

Fatty Acids in Six Small Pelagic Fish Species and Their Crustacean Prey from the Mindanao Sea, Southern Philippines

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Abstrak: Asid lemak adalah penting untuk kesihatan manusia dan berguna dalam analisis jaringan makanan marin, namun demikian maklumat tentang organisma pelagik tropikal adalah kurang. Enam spesies ikan pelagik kecil zooplanktivorous (*Decapterus kurroides*, *Decapterus macarellus*, *Selar crumenophthalmus*, *Sardinella lemuru*, *Spratilloides gracilis* dan *Stolephorus insularis*) dan empat mangsa krustasia zooplanktonik ikan tersebut [tiga spesies sergestoid (*Acetes erythraeus*, *Acetes intermedius* dan *Lucifer penicillifer*) dan satu spesies kopepoda kalonoid (*Acartia erythraea*)] telah dikumpul dari Laut Mindanao, dan kandungan asid lemak haiwan-haiwan tersebut telah diprofil. Profil-profil yang terhasil menunjukkan 17 asid lemak yang spesifik kepada spesies tertentu dan 9 {asid miristik [C14:0], asid palmitik [C16:0], asid stearik [C18:0]; asid palmitoleik [C16:1], asid oleik [C18:1n9c], asid linoleik [C18:2n6c], asid linolenik [C18:3n3], asid eikosapentaenoik (EPA) [C20:5n3] dan asid dokosaheksaenoik (DHA) [C22:6n3]} yang am bagi semua spesies. Analisis kluster dan penskalaan multidimensi bukan-metrik (NMDS) asid lemak telah menunjukkan persamaan yang tinggi dalam profil semua spesies, tetapi kluster yang berasingan telah diperolehi untuk ikan dan zooplankton. Jumlah kandungan *n*-3 asid lemak spesies makrel (*D. macarellus*, *D. kurroides* dan *S. crumenophthalmus*) menyamai pemangsa Acetes. Kopepoda *A. erythraea* dan sergestoid *L. penicillifer* telah menunjukkan nilai nisbah EPA:DHA yang paling rendah, yang besar kemungkinan akibat tabiat pemakanan phytoplanktivorous mereka, namun demikian nilai tertinggi nisbah tersebut dalam Acetes mencadangkan kemasukan detritus tumbuhan dalam diet mereka. Nilai DHA seakan mengesahkan pautan trofik antara kopepoda, Acetes dan spesies makrel.

Kata kunci: Asid Lemak, Jaringan Makanan, Tropikal, Zooplankton, Ikan Pelagik Kecil, Laut Mindanao

Abstract: Fatty acids are important in human health and useful in the analysis of the marine food web, however information on tropical pelagic organisms is scarce. Six zooplanktivorous small pelagic fish species (*Decapterus kurroides*, *Decapterus macarellus*, *Selar crumenophthalmus*, *Sardinella lemuru*, *Spratilloides gracilis* and *Stolephorus insularis*) and four of their zooplanktonic crustacean prey [three sergestoid species (*Acetes erythraeus*, *Acetes intermedius* and *Lucifer penicillifer*) and one calanoid copepod (*Acartia erythraea*)] were collected from the Mindanao Sea, and their fatty acids were profiled. The resulting profiles revealed 17 fatty acids that were specific to certain species and 9 {myristic acid [C14:0], palmitic acid [C16:0], stearic acid [C18:0]; palmitoleic acid [C16:1], oleic acid [C18:1n9c], linoleic acid [C18:2n6c], linolenic acid [C18:3n3], eicosapentaenoic acid (EPA) [C20:5n3] and docosahexaenoic acid (DHA) [C22:6n3]} that were common to all species. Cluster analysis and non-metric multidimensional scaling (NMDS) of fatty acids indicate a high similarity in profiles in all species, but separate fish and zooplankton clusters were obtained. Mackerel species (*D. macarellus*, *D. kurroides* and *S. crumenophthalmus*) had concentrations of total *n*-3 fatty acids that match those of

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their *Acetes* prey. The copepod *A. erythraea* and the sergestoid *L. penicillifer* exhibited the lowest values of the EPA:DHA ratio, which was most likely due to their phytoplanktivorous feeding habits, but the occurrence of the highest values of the ratio in *Acetes* suggests the inclusion of plant detritus in their diet. DHA values appear to affirm the trophic link among copepod, *Lucifer*, *Acetes* and mackerel species.

Keywords: Fatty Acids, Food Webs, Tropical, Zooplankton, Small Pelagic Fishes, Mindanao Sea

INTRODUCTION

Fatty acids are fundamental biomolecules and have been used as trophic biomarkers in marine food web analysis (Elsdon 2010; El-Sabaawi *et al.* 2009; Hall *et al.* 2006). The concept is based on the assumption that certain fatty acids, specifically polyunsaturated fatty acids (PUFA), can only be biosynthesised by certain species of phytoplankton and macroalgae and can be traced as essential dietary components to higher trophic levels such as zooplankton (van der Meeren *et al.* 2008) and fish (Jackson *et al.* 2007). Fatty acid biomarkers complement the use of carbon and nitrogen stable isotopes (Boecklen *et al.* 2011) and DNA-based techniques (Traugott *et al.* 2013) in trophic ecological studies.

Fatty acids, particularly omega-3 types, in many marine food organisms are now recognised as one of the benefits provided by marine biodiversity to humans (Lloret 2010). More attention is focused on two omega-3 PUFA, docosahexaenoic acid (DHA) and eicosahexaenoic acid (EPA), because they are known to be crucial to human nutrition and health as a result of their influence on the proper functioning of cardiovascular, renal, nervous, immune and reproductive systems in humans (Sahena *et al.* 2009; Crawford *et al.* 1999). The PUFAs are also important in the growth, feeding and reproduction aspects of the aquaculture (Sargent *et al.* 2002) and the food supplement industries (Sahena *et al.* 2009).

Levels of fatty acids can be variable: for example, fish PUFA is usually stored in most organs except during the spawning season, when PUFA is mobilised (Saito *et al.* 1999). It can also be modified in association with migratory behaviour (Osako *et al.* 2006). However, high DHA levels may be maintained year-round in some fish species (Osako *et al.* 2006). Apart from seasonal variation, levels of fatty acids also differ between latitudes, with species from high latitudes having higher amounts of PUFA than tropical low-latitude species (Huynh & Kitts 2009; Garrido *et al.* 2008).

Most studies on fatty acids in tropical fish have focused on coral reef fish (e.g., Osako *et al.* 2006; Saito *et al.* 1999; Belling *et al.* 1997) and potential species for aquaculture (Mohd Yusof *et al.* 2010; Ogata *et al.* 2004). The limited information available for tropical pelagic fishes is confined to species belonging to the Family Scombridae, particularly *Thunnus tonggol*, *Thunnus thynnus*, *Thunnus alalunga*, *Thunnus albacares*, *Euthynnus pelamis*, *Euthynnus affinis*, *Auxis rochei* and *Auxis thazard* of the Tribe Thunnini (Osako *et al.* 2009; Saito *et al.* 2005), and, recently, the sardine *Sardinella lemuru* (Khoddami *et al.* 2009).

Pelagic finfishes contribute significantly to the diet of many people of Southeast Asia [Food and Agriculture Organization (FAO) 2013]. In the Philippines alone, the population relies on marine fish for approximately 50% of their protein, and this reliance could reach up to 80%, particularly in municipal coastal areas (Savina & White 1986). In 2003, pelagic finfishes including tuna, sardines and round scads contributed an estimated total of US\$20 million to the Philippine economy [Bureau of Agricultural Statistics (BAS) 2011]. In this study, fatty acids were analysed in six zooplanktivorous small pelagic finfishes and four of their known zooplankton prey collected from the Mindanao Sea, southern Philippines to provide baseline information and infer the trophic positions of these species in the food chain. The association between fish and prey with respect to their fatty acid profiles was analysed using multivariate analysis. Possible feeding habits of the species analysed are inferred using known fatty acid trophic biomarkers.

MATERIALS AND METHODS

Collection of Samples

Samples for this study were collected between April and May 2009 from coastal marine waters off Dipolog City (8.549°N–8.554°N, 123.218°E–123.259°E), which is located on the southwestern part of the Mindanao Sea, Republic of the Philippines. Details of sampled species are shown in Table 1. Fresh samples of six fish species (*Decapterus kurroides*, *Decapterus macarellus*, *Selar crumenophthalmus*, *S. lemuru*, *Spratilloides gracilis* and *Stolephorus insularis*) common to the Mindanao Sea were purchased from local artisanal fishers immediately after landing. All fish species were collected by hook and line, which kept their bodily damage to a minimum. Muscle tissue with intact skin was filleted out from the shoulder to tail of individual fish and immediately placed in sealed Styrofoam containers, frozen at –20°C within 10–15 min and stored at –80°C until lipid extraction. Samples from four individual fish were pooled to represent one composite sample of a species. The calanoid copepod, *Acartia erythraea* and the sergestid *Lucifer penicillifer* were collected using a 279 µm mesh (General Oceanics, Florida, USA) conical plankton net with a mouth diameter of 0.32 m and a closed cod-end, which minimises damage to the animals (Omori & Ikeda 1984). Zooplankton samples were collected by horizontal towing for 3 minutes at the lowest running speed (1.5 knots) of a motorised outrigger canoe. Bulk plankton samples, which were dominated by the two target zooplankton species, were carefully filtered through a net with 1 mm nylon mesh. Target species were immediately sorted out, frozen and stored in the same manner as the fish samples. The two sergestid species of *Acetes* (*A. erythraeus* and *A. intermedius*) were collected using a fisherman-designed 4 mm mesh triangular push-net that was mounted astern on a motorised outrigger canoe with the apex of the triangle pointing towards the boat. *Acetes* collection was achieved by submerging the entire net at a subsurface depth while the canoe ran at its lowest speed. *Acetes* samples were frozen and stored in the same manner as the fish and other zooplankton samples. All frozen fish and zooplankton samples were freeze dried

prior to lipid extraction and fatty acid analysis, which were performed within two weeks after sample collection.

Table 1: Common name, scientific name and mean length of the zooplankton and finfish species analysed in this study.

| Common name | Scientific name | Length (cm) | Number of individuals per sample |
|-----------------------------|--------------------------------------------------------|-------------|----------------------------------|
| Finfish | | | |
| Big eye scad | <i>Selar crumenophthalmus</i> (Bloch, 1793) | 15.7–18.6 | 5 |
| Mackerel scad | <i>Decapterus macarellus</i> (Cuvier, 1833) | 13.6–14.9 | 5 |
| Redtail scad | <i>Decapterus kurroides</i> (Bleeker, 1855) | 10.8–12.6 | 10 |
| Bali sardine | <i>Sardinella lemuru</i> (Bleeker, 1853) | 10.3–13.8 | 12 |
| Silver-stripe round herring | <i>Spratilloides gracilis</i> (Temm. & Schlegel, 1846) | 7.9–8.7 | 24 |
| Gold estuarine anchovy | <i>Stolephorus insularis</i> (Hardenberg, 1933) | 6.9–7.7 | 24 |
| Zooplankton | | | |
| Ghost shrimp | <i>Acetes erythraeus</i> (Nobili, 1905) | 2.0–4.1 | 182–195 |
| Ghost shrimp | <i>Acetes intermedius</i> (Omori, 1975) | 1.8–2.7 | 210–265 |
| Ghost shrimp | <i>Lucifer penicillifer</i> (Hansen, 1919) | 0.95–1.10 | 490–550 |
| Calanoid copepod | <i>Acartia erythraea</i> (Giesbrecht, 1892) | 0.049–0.054 | 95–116.6 ^a |

Note: Fish length is from tip of snout to the base of the tail peduncle; shrimp length is from the tip of rostrum to the tip of telson; copepod length is from the median anterior tip to the median posterior tip of the prosome; a – values × 1000

Fatty Acid Analysis

Lipids were extracted from a homogenised sample by a modified one-phase chloroform-methanol-water (CHCl₃-MeOH-H₂O) following the Bligh and Dyer (1959) method. The fatty acid composition of the animals was analysed after saponification and esterification of an aliquot of the total lipid extract. An aliquot of the total solvent extract was treated with methanol-hydrochloric acid-chloroform under nitrogen at –80°C for 2 hours to form fatty acid methyl ester (FAME). Following the addition of water, FAME and free sterols were extracted into hexane/chloroform, transferred to vials, reduced under a stream of nitrogen and stored in chloroform. Identification and quantification of FAME compounds was performed using a gas chromatograph (GC-Shimadzu-148, Shimadzu Corporation, Kyoto, Japan) equipped with a 100 m, 0.25 mm inner diameter SP2560 fused silica capillary column. FAME was detected through a flame ionisation detection system, and retention time and mass spectral data were compared with those of the external standard Supelco 37 Component FAME Mixture (10 mg/ml in methylene chloride) (Sigma-Aldrich, Pennsylvania, USA). The fatty acid profile was determined as FAME concentration, which was expressed as percentage of total fatty acids computed from the conversion of area percentage to weight percentage with correction factors.

Data Analysis

The concentrations of the different fatty acids from two replicate composite samples were computed as average \pm standard deviation. The fatty acid profile among species was analysed using log (x+1) transformed values of fatty acid concentrations, and the Bray-Curtis similarity index (average linkage) with the analysis of similarity (ANOSIM) Global R statistic was computed using the PRIMER-E software (Clarke & Warwick 2001). The ANOSIM statistic was used to test the significance of the variation in the structure of the fatty acid profiles of the different species. Non-parametric statistical tests available in SPSS for Windows version 11 software (SPSS Inc. 2002) were used to test differences in fatty acid levels among species (Kruskal-Wallis H test) and between two species (Mann-Whitney U test).

RESULTS AND DISCUSSION

Seventeen types of fatty acids were observed in this study (Table 2). The highest values of total saturated fatty acids (SFAs) were comparable ($H = 9.64$, $df = 9$, $p > 0.05$) in all species. The palmitic SFA showed highest values among all fatty acids and this was consistent in all species examined. The two anchovy species (*S. insularis* and *S. gracilis*), however, appeared to have the highest levels of palmitic acid ($H = 18.73$, $df = 9$, $p < 0.04$). The second most abundant SFA was stearic acid, which was highest among the three scad species (*S. crumenophthalmus*, *D. macarellus* and *D. kurroides*) ($H = 18.41$, $df = 9$, $p < 0.05$). These two SFAs have been reported to have the highest concentrations in fishes (Elsdon 2010; Sahena *et al.* 2009), *Acetes* (Montaño *et al.* 2001) and in copepods (van der Meer *et al.* 2008). The predominance of both fatty acids has been attributed to their use as a major source of energy for metabolism and growth (Sargent *et al.* 2002). Hale (1984) reported the highest palmitic and stearic acids concentration for the round scad *Decapterus punctatus* from the Atlantic Bight and Gulf of Mexico. Turan *et al.* (2007) reported highest palmitic acid values for anchovy meal from Turkish waters, but their values are two-fold lower than the values in this study. Although they did not explain their findings, Zlatanov and Laskaridis (2007) reported highest levels of palmitic acid in the anchovy *Sprattelloides gracilis* compared to the sardine and picarel fish species examined, and their reported value of 38.85% is comparable to the values reported in this study. Fishes from warm waters tend to show high levels of palmitic and stearic acids compared to those from cold waters. This difference is due to metabolic differences between cold and warm water species, because these fatty acids are not usually subject to differences in diet (Ackman & Eaton 1966 as cited by Huynh & Kitts 2009).

The monounsaturated fatty acids (MUFA) were the third most abundant fatty acids, with highest values for oleic acid (Table 2). This is in agreement with findings in copepod (Olivotto *et al.* 2010), *Acetes* (Montaño *et al.* 2001) and fish fatty acid profiles (Elsdon 2010; Huynh & Kitts 2009; Sahena *et al.* 2009; Sirot *et al.* 2008). Oleic MUFA is naturally occurring in large concentrations in many marine organisms, which can also synthesise this MUFA *de novo* (Sargent *et al.*

2002). The three mackerel species showed slightly higher amounts compared to the rest of species analysed ($U = 83.50$, $df = 1$, $p < 0.0001$). Hale (1984) reported highest levels of the oleic MUFA in *D. punctatus*, and the values reported are comparable to the values reported in this study. The MUFA were lowest among the small zooplankton species (*A. erythraea* and *L. penicillifer*) ($H = 18.81$, $df = 9$, $p < 0.03$) which is in agreement with the findings of van der Meeren *et al.* (2008) where total MUFA is twofold lower in concentration than total SFA and fivefold lower than total PUFA.

Except for *A. intermedius*, DHA in the rest of analysed species showed the highest concentration among PUFA, followed by EPA (Table 2). Higher EPA than DHA concentrations in *A. intermedius* may be a species-specific attribute. These two PUFAs are also known as highly unsaturated fatty acids (HUFA). The copepod *A. erythraea* was found to have the highest DHA concentration, followed by the two *Decapterus* species, and the third highest was shown in *A. erythraeus* ($H = 18.62$, $df = 9$, $p < 0.03$). Overall, copepods show the highest levels of PUFA, which makes them ideal as live food in fish larviculture (Ajiboye *et al.* 2010; Sargent *et al.* 2002). The DHA concentrations in the two *Decapterus* species were twice as low as those reported by Hale (1984) for the congener *D. punctatus*. Species differences may explain the contrasting DHA levels of *A. intermedius* and *A. erythraeus* ($U = 6.00$, $df = 1$, $p < 0.05$), but the levels found in the latter species is comparable to those in *Acetes* sp. obtained by Montaña *et al.* (2001). However, in terms of total PUFA, the highest values were shown by the two *Acetes* species and the copepod *A. erythraea*, while the lowest values were observed in the anchovy *S. insularis* and *S. lemuru* ($H = 18.70$, $df = 9$, $p < 0.03$). The low levels in the latter fish species may be explained by seasonality in PUFA levels (Khoddami *et al.* 2009; Gopakumar 1974). Feeding on copepods by *Acetes* may explain their highest total PUFA levels (Metillo 2011). The copepod *A. erythraea* exhibited the highest DHA, which could possibly be traced back to its primary consumer trophic position of grazing on primary producers (El-Sabaawi *et al.* 2009). The species showed <1 EPA:DHA ratio, which suggests that this species most likely feeds more on flagellates such as pico- and nanoflagellates (Elsdon 2010; El-Sabaawi *et al.* 2009). Flagellates have been considered to contribute significantly to the diet and elevated levels of PUFA in copepods (van der Meeren *et al.* 2008). Except for the two *Acetes* species and all fish species, the two microparticulate feeding copepod and *L. penicillifer* have <1 EPA:DHA ratio, which indicates a feeding ecology traceable to a mainly flagellates-based primary production or the microbial loop system.

Cluster analysis of the relative amounts of these fatty acids showed a high similarity ($>80\%$) among species, but two groups were identified in the dendrogram with one cluster composed of fish and the other of zooplankton [Fig. (1a)]. The latter had higher concentration of PUFA than in fish. The two zooplankton sub-groups were composed of two *Acetes* species and the microparticulate feeding copepod and *L. penicillifer*, while the three fish sub-clusters comprise of mackerels, anchovies and the sardine species *S. lemuru*. The non-metric multidimensional scaling (NMDS) output clearly differentiated three distinct groups, which suggest the possible trophic linkage between these groups where the two *Acetes* species assume an intermediate trophic position between

scombrid fish species and small zooplankton [Fig. (1b)]. *Acetes* are known prey of scombrids (Xiao & Greenwood 1993), while *Acetes* themselves feed on copepods (Metillo 2011) and possibly on *L. penicillifer*. The three zooplanktivorous clupeiform fish species are known copepod feeders, and possibly also feed on the larval stages of *Acetes*. The NMDS plot shows that these fish species are on the same level as the two small zooplankton species. This study demonstrates that fatty acids are useful trophic biomarkers, however other techniques including stable isotopes analysis and DNA-based techniques (Traugott *et al.* 2013; Boecklen *et al.* 2011) may also be helpful as alternative approaches in elucidating trophic linkages among species analysed in this study.

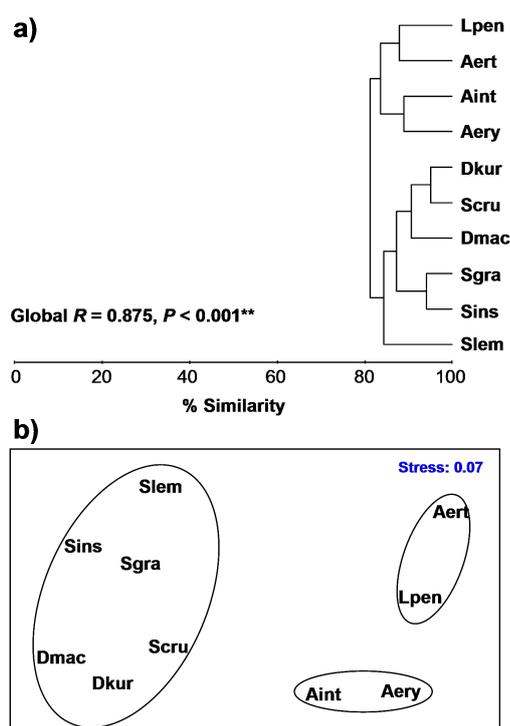


Figure 1: Multivariate analysis output of the fatty acid profiles of the six fish and four zooplankton species analysed in this study: a) Bray-Curtis dendrogram for the fatty acid profiles with highly significant ANOSIM Global R statistic; b) NMDS plot with excellent goodness of fit (stress = 0.07) (species codes: Slem – *Sardinella lemuru*, Sins – *Stolephorus insularis*, Sgra – *Spratilloides gracilis*, Dmac – *Decapterus macarellus*, Scru – *Selar crumenophthalmus*, Dkur – *Decapterus kurroides*, Aery – *Acetes erythraeus*, Aint – *Acetes intermedius*, Aert – *Acartia erythraea*, Lpen – *Lucifer penicillifer*).

Table 2: Mean (±SD) concentration (% of total fatty acids) of fatty acids in the four zooplankton and six small pelagic finfish species.

| Fatty acid as methyl ester | Zooplankton | | | | | | Small pelagic finfish | | | | | |
|----------------------------|------------------------------------|--------------------------------------|------------------------------------|-----------------------------------|----------------------------------------|-----------------------------------------|------------------------------------|---------------------------------------------|-------------------------------------|------------------------------------|--|--|
| | <i>Acartia erythraea</i> (copepod) | <i>Lucifer penicillifer</i> (shrimp) | <i>Acetes intermedius</i> (shrimp) | <i>Acetes erythraeus</i> (shrimp) | <i>Stolephorus insularis</i> (anchovy) | <i>Spratelloides gracilis</i> (anchovy) | <i>Sardinella lemuru</i> (sardine) | <i>Selar crumenoph.</i> ^a (scad) | <i>Decapterus macarellus</i> (scad) | <i>Decapterus kurroides</i> (scad) | | |
| C12:0 | 3.22±0.03 | trace | 0.23±0.01 | trace | 0.90±0.02 | 1.10±0.04 | 0.43±0.14 | ND | ND | ND | | |
| C14:0 | 5.79±0.03 | 2.60±0.00 | 3.75±0.35 | 3.55±0.07 | 7.50±0.04 | 7.70±0.02 | 15.30±0.21 | 6.00±0.18 | 5.30±0.18 | 4.30±0.03 | | |
| C15:0 | 1.32±0.02 | 1.49±0.02 | 0.82±0.04 | 1.30±0.01 | 1.84±0.04 | 2.03±0.02 | 1.12±0.02 | 1.80±0.04 | 1.12±0.00 | 1.24±0.06 | | |
| C16:0 | 31.92±0.03 | 33.86±0.93 | 36.95±0.35 | 33.45±1.34 | 48.10±1.02 | 43.70±0.61 | 37.60±0.87 | 39.00±0.05 | 40.70±0.98 | 40.30±0.97 | | |
| C17:0 | 3.02±0.35 | 3.95±0.11 | 2.15±0.03 | 1.96±0.02 | 1.32±0.04 | 1.21±0.03 | 1.59±0.06 | 2.33±0.05 | 0.96±0.13 | 1.04±0.10 | | |
| C18:0 | 12.58±0.14 | 11.55±0.25 | 10.65±0.64 | 9.15±0.07 | 11.30±0.13 | 11.40±0.55 | 9.40±0.53 | 17.00±0.21 | 13.20±0.08 | 18.90±0.63 | | |
| C20:0 | 0.86±0.01 | 1.20±0.11 | 0.44±0.04 | 0.52±0.16 | 0.48±0.03 | 0.60±0.02 | 0.42±0.02 | 0.33±0.00 | 0.44±0.11 | 0.42±0.01 | | |
| C22:0 | 2.54±0.12 | 2.08±0.20 | 0.36±0.00 | 0.83±0.20 | 0.37±0.03 | 0.36±0.01 | 0.27±0.00 | 0.33±0.04 | 0.25±0.01 | 0.30±0.01 | | |
| C24:0 | 5.07±0.77 | 10.94±1.40 | 0.36±0.32 | 0.90±0.09 | 0.58±0.14 | 0.65±0.11 | 0.60±0.02 | 0.63±0.00 | 0.46±0.03 | 0.37±0.01 | | |
| C16:1 | 1.69±0.11 | 2.57±0.41 | 10.54±0.60 | 7.16±1.00 | 5.12±0.11 | 4.51±0.03 | 8.89±0.32 | 5.37±0.04 | 4.70±0.04 | 3.63±0.03 | | |
| C18:1n-9 | 5.66±0.04 | 4.56±0.21 | 4.85±0.64 | 8.20±0.00 | 7.40±0.13 | 5.60±0.03 | 7.30±0.08 | 8.20±0.53 | 15.60±0.53 | 8.20±0.62 | | |
| C24:1 | trace | 1.60±0.13 | 6.35±0.53 | 4.17±0.35 | 4.86±0.07 | 5.01±0.03 | 3.83±0.12 | 4.01±0.17 | ND | 3.89±0.04 | | |
| C18:2n-6 | 4.29±0.07 | 2.66±0.26 | 1.55±0.21 | 3.35±0.64 | 1.40±0.04 | 1.60±0.22 | 1.20±0.63 | 1.30±0.17 | 0.90±0.06 | 0.90±0.07 | | |
| C18:3n-3 | 0.97±0.06 | 1.58±0.12 | 0.60±0.00 | 2.60±0.28 | trace | 0.30±0.01 | 0.40±0.13 | 0.20±0.00 | 0.40±0.04 | 0.50±0.00 | | |
| C20:3n-3 | trace | 3.27±0.33 | 1.06±0.06 | 1.46±0.43 | 1.13±0.04 | 1.15±0.11 | 1.33±0.03 | 1.51±0.01 | 0.93±0.04 | 1.01±0.01 | | |
| C20:5n-3 (EPA) | 1.39±0.05 | trace | 9.15±0.21 | 8.65±0.07 | 1.80±0.10 | 1.81±0.04 | 2.60±0.06 | 2.40±0.08 | 2.50±0.08 | 1.90±0.20 | | |
| C22:6n-3 (DHA) | 13.48±0.24 | 7.26±0.48 | 7.50±0.71 | 12.00±0.67 | 5.40±1.35 | 10.60±0.23 | 5.00±0.43 | 9.00±0.21 | 12.40±0.32 | 12.60±0.53 | | |
| Total SFA | 66.32 | 67.67 | 55.71 | 51.66 | 72.39 | 68.75 | 66.73 | 67.42 | 62.43 | 66.87 | | |
| Total MUFA | 7.35 | 8.73 | 21.74 | 19.53 | 17.38 | 15.12 | 20.02 | 17.58 | 20.3 | 15.72 | | |
| Total PUFA | 20.13 | 14.77 | 19.86 | 28.06 | 9.73 | 15.46 | 10.53 | 14.41 | 17.13 | 16.91 | | |
| Total n-3 | 15.84 | 12.11 | 18.31 | 24.71 | 8.33 | 13.86 | 9.33 | 13.11 | 16.23 | 16.01 | | |
| Total n-6 | 4.26 | 2.66 | 1.55 | 3.35 | 1.4 | 1.6 | 1.2 | 1.3 | 0.9 | 0.9 | | |
| EPA:DHA | 0.1 | 0 | 1.22 | 0.72 | 0.33 | 0.17 | 0.52 | 0.27 | 0.2 | 0.15 | | |

Note: SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; ND = not detected; trace = <0.001%; a = *crumenophthalmus*; common names of species are in parenthesis

CONCLUSION

Fatty acid profiles are similar among species but specific fatty acid types and their relative concentrations differed among species. Palmitic and stearic SFA fatty acids were highest in anchovy and scad species, respectively. Oleic MUFA was highest in scad and lowest in zooplankton. The DHA PUFA was highest among zooplankton, *Acetes* and scad. Ratios of EPA:DHA may be useful in deducing the trophic linkage among species in support of the use of fatty acids as trophic biomarkers.

ACKNOWLEDGEMENT

We are deeply grateful to the National Research Council of the Philippines, Department of Science and Technology for the research fund (NRCP # J-58); Dr. Maria Tranquilina Rachel Dolota Sanchez-Metillo for copy editing the manuscript; and the two anonymous reviewers for the constructive comments that further improved the quality of our manuscript.

REFERENCES

- Ackman R G and Eaton C A. (1966). Some commercial Atlantic herring oils: Fatty acid composition. *Journal of the Fisheries Research Board of Canada* 23(7): 911–917.
- Ajiboye O O, Yakubu A F, Adams T E, Olaji E D and Nwogu N A. (2010). A review of the use of copepods in marine fish larviculture. *Reviews in Fish Biology and Fisheries* 21(2): 225–246.
- Boecklen W J, Yarnes C T, Cook B A and James A C. (2011). On the use of stable isotopes in trophic ecology. *Annual Review of Ecology, Evolution and Systematics* 42(1): 411–440.
- Bureau of Agricultural Statistics (BAS). (2011). *Fisheries: Value of production*. Philippines: Department of Agriculture. <http://countrystat.bas.gov.ph/selection.asp> (accessed on 20 April 2013).
- Belling G B, Abbey M, Campbell J H and Campbell G R. (1997). Lipid content and fatty acid composition of 11 species of Queensland (Australia) fish. *Lipids* 32(6): 621–625.
- Bligh E G and Dyer W J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* 37(8): 911–917.
- Clarke K R and Warwick R M. (2001). *Change in marine communities: An approach to statistical analysis and interpretation*, 2nd ed. Plymouth: Plymouth Marine Laboratories.
- Crawford M A, Bloom M, Broadhurst C L, Schmidt W F, Cunnane S C, Galli C, Gehbremeskel K, Linseisen F, Lloyd-Smith J and Parkington J. (1999). Evidence for the unique function of docosahexaenoic acid during the evolution of the modern hominid brain. *Lipids* 34(Suppl. 1): S39–S47.
- El-Sabaawi R, Dower J F, Kainz M and Mazumder A. (2009). Characterizing dietary variability and trophic positions of coastal calanoid copepods: Insight from stable isotopes and fatty acids. *Marine Biology* 156(3): 225–237.
- Elsdon T S. (2010). Unraveling diet and feeding histories of fish using fatty acids as natural tracers. *Journal of Experimental Marine Biology and Ecology* 386(1–2): 61–68.

- Food and Agriculture Organization (FAO). (2013). *Food balance sheet of fish and fishery products in live weight and fish contribution to protein supply*. Rome: Fisheries and Aquaculture Department, FAO. ftp://ftp.fao.org/FI/STAT/summary/FBS_bycontinent.pdf (accessed on 20 April 2013).
- Garrido S, Rosa R, Ben-Hamadou R, Cunha M E, Chícharo M A and van der Lingen C D. (2008). Spatio-temporal variability in fatty acid trophic biomarkers in stomach contents and muscle of Iberian sardine (*Sardina pilchardus*) and its relationship with spawning. *Marine Biology* 154(6): 1053–1065.
- Gopakumar K. (1974). Fatty acid make up of lipids of oil sardine (*Sardinella longiceps*) in relation to seasons. *Journal of the Marine Biological Association of India* 16(3): 830–833.
- Hale M B. (1984). Proximate chemical composition and fatty acids of three small coastal pelagic species. *Marine Fisheries Review* 46(1): 19–21.
- Hall D, Lee S Y and Meziane T. (2006). Fatty acids as trophic tracers in an experimental estuarine food chain: Tracer transfer. *Journal of Experimental Marine Biology and Ecology* 336(1): 42–53.
- Huynh M D and Kitts D D. (2009). Evaluating nutritional quality of pacific fish species from fatty acid signatures. *Food Chemistry* 114(3): 912–918.
- Jackson G D, Bustamante P, Cherel Y, Fulton E A, Grist E P M, Jackson C H, Nichols P D, Pethybridge H, Phillips K, Ward R D *et al.* (2007). Applying new tools to cephalopod trophic dynamics and ecology: Perspectives from the Southern Ocean Cephalopod Workshop, February 2–3 2006. *Reviews in Fish Biology and Fisheries* 17(2–3): 79–99.
- Khoddami A, Ariffin A A, Bakar J and Ghazali H M. (2009). Fatty acid profile of the oil extracted from fish waste (head, intestine and liver) (*Sardinella lemuru*). *World Applied Sciences Journal* 7(1): 127–131.
- Lloret J. (2010). Human health benefits supplied by Mediterranean marine biodiversity. *Marine Pollution Bulletin* 60(10): 1640–1646.
- Metillo E B. (2011). Feeding ecology of *Acetes intermedius* Omori 1975 (Crustacea, Decapoda, Sergestidae) in Iligan Bay, Philippines. *Zoological Studies* 50(6): 725–736.
- Mohd-Yusof N Y, Monroig O, Mohd-Adnan A, Wan K-L and Tocher D R. (2010). Investigation of highly unsaturated fatty acid metabolism in the Asian sea bass, *Lates calcarifer*. *Fish Physiology and Biochemistry* 36(4):827–43.
- Montañó N, Gavino G and Gavino V C. (2001). Polyunsaturated fatty acids of some traditional fish and shrimp paste condiments of the Philippines. *Food Chemistry* 75(2): 155–158.
- Olivotto I, Tokle N E, Nozzi V, Cossignani L and Carnevali O. (2010). Preserved copepods as a new technology for the marine ornamental fish aquaculture: A feeding study. *Aquaculture* 308(3–4): 124–131
- Omori M and Ikeda T. (1984). *Methods in marine zooplankton ecology*. New York: John Wiley & Sons.
- Ogata H Y, Emata A C, Garibay E S and Furuita H. (2004). Fatty acid composition of five candidate aquaculture species in Central Philippines. *Aquaculture* 236(1–4): 361–375.
- Osako K, Saito H, Hossain M A, Kuwahara K and Okamoto A. (2006). Docosahexaenoic acid levels in the lipids of spotted mackerel *Scomber australasicus*. *Lipids* 41(7): 713–720.
- Osako K, Saito H, Weng W, Kuwahara K and Tanaka M. (2009). Lipid characteristics of coastal migratory *Sarda orientalis* tissues. *Fisheries Science* 75(4):1055–1066.
- Sahena F, Zaidul I S M, Jinap S, Saari N, Jahurul H A, Abbas K A and Norulaini N A. (2009). PUFAs in fish: Extraction, fractionation and importance in health. *Comprehensive Review in Food Science and Food Safety* 8(2): 59–74.

- Saito H, Seike Y, Ioka H, Osako K, Tanaka M, Takashima A, Keriko J M, Kose S and Souza J C R. (2005). High docosahexaenoic acid levels in both neutral and polar lipids of a highly migratory fish: *Thunnus tonggol* (Bleeker). *Lipids* 40(9): 941–953.
- Saito H, Yamashiro R, Alasalvar C and Konno T. (1999). Influence of diet on fatty acids of three subtropical fish, Subfamily Caesioninae (*Caesio diagramma* and *C. tile*) and Family Siganidae (*Siganus canaliculatus*). *Lipids* 34(10): 1073–1082.
- Sargent J R, Tocher, D R and Bell J G. (2002). The lipids. In J E Halver and R W Hardy (eds.). *Fish nutrition*, 3rd ed. San Diego: Academic Press, 181–257.
- Savina G C and White A T. (1986). Reef fish yield and non-reef catch of Pamilacan Island, Bohol, Philippines. In J L MacLean, L B Dizon and L V Hosillos (eds.). *The First Asian Fisheries Forum*. Manila: Asian Fisheries Society, 497–500.
- Sirot V, Oseredczuk M, Bemrah-Aouachria N, Volatier J-L and Leblanc J-C (2008). Lipid and fatty acid composition of fish and seafood consumed in France: CALIPSO study. *Journal of Food Composition and Analysis* 21(1): 8–16.
- SPSS. (2002). *SPSS for Windows version 11*. Chicago: SPSS Inc.
- Traugott M, Kamenova S, Ruess L, Seeber J and Plantegenest M. (2013). Empirically characterising trophic networks: What emerging DNA-based methods, stable isotope and fatty acid analyses can offer. In G Woodward and D A Bohan (eds.). *Advances in ecological research*, vol. 49. London: Elsevier Ltd., 177–224.
- Turan H, Kaya Y and Erkoyuncu I. (2007). Protein and lipid content and fatty acid composition of anchovy meal produced in Turkey. *Turkish Journal of Veterinary & Animal Science* 31(2): 113–117.
- van der Meer T, Olsen R E, Hamre K and Fyhn H J. (2008). Biochemical composition of copepods for evaluation of feed quality in production of juvenile marine fish. *Aquaculture* 274(2–4): 375–397.
- Xiao Y and Greenwood J G. (1993). The biology of *Acetes* (Crustacea: Sergestidae). In A D Ansell, R N Gibson and M Barnes (eds.). *Oceanography and marine biology annual review*, vol. 31. London: UCL Press Ltd., 259–444.
- Zlatanov S and Laskaridis K. (2007). Seasonal variation in the fatty acid composition of three Mediterranean fish – sardine (*Sardina pilchardus*), anchovy (*Engraulis encrasicolus*) and picarel (*Spicara smaris*). *Food Chemistry* 103(3): 725–728.