Influence of growth on fatty acid composition of the moon wrasse *Thalassoma lunare* collected in coral reef habitats of the Malaysian South China Sea

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Abstract: The composition of fatty acids in fishes can serve as trophic biomarkers in the analysis of marine food webs because fatty acids can be traced as essential dietary components to higher trophic levels; however, information on tropical coral fishes is scarce. In order to understand the feeding ecology of coral reef fish, fatty acid composition and levels were examined in the moon wrasse *Thalassoma lunare* collected in the Malaysian South China Sea. Fatty acid composition was predominantly saturated fatty acids (SAFA) (62.5 %), followed by monounsaturated fatty acids (MUFA) (33.8 %) and polyunsaturated fatty acids (PUFA) (3.7 %). The most abundant fatty acids in SAFA, MUFA and PUFA were palmitic acid (C16:0), oleic acid (C18:1ω9c) and elaidic acid (C18:1ω9t) and linolenic acid (C18:3n3) and EPA (C20:5n3), respectively. Fatty acid concentrations, especially in SAFA and MUFA, changed with fish growth, although PUFA did not change in fish growth. Such differences might be caused by differences in the diet of the fish accompanying the growth.

Key words: Coral reef fish, fatty acid, feeding, habitat ecology, South China Sea.

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Introduction

Coral reefs serve as habitat for many commercially important species targeted for fishing. Southeast Asian coral reefs have the highest levels of biodiversity for the world's marine ecosystems and are recognised as the global centre for coral reefs (Burke *et al.* 2002). More than 30 % of the coral reefs on earth are located in the seas of Southeast Asia (Burke *et al.* 2002). However, many coral areas are currently under threat from coastal development, marine pollution, overexploitation of marine resources and land pollution and erosion (Arai *et al.* 2016).

Recently, Ambak *et al.* (2010) and Chong *et al.* (2010) listed 2243 and 1951 fish species, respectively, in Malaysian waters. Those reports suggest that Malaysia has one of the highest and richest diversity of fish in the world (Arai 2015). In fact, Malaysia is identified as one of the world's 12

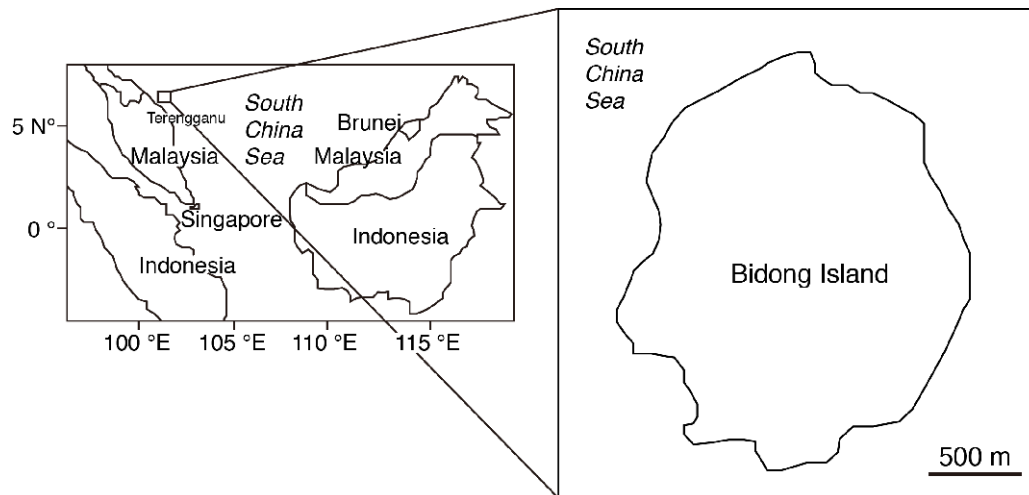*Corresponding Author*; e-mail: takaomi.arai@ubd.edu.bn

Fig. 1. Map showing the location of the study site at the Bidong Island in Malaysian South China Sea, off the Terengganu State in the east coast of Peninsula Malaysia.

mega-diversity centers and is one of the six countries of the Coral Triangle, which boasts the most diverse and richest coral reefs in the world with more than 1000 species of coral reef fishes (Chong *et al.* 2010). Although there are several studies about the taxonomy and distribution of coral fish species in Malaysian waters, few studies have examined the life history, ecology and reproduction of these species in comparison to other coral reef areas in the Indo-Pacific region.

Information regarding the feeding ecology, habitat use, and migration of coral reef fishes are needed to fully understand their life history. Recently, the signature of fatty acid analysis has been increasingly used to study the diet of a number of marine species (e. g. Arai *et al.* 2015a, b; Couturier *et al.* 2013; Daly *et al.* 2010; Stowasser *et al.* 2012). Fatty acid composition is recognized as a potential trophic biomarker that can indicate fish trophic position and diets in the marine environment (Stowasser *et al.* 2012). 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 fatty acids 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).

The moon wrasse, *Thalassoma lunare*, is common in reefs distributed throughout the tropical Indo-Pacific (Randall & Lim 2000). It resides on coral reefs, lagoon, coastal reefs and surrounding areas at depths from 1 to 20 m. The fish is carnivorous and tends to prey on fish eggs and small sea-floor dwelling invertebrates (Randall & Lim 2000). *T. lunare* is commonly regarded as a highly opportunistic predator, preying upon a wide range of food sources depending on sporadic events that may influence prey vulnerability or availability (Connell 1998; Holmes *et al.* 2012). While they have been shown to feed actively on juvenile reef fish in manipulated tank experiments (Beukers & Jones 1997; Holmes & McCormick 2010), evidence of hunting and feeding on the same prey under natural conditions is rare. The moon wrasse can reach 45 cm in total length (Randall & Lim 2000). Although the fish can be commonly found in coral reefs in the tropical Indo-Pacific region, its life history is not well understood.

In the present study, fatty acid analyses were used to investigate the feeding ecology of the moon wrasse, *Thalassoma lunare*, collected in Malaysian South China Sea. To understand the changes of fatty acid compositions in accordance with the growth, fatty acid signatures were compared among fish within various size classes.

Material and methods

All specimens of the moon wrasse, *Thalassoma lunare* were collected in reef areas at the Bidong Island in the South China Sea, Malaysia (Latitude 5.62°, Longitude 103.07°) between 27 and 28 October 2014 (Fig. 1). Bidong Island is located off Terengganu State on the east coast of Peninsular Malaysia, known for its history as Vietnamese

Table 1. Fatty acid composition in livers of the moon wrasse *Thalassoma lunare* collected at the Bidong Island, Malaysian South China Sea.

Fatty acids	mean ± SD	range
SAFA		
C14:0	13.5 ± 3.6	9.7-20.6
C16:0	48.9 ± 13.3	28.2-62.9
C18:0	0.0 ± 0.0	0.0
C20:0	0.03 ± 0.05	0.0-0.1
∑SAFA	62.5 ± 11.1	47.3-74.3
MUFA		
C16:1	6.4 ± 2.2	2.8-10.4
C17:1	0.8 ± 0.6	0.1-1.8
C18:1ω9c	11.0 ± 5.8	0.0-18.5
C18:1ω9t	14.3 ± 8.5	6.7-30.2
C20:1	1.5 ± 1.4	0.0-3.2
∑MUFA	33.8 ±9.6	24.2-49.3
PUFA		
C18:3n3	1.3 ± 0.6	0.7-2.3
C18:3n6	0.3 ± 0.2	0.1-0.7
C20:3n3	0.4 ± 0.3	0.1-0.9
C20:5n3 (EPA)	1.2 ± 1.5	0.0-4.5
C22:6n3 (DHA)	0.5 ± 0.5	0.1-1.7
∑PUFA	3.7 ± 2.6	1.0-8.8

refugee settlement. All fishes were collected with fish traps and hook and line. After collecting, all fishes were immediately stored in an ice chest, brought back to laboratory, kept in -20 °C freezer, and fatty acid analyses were conducted within one month. A total of twelve fish samples were evaluated; for each fish total length (TL) and body weight (BW) were recorded and the liver was removed to determine the weight. The stomach for each fish was dissected for the stomach content analyses.

Liver samples of *Thalassoma lunare* were analysed for fatty acid composition following the one step method (Arai *et al.* 2015c, d, e). Three replicates of each liver and tissue samples were mixed with 4 ml of hexane and 1 ml of internal standard solution in a 50 ml centrifuge tube. After adding 2 ml of 14 % BF3 in methanol, the tube was flushed with nitrogen gas. The capped tube was heated on a hot plate at 100 °C for 120 minutes. One ml of hexane was added followed by 2 ml of distilled water. The tube was then shaken vigorously for 1 min and centrifuged for 3 min at 2500 rpm.

Samples were then analyzed using a GC-FID (GC 14-B, Shimadzu). Separation was performed with an FFAP-polar capillary column (30 m × 0.32 mm internal diameter, 0.25 µm film thickness). Hydrogen was used as a carrier gas. After injection at 60 °C, the oven temperature was raised to 150 °C at a rate 40 °C min^{-1}, then to 230 °C at 3 °C min^{-1}, and finally held constant for 30 min. The flame ionization was held at 240 °C. Peaks were identified by comparing their retention times with those of authentic standards (Supelco Inc.). Fatty acids were designated as an n:pωx, where n is the number of carbon atoms in the aliphatic chain, p is the number of double bonds and x is the position of the first double bond from the terminal methyl group. The analytical precision for samples was generally < 5 % for total amounts and major components.

Fatty acid concentrations (mg g^{-1} dry weight) were calculated by comparing the peak area of fatty acid in the sample with the peak area of internal standard. The percentage for each fatty acid was converted from the area of chromatogram peaks. The composition is expressed as percentage of total fatty acids (Table 1).

The significance of the correlation coefficient and the regression slope in relationships between fatty acid compositions in the livers and TL and BW were determined using a t-test (Sokal & Rohlf 1995).

Results

Biological characteristics such as TL, BW and liver weight of the moon wrasse, *Thalassoma lunare* ranged from 12.7 cm to 24.2 cm, from 24.2 g to 123.4 g and from 0.12 g to 1.92 g, respectively. The stomach content for each fish was observed for all samples. However, in all fish, prey organisms could not be identified under macro- and micro-observations because the items were well digested.

Saturated fatty acids (SAFA) were the most abundant accounting for 47.3 % to 74.3 % of the fatty acids (Table 1). Palmitic acid (C16:0) was the most abundant saturated fatty acid ranging from 28.2 % to 62.9 % followed by myristic acid (C14:0) (Table 1).

Monounsaturated fatty acids (MUFA) were the next most prevalent making up 24.2 % to 49.3 % of the fatty acids (Table 1). Of all the MUFA, oleic acid (C18:1ω9c) and elaidic acid (C18:1ω9t) were the most abundant followed by palmitoleic acid (C16:1) (Table 1).
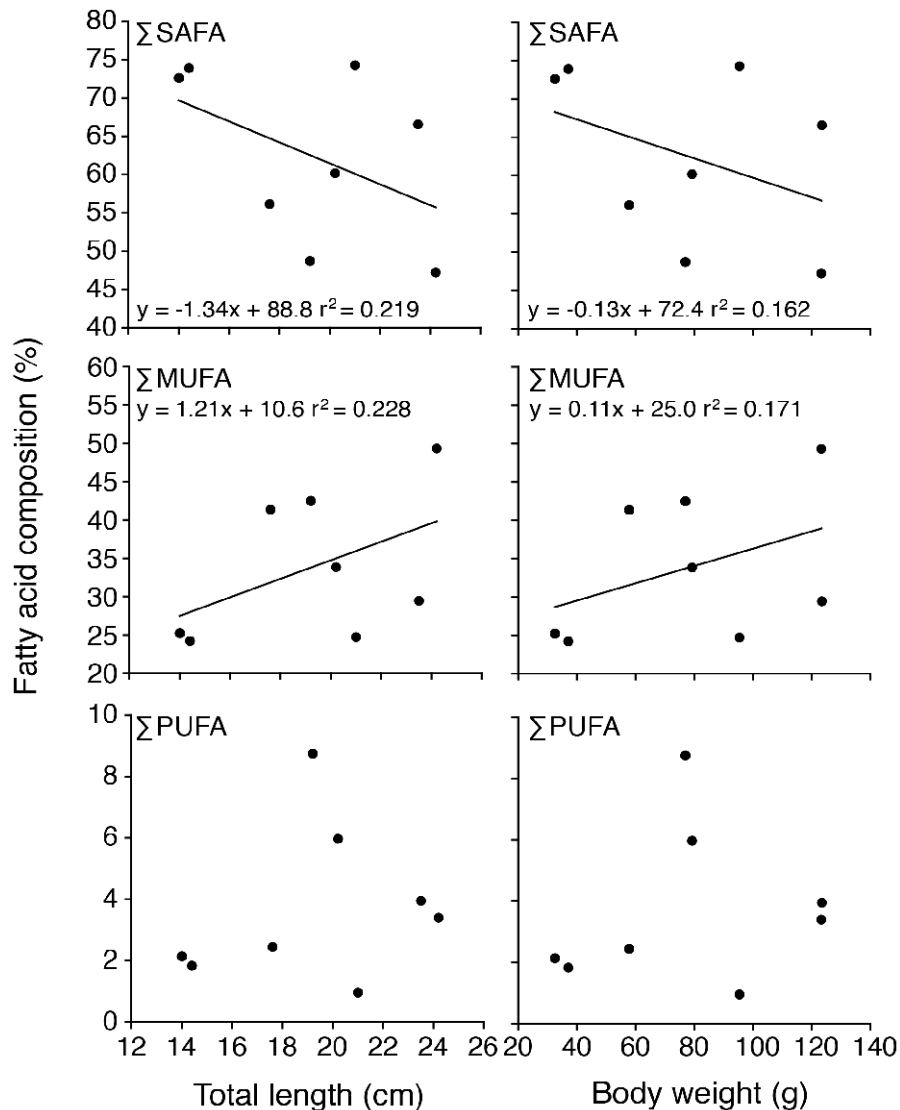
The proportion of polyunsaturated fatty acids

Fig. 2. Relationships between liver fatty acid compositions (∑SAFA, ∑MUFA, and ∑PUFA) and the total length (TL) and body weight (BW) of the moon wrasse *Thalassoma lunare* collected at the Bidong Island in Malaysian South China Sea.

(PUFA) was accordingly low ranged from 1.0 % to 8.8 % (Table 1). Linolenic acid (C18:3n3) ranged from 0.7 % to 2.3 %, followed by EPA (C20:5n3) and DHA (C22:6n3) (Table 1).

Fatty acid composition changed with fish size (Fig. 2). Significantly negative relationships were found between ∑SAFA and TL and BW (t-test, n = 7, t = -9.22 - -12.0, $P < 0.0001$). And, significantly positive relationships were found between ∑MUFA and TL and BW (t - test, n = 7, t = -1.84 - -4.89, $P < 0.05 - 0.005$) (Fig. 2). However no significant relationships were found between ∑PUFA and TL and BW (t-test, n = 7, t = 7.06 - 10.9, p > 0.05) (Fig. 2).

Discussion

Although we did not conduct fatty acid analyses for potential prey organisms, fatty acid composition was different depending on the fish growth in the moon wrasse *Thalassoma lunare* (Fig. 2). Differences in individual fatty acid profiles in relation to development, food habits and habitat use, temperature and salinity have been reported in different fish species (e. g. Couturier *et al.* 2013; Daly *et al.* 2010; Stowasser *et al.* 2012). Although the mechanism of lipid deposition in the liver of fish fed diets was still indeterminate, fatty acid synthesis was regulated by the liver X receptor

suggesting the profiles of the liver reflected the diets of fish (Peng *et al.* 2014). However, differences in fatty acid composition and levels in relation to fish growth in wild fish species have not been well reported. It is likely that such differences might be caused by differences in the diet of the moon wrasse *Thalassoma lunare* accompanying the growth.

SAFA were the most abundant fatty acids and the palmitic SAFA had the highest values among all fatty acids. The palmitic SAFA has been reported to have the highest concentration in other marine fish species (Elsdon 2010; Sahena *et al.* 2009). The predominance of the palmitic SAFA 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 acid level for the round scad *Decapterus punctatus* from the Atlantic Bight and Gulf of Mexico. 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 (Huynh and Kitts 2009).The moon wrasse *Thalassoma lunare* was collected in the South China Sea in tropical waters in the present study, and thus the fish might have higher palmitic and stearic acid levels. The higher ∑ SAFA found in smaller fishes might suggest a higher metabolism rate than that of larger fishes during growth.

MUFA were the second most abundant fatty acids, and oleic acid was the highest composition among MUFA. This is in agreement with findings in copepod (Olivotto *et al.* 2010), Acetes (Montaño *et al.* 2001) and fish fatty acid profiles (Elsdon 2010; Huyn and 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 synthesize this MUFA de novo (Sargent *et al.* 2002). High proportions of MUFAs in marine predators are generally derived from marine zooplankton, in particular calanoid copepods, such as *Calanoides acutus* and *Calanus propinquus* in Antarctic waters (Pond *et al.* 2012). *Dissostichus eleginoides*, the species with the highest levels of MUFAs, has previously been classified as an opportunistic predator mainly feeding on fish, squid and, to a smaller degree, on *Euphausia superba* (Antarctic krill) (e.g. Collins *et al.* 2007; Pilling *et al.* 2001). The higher level of MUFAs found in *Thalassoma lunare* might suggest that the fish might feed on copepods as one of its potential prey organisms during their life history.

Stomach content analysis is the basic and conventional approach used to assess fish diet (Cortés 1997). However, in this study the prey organisms in stomachs could not be identified in *Thalassoma lunare*. Nevertheless stomach content analysis have some limitations because this technique only provides recent feeding and may not accurately reflect the composition of prey items that contribute most significantly to its general diet (Couturier *et al.* 2013). Fatty acid signature has been increasingly used to study the diet of a number of marine species such as Caribbean grunts (Haemulidae) and snappers (Lutjanidae) (Cocheret de la Morinie`re *et al.* 2003), black bream *Acanthopagrus butcheri* (Elsdon 2010) and reef manta ray *Manta alfredi* (Couturier *et al.* 2013). The present study suggests that the diets of the coral fish species *Thalassoma lunare* change with the fish growth, although no relationship was found between ∑ PUFA and somatic growth in *Thalassoma lunare*. Further studies are needed on various organisms in the coral reef ecosystem using fatty acid signature to provide a better understanding of the life history and ecology of *Thalassoma lunare*.

Acknowledgement

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