amount of total lipid in the yolk-sac-resorbed larvae were lower than ($P < 0.05$) than in fertilised eggs and newly hatched larvae.

The percentage contribution of fatty acids to total lipid, and the percentage of saturated (saturates), monounsaturated (monoenes) and polyunsaturated (PUFA) fatty acids in identified fatty acids in total lipid, at different developmental stages in trout cod and Murray cod are shown in *figure 1*. In both trout cod and Murray cod the fatty acids accounted for more than 80 % of the total lipid in all the developmental stages investigated. In trout cod, the percentage of fatty acids decreased ($P < 0.05$) during development from egg to yolk-sac-resorbed larva. In Murray cod on the other hand, the percentage of fatty acids in total lipid in newly hatched larvae was lower ($P < 0.05$) than in the other two developmental stages investigated. In both species, in all the developmental stages studied, PUFA accounted for more than 50 % of the fatty acids in total lipid, and the contribution of n-3 and n-6 series fatty acids to the pool was higher than saturates and/or monoenes. Also, the percentage changes in saturates, monoenes and PUFA (n-3 and n-6) in total fatty acid pool with development were not significant. The n-3 to n-6 ratio during ontogeny ranged between 1.15–1.24 and 1.07–1.19 in trout cod and Murray cod, respectively.

3.3. Changes in individual fatty acids

In the two Percichthyid fishes, 19 fatty acids were identified and quantified for all the developmental stages investigated. In addition, three other fatty acids [20:4(n-3), 18:3(n-6), and 22:5(n-6)] occurred in trace amounts only. The changes in individual fatty acids in total lipid, expressed in µg per mg total lipid, are given in *table I*. In both trout cod and Murray cod the fatty acids that occurred in highest abundance, through all the developmental stages, in order, were docosahexaenoic acid [DHA; 22:6(n-3)], arachidonic acid [AA; 20:4(n-6)], oleic acid [18:1(n-9)] and palmitic acid (16:0), all of which exceeded 100 µg·mg total lipid^{-1} in most instances (*table I*). In trout cod, 11 fatty acids decreased significantly ($P < 0.05$) during ontogeny from egg to yolk-sac-resorbed larva, whereas in Murray cod, only one fatty acid [16:1(n-7)] decreased significantly. Indeed, in Murray cod, the amount of 20:4(n-6) in total lipid increased ($P < 0.05$) during ontogeny. With development the amount of saturates and monoenes decreased ($P < 0.05$) in trout cod but not in Murray cod (*table I*).

The changes in fatty acid composition when expressed in absolute amounts (µg·egg^{-1} or larva^{-1}) were more pronounced, particularly in the case of trout cod (*table II*). In general, the same trends were evident as previously (*table I*) except in a few instances when the differences were more pronounced. For example, in trout cod, all the individual PUFA decreased ($P < 0.05$) during ontogeny. In Murray cod, however, the only PUFA that decreased during embryogenesis were 18:2(n-6) and 18:3(n-3) (*table II*).

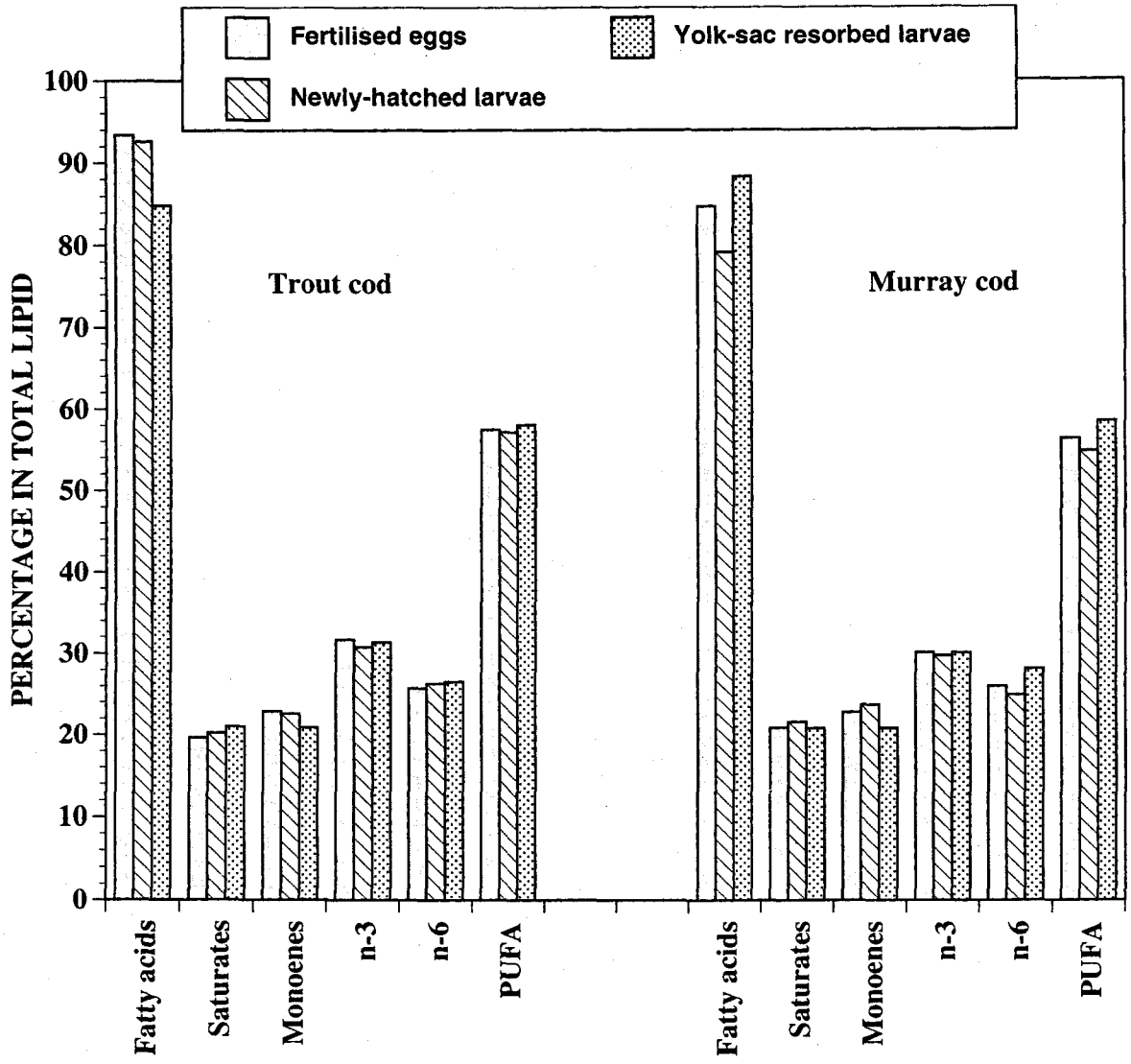

Figure 1. Mean percentage contribution of identified fatty acids to total lipids, and the mean percentage of saturated (saturates), monounsaturated (monoenes), n-3, n-6 series and polyunsaturated fatty acids (PUFA) of the fatty acid pool in total lipid at different developmental stages in trout cod and Murray cod. For clarity, the error bars have been omitted from the figure. For any one parameter, in either species, the differences between developmental stages were not significant, except in the cases of total fatty acids in trout cod when the percentage in yolk-sac-resorbed larvae was lower ($P < 0.05$) than that in other stages, whilst in Murray cod, it was lower in newly hatched larvae ($P < 0.05$) than in the other two stages studied.

4. DISCUSSION

Trout cod and Murray cod are closely related species with comparable habitat preferences, food habits, and are single spawners and lay adhesive eggs [34]. It has been shown that in both species the protein content of eggs and larvae tended to decrease with development but not the total lipid [26]. During embryogenesis, until the onset of exogenous feeding, all nutrients needed for growth, cellular and organ differentiation and metabolism have to originate from yolk reserves. The observed change suggests that both trout cod and Murray cod utilise predominantly the proteins and/or other nitrogenous sources for the above purposes. Fish species vary in their energy utilisation strategies during development; fertilised eggs of some species obtain their energy requirements from protein and others from lipids [21, 35].

Eggs of both trout cod and Murray cod do not have oil globules. Generally, eggs with oil globules are richer in lipids, and tend to utilise lipids during early embryonic development [35]. However, there are ex-

Table I. The mean amount of individual fatty acids in μg per mg total lipid (± SE) in eggs and/or larva of trout cod and Murray cod during development. Individual fatty acids with the same superscript, for each species, are not significantly different ($P > 0.05$) between developmental stages. Fe – fertilized eggs; Nhl – newly hatched larvae; Ysrl – yolk-sac-resorbed larvae. 18:3(n-6), 20:4(n-3) and 22:5 (n-6) were found in trace amount. 16:1(n-9) and 20:1(n-9) were not detected.

Fatty acid	Trout cod			Murray cod		
	Fe	Nhl	Ysrl	Fe	Nhl	Ysrl
14:0	4.9 ^{a} ± 0.6	4.1 ^{ab} ± 0.9	3.3^{a} ± 0.4	3.9 ± 1.2	2.4 ± 0.2	4.3 ± 0.3
16:0	105.2 ^{ab} ± 1.8	107.4^{b} ± 1.4	101.5 ^{a} ± 1.6	102.3 ± 3.0	98.4 ± 2.2	104.3 ± 3.2
18:0	72.1 ± 1.7	74.9 ± 1.2	72.9 ± 1.0	70.4 ± 2.4	69.2 ± 2.4	74.2 ± 2.4
20:0	0.8 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.8 ^{ab} ± 0.0	0.6 ^{a} ± 0.1	1.2 ^{b} ± 0.1
Σ saturates	183.2 ^{ab} ± 3.2	187.4 ^{b} ± 3.2	178.7 ^{a} ± 2.5	177.7 ± 4.6	170.9 ± 3.8	184.1 ± 5.4
16:1n-7	36.0 ^{b} ± 2.4	32.7 ^{b} ± 2.9	20.5 ^{a} ± 1.3	32.9 ^{b} ± 2.9	27.2 ^{ab} ± 1.8	24.9 ^{a} ± 1.6
18:1n-9	114.2 ^{b} ± 1.3	117.0 ^{b} ± 1.0	107.4 ^{a} ± 1.0	117.5 ± 2.5	114.4 ± 1.4	113.6 ± 2.5
18:1n-7	56.8 ^{b} ± 2.5	52.9 ^{b} ± 2.8	44:8 ^{a} ± 2.4	47.0 ± 2.8	43.6 ± 1.4	41.8 ± 1.4
20:1n-9	7.4 ^{b} ± 0.6	7.0 ^{b} ± 0.6	5.3 ^{a} ± 0.4	3.7 ^{b} ± 0.2	2.7 ^{a} ± 0.2	3.6 ^{b} ± 0.2
Σ monoenes	214.6 ^{b} ± 5.8	209.7 ^{b} ± 7.1	178.2 ^{a} ± 4.7	193.1 ± 7.8	188.0 ± 3.5	184.1 ± 5.3
18:2n-6	56.4 ^{b} ± 4.0	51.2 ^{b} ± 3.0	36.7 ^{a} ± 2.2	63.6 ± 4.7	55.1 ± 4.4	53.4 ± 0.8
18:3n-3	8.9 ^{b} ± 0.7	7.4 ^{ab} ± 0.4	6.3 ^{a} ± 0.8	11.0 ± 3.4	9.0 ± 2.5	7.6 ± 1.6
18:4n-3	2.4 ^{b} ± 0.1	1.7 ^{a} ± 0.1	1.2 ^{a} ± 0.0	3.7 ± 0.3	3.0 ± 0.4	2.8 ± 0.4
20:2n-6	5.5 ± 0.6	4.8 ± 0.4	4.5 ± 0.4	4.1 ± 0.8	3.5 ± 0.5	3.7 ± 0.5
20:3n-3	27.5 ^{b} ± 2.3	19.4 ^{a} ± 1.0	31.3 ^{b} ± 2.2	28.3 ^{ab} ± 1.7	35.2 ^{b} ± 3.1	26.5 ^{a} ± 3.0
20:3n-6	20.2 ^{b} ± 1.1	18.9 ^{b} ± 0.9	15.2 ^{a} ± 1.0	17.9 ± 2.8	14.2 ± 2.1	17.4 ± 2.8
20:4n-6	135.2 ± 7.7	145.5 ± 7.6	146.9 ± 4.0	118.2 ^{a} ± 7.3	110.2 ^{a} ± 9.4	154.1 ^{b} ± 12.1
20:5n-3	29.4 ± 3.0	31.1 ± 2.4	24.9 ± 1.4	19.8 ± 2.9	18.8 ± 2.8	21.1 ± 1.5
22:4n-6	22.5 ± 1.6	22.7 ± 1.2	21.9 ± 1.3	16.5 ^{ab} ± 0.9	13.5 ^{a} ± 1.1	19.8 ^{b} ± 1.9
22:5n-3	69.1 ^{b} ± 5.7	66.9 ^{b} ± 4.6	48.5t^{a} ± 2.5	48.7 ± 5.6	38.0 ± 4.2	43.5 ± 5.4
22:6n-3	158.4 ± 10.2	158.7 ± 8.2	155.7 ± 5.7	143.8 ± 10.9	130.6 ± 11.8	164.2 ± 10.4
Σ n-3	296.4 ± 18.0	285.4 ± 15.0	267.5 ± 8.4	255.4 ± 18.3	234.7 ± 17.2	265.9 ± 13.3
Σ n-6	240.0 ± 14.2	243.2 ± 12.5	225.3 ± 8.1	220.5 ^{ab} ± 11.2	197.0 ^{a} ± 14.2	248.5 ^{b} ± 15.7
Σ PUFA	536.4 ± 30.9	528.6 ± 26.6	492.9 ± 16.1	476.0 ± 28.7	431.8 ± 30.6	514.5 ± 27.7

ceptions to this rule and these have been discussed by Desvilettes et al. [16]. Trout cod and Murray cod eggs tend to conform to the general pattern, in that eggs lacking oil globules, are relatively low in total lipid (6.7 and 7.4 %, respectively [26]), and lipids are sparsely utilised in early ontogeny. On the other hand, in gilthead sea bream, *Sparus aurata*, free amino acids (FAA) were the main energy substrate during egg stage, and fatty acids of neutral lipids provided the main metabolic fuel after hatching [40]. At the other end of the spectrum are species which use FAA as the primary source of energy during embryogenesis such as, for example, Atlantic cod and Atlantic halibut [22, 24]. Clearly, with respect to freshwater species, we are far from discerning general patterns of energy utilisation during early ontogeny in relation to egg characteristics unlike in the case of marine species [23], and therefore, this warrants further investigation.

With regard to fatty acids, in relation to early development in fish, apart from a few exceptions [15, 59], the great majority of studies have been carried out on marine species. In both trout cod and Murray cod, PUFA (both n-3 and n-6) was the predominant group of fatty acids in total lipid, and accounted for over 50 % of the fatty acids in the total lipid in all the developmental stages investigated. Predominance of PUFA in total lipid in eggs and larvae has also been reported for chinook salmon [4], Atlantic halibut [19], whitefish [49] and pike [16]. On the other hand, species in which lipids are utilised as the major energy source during ontogeny, saturates and monoenes have been found to be the predominant group of fatty acids, as in Senegal sole [55] and striped bass [9, 27], respectively. The n-3 to n-6 ratio in trout cod and Murray cod during developmental stages is typical of freshwater fish [29].

With regard to the fatty acid profiles of eggs and larvae of fish, there are conflicting observations with respect to those which occur in small amounts (< 0.1 % of total lipid). For example, in both trout cod and Murray cod, in all the developmental stages investigated, 22:4(n-6) was found in significant amounts and 22:5(n-6) was detected only in trace amounts. This observation is in agreement with that of Anderson et al. [2] on the eggs of six Australian fish species (including Murray cod). On the other hand, 22:5(n-6) has not been reported in all species investigated. For example, 22:5(n-6) was reported in eggs and larvae of Atlantic halibut [8] and Senegal sole [38] but not in Atlantic cod [21], turbot [47] and pike [15]. Similarly, 18:3(n-6) has not always been reported in eggs and larvae of fish, and when present, it has accounted for less than 0.5 % of fatty acids in total lipid [8, 21]. Observations on 20:3(n-3) and 20:4(n-3) are also conflicting, the former being reported in Senegal sole [38], turbot [47] and the two Percichthyid

Table II. The mean amount of individual fatty acids (± SE) in µg per egg and/or larva during development in trout cod and Murray cod. For each species, individual fatty acids with the same superscript, occur in quantities that are not significantly different ($P > 0.05$) between developmental stages from each other. Fe – fertilized eggs; Nhl – newly hatched larvae; Ysrl – yolk-sac-resorbed larvae.

Fatty acid	Trout cod			Murray cod		
	Fe	Nhl	Ysrl	Fe	Nhl	Ysrl
14:0	5.4 ^{b} ± 0.6	4.1 ^{ab} ± 0.7	2.5 ^{a} ± 0.34	4.1 ± 1.3	2.9 ± 0.4	3.6 ± 0.3
16:0	118.6 ^{b} ± 3.5	116.3^{b} ± 4.9	76.9 ^{a} ± 2.6	100.9 ^{ab} ± 4.4	113.2 ^{b} ± 7.7	90.1 ^{a} ± 6.0
18:0	81.7 ^{b} ± 3.7	81.4 ^{b} ± 3.9	55.2 ^{a} ± 1.8	69.1 ^{a} ± 1.8	78.5 ^{b} ± 2.1	63.8 ^{a} ± 3.4
20:0	0.9 ^{ab} ± 0.0	1.0 ^{b} ± 0.1	0.7 ^{a} ± 0.0	0.8 ± 0.0	0.7 ± 0.1	1.0 ± 0.0
Σ saturates	206.7 ^{b} ± 7.0	202.9 ^{b} ± 8.8	135.4 ^{a} ± 4.4	175.0 ^{ab} ± 6.5	195.4 ^{b} ± 10.1	158.6 ^{a} ± 9.4
16:1n-7	40.3 ^{b} ± 2.7	34.8 ^{b} ± 2.7	15.4 ^{a} ± 1.0	32.2 ^{b} ± 3.3	31.6 ^{b} ± 3.4	21.8 ^{a} ± 2.3
18:1n-9	128.8^{b} ± 3.9	126.8 ^{b} ± 5.4	81.3 ^{a} ± 2.4	115.7 ^{b} ± 3.8	131.1 ^{b} ± 6.9	98.3 ^{a} ± 6.4
18:1n-7	63.9^{c} ± 3.1	56.6 ^{b} ± 2.7	33.6 ^{a} ± 1.6	46.4 ^{ab} ± 3.5	50.3 ^{b} ± 3.9	36.4 ^{a} ± 2.8
20:1n-9	8.3 ^{c} ± 0.7	7.4^{bc} ± 0.5	4.0 ^{a} ± 0.3	3.6 ^{b} ± 0.2	3.0 ^{a} ± 0.1	3.0 ^{a} ± 0.1
Σ monoenes	241.5 ^{b} ± 8.5	225.8 ^{b} ± 9.6	134.5 ^{a} ± 4.2	198.4 ^{b} ± 9.5	216.0 ^{b} ± 13.7	159.7 ^{a} ± 11.4
18:2n-6	62.6 ^{b} ± 3.6	54.3 ^{b} ± 2.3	27.2 ^{a} ± 1.0	63.1 ^{b} ± 5.9	64.1^{b} ± 8.0	46.4 ^{a} ± 3.2
18:3n-3	9.9 ^{b} ± 0.8	7.9 ^{b} ± 0.4	4.6 ^{a} ± 0.5	11.2 ^{b} ± 3.7	11.2 ^{b} ± 3.8	6.9 ^{a} ± 1.8
18:4n-3	2.7 ^{c} ± 0.1	1.8 ^{b} ± 0.0	0.9 ^{a} ± 0.0	3.6 ^{b} ± 0.3	3.3 ^{ab} ± 0.4	2.4 ^{a} ± 0.3
20:2n-6	6.0 ^{b} ± 0.5	5.0 ^{b} ± 0.2	3.3 ^{a} ± 0.2	4.1 ± 0.9	4.2 ± 0.8	3.3 ± 0.6
20:3n-3	32.1 ^{b} ± 3.6	21.0 ^{a} ± 1.3	23.5 ^{a} ± 1.4	28.2 ^{a} ± 2.3	41.0 ^{b} ± 4.8	24.0 ^{a} ± 3.7
20:3n-6	23.0 ^{b} ± 1.7	20.4 ^{b} ± 1.1	11.4 ^{a} ± 0.7	17.2 ± 2.5	15.4 ± 1.9	14.6 ± 2.5
20:4n-6	151.3 ^{b} ± 8.9	155.3 ^{b} ± 7.2	110.4 ^{a} ± 2.7	115.8 ± 6.4	124.8 ± 9.7	129.6 ± 6.0
20:5n-3	33.6 ^{b} ± 3.8	33.2 ^{b} ± 2.4	18.9 ^{a} ± 1.2	19.7 ± 3.3	22.1 ± 4.9	18.3 ± 1.9
22:4n-6	25.1 ^{b} ± 1.7	24.2 ^{b} ± 1.1	16.3 ^{a} ± 0.7	16.1 ± 0.6	15.0 ± 0.5	16.6 ± 1.4
22:5n-3	77.7 ^{b} ± 7.1	70.9 ^{b} ± 3.6	36.4 ^{a} ± 2.0	47.2 ± 4.8	44.2 ± 3.0	36.7 ± 4.5
22:6n-3	178.3 ^{b} ± 13.1	169.8 ^{b} ± 8.0	116.8 ^{a} ± 4.3	141.9 ± 12.5	150.2 ± 17.1	139.3 ± 6.6
Σ n-3	334.5 ^{b} ± 24.2	304.9 ^{b} ± 14.0	201.3 ^{a} ± 6.2	252.0 ± 21.1	270.3 ± 27.7	227.7 ± 13.6
Σn-6	271.3 ^{b} ± 15.3	259.3 ^{b} ± 11.3	186.8 ^{a} ± 4.2	216.5 ± 10.8	224.0 ± 16.7	210.7 ± 10.3
Σ PUFA	602.7 ^{b} ± 38.8	564.2 ^{b} ± 24.2	370.1 ^{a} ± 18.9	468.6 ± 31.6	494.4 ± 44.0	438.5 ± 22.4

fishes studied here. These fatty acids, however, were not found in Atlantic cod [54] nor in a previous study on six Australian fish species [2].

In most fish species, the fatty acids that are found in highest amounts are generally 16:0 in herring [53] and Senegal sole [55], 18:1(n-9) in chinook salmon [4] and striped bass [9, 27], and 22:6(n-3) in almost all other species that have been investigated, including trout cod and Murray cod. However, it is interesting to note that the proportion of 20:4(n-6) in total lipid in eggs and developing larvae of trout cod and Murray cod was considerably higher than that reported for other fish species. One reason for this may be the feeding of broodstock with ox liver 2 months prior to spawning.

Furthermore, as development proceeded the amount of 20:4(n-6) in total lipid increased in both species, and significantly so in Murray cod. This may suggest that broodstock diet may not necessarily be the only factor responsible for an elevated level of 20:4(n-6) in the two Percichthyid species investigated. Such increases were concomitant with decreases in 18:2(n-6) and 20:3(n-6) but not significantly in Murray cod. This may be indicative of biosynthesis of 20:4(n-6) during early ontogeny of Percichthyid fish. 20:4(n-6) has been shown to be a precursor for the synthesis of prostaglandins in marine fish [44]. There is no reason to believe that freshwater fish are different in this regard. Similarly and more recently, the influence of 20:4(n-6) on growth and pigmentation in the Japanese flounder *Paralichthys olivaceus* has been demonstrated [18]. The possible conservation and likely bioconversion of other homologues to 20:4(n-6) may be an indication of its essentiality in later development in trout cod and Murray cod, and its possible use structurally.

It was also evident that in the two Percichthyid species DHA was conserved in early ontogeny. In trout cod, the amount of 18:3(n-3), 18:4(n-3) and 22:5(n-3), all of which are generally conceived to be precursors of DHA, decreased significantly with development. The corresponding decreases in Murray cod were not statistically significant, however. According to Sargent [43], the conclusions of which were based mostly on marine and anadromous fish, major n-3 HUFA of all fish eggs are DHA and 20:5(n-3), generally found in a ratio of approximately 2:1. However, in trout cod and Murray cod, the above ratio was 5.4:1 and 7.3:1, respectively, and remained almost unchanged throughout development. It has been shown that in fish, of all the n-3 PUFA, DHA has the generalised function in maintaining structural and functional integrity of cell membranes [43]. DHA is also known to be essential for the development of the brain and the retina in marine fish [5, 37]. As such, one would expect to encounter high levels of this fatty acid in fish tissues, as observed in the two Percichthyids. On the other hand, in Senegal sole [55], significant reduction of DHA, 20:5(n-3) and 22:5(n-3) were observed with development. It may be that different fish species

and/or species groups have different strategies in the utilisation of n-3 PUFA.

The fatty acid requirements of freshwater species, apart from a few exceptions are known to be different from marine fish. In general, freshwater fish have an essentiality for both n-3 and n-6 PUFA, in the form of 18:3(n-3) and 18:2(n-6), whereas marine fish require C20 and C22 HUFA [29, 44]. The fatty acid composition of eggs and associated changes in composition during development are thought to be indicative of the fatty acid requirements for growth and well being [43]. Based on the retention of C20 and C22 HUFA of both n-3 and n-6 series by developing eggs and larvae of trout cod and Murray cod, it may be concluded that these species have an equally important requirement for n-6 as for n-3. Based on early studies on freshwater fish, the consensus has been that freshwater fish require 18:2(n-6) and 18:3(n-3) as they have the ability to convert these to the higher homologues [33, 51].

In both Percichthyid fishes, there was a tendency to retain PUFA. Such selective retention of yolk PUFA through early development is not uncommon in freshwater fish, as observed for steelhead trout [28], goldfish [59] and pike [15]. On the other hand, Henderson et al. [30] demonstrated that pike was unable to synthesise 20:4(n-6) and 20:5(n-3) from 18:2(n-6) and 18:3(n-3), respectively, and suggested that pike may require the former fatty acids preformed in the diet. Desvilettes et al. [15] based on their study on larval pike suggested that larval pike may be able to biosynthesise 22:6(n-3) and that this trait may have developed to counteract rather unpredictable changes in abundance of microcrustacean food resources in nature, which are its main sources of C20 and C22 HUFA. First feeding larvae of trout cod and Murray cod [34, 42] feed on microcrustaceans, and the conditions that these fish have to face in nature are possibly not that different to those of pike. It is, therefore, conceivable that the hypothesis is extendable to trout cod and Murray cod, two top order carnivores in Australian freshwaters, but requires further study for confirmation of the hypothesis. However, and in spite of the possible similarity between top-order freshwater carnivores and marine fish [45], in their requirements for C20 and C22 in the diet, the overall fatty acid profiles of the former appear to conform to those typical of freshwater fish with a n-3 to n-6 ratio less than 4.7 [29].

In conclusion, this study confirms that broad differences occur in the fatty acid profile, in relation to early ontogeny, in freshwater and marine fish species. The study also indicates that during early ontogeny top-order, freshwater carnivores may have different biosynthesising capabilities of PUFA from fish with other feeding habits.

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