

Proximate Composition, Fatty Acid Profile, and Microplastic Contamination of Edible Odonate Larvae (Aeshnidae: *Anax* sp.) in Rice Fields

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Abstract

Although the human consumption of edible insects is a culturally well documented practice, the nutritional literature of aquatic insects has not been completely covered. The present study was conducted with the aim of evaluating the proximate composition, fatty acid profiles, and microplastic contamination of edible odonate larvae (Aeshnidae: *Anax* sp.). Nutrient analysis showed that the proximate composition was a good source of protein (65.70 g/100g dry weight). The major fatty acids were oleic (1.08 g/100g DW) and palmitic acid (1.01 g/100g DW). The long-chain polyunsaturated fatty acid profile showed an abundance of linoleic, alpha-linolenic, and eicosapentaenoic acids. Total microplastics content found 694 items in the gastrointestinal tract, with a mean abundance of 11.57 items/individual. Five distinct polymers, including polyethylene, polyamide, polypropylene, polyethylene terephthalate, and cellulose, were identified through chemical analysis of FTIR spectra. Future research should be conducted regarding a comprehensive nutritional study to as method for a nutritional reference on food safety and security.

Keywords: Edible aquatic insect, Odonata, Proximate composition, Fatty acid, Microplastics, Rice field

1. Introduction

Food security concerns are rising due to the expected increase in the world population growing to 9.6 billion by the year 2050 (UN, 2019). These concerns are influencing research into several alternate human dietary sources. From this perspective, edible insects as early as 1975 (Meyer-Rochow, 1975) were considered to contribute to the food security of the world based on their nutritional value and sustainability of the production system. Globally, approximately 2,000 insect species of 14 orders are considered edible (Mitsuhashi, 2008; Kouřimská and Adámková, 2016). Insects are one of the most diverse and abundant groups in the ecosystem because they are able to exist in and adapt to a variety of terrestrial and aquatic ecosystems. They also have a high reproductive capacity (Das and Hazarika, 2019). The consumption of insects has been recorded throughout history and throughout the world (FAO, 2013). Consuming insects, also known as entomophagy, is a good source of amino acids and fatty acid profiles as well as protein (20–76% of dry matter), fat (2–50% of dry matter) (Kouřimská and Adámková, 2016), including minerals and vitamins (Chakravorty *et al.*, 2011; Ghosh *et al.*, 2017). Beetles (Coleoptera) (31%) are the most widely consumed edible insects in the world, followed by caterpillars (Lepidoptera) (18%), bees, wasps,

and ants (Hymenoptera) (14%) (van Huis, 2013; Macadam and Stockan, 2017).

Prior research has recorded the nutrient composition of dragonfly larvae (Odonata) (Feng *et al.*, 2001; Mozhui *et al.*, 2020; Narzari and Sarmah, 2017; Xiaoming *et al.*, 2010), but only a few species have been investigated. In Thailand, Odonata is a common insect found throughout the country, especially in rice paddies and other wetlands. The practice of consuming dragonfly larvae is common throughout Thailand, particularly in the north and northeast. Odonata species eaten in Northeast Thailand are Aeshnidae (*Aeshna* sp.), Coenagrionidae (*Ceriagrion* sp.), Corduliidae (*Epophtalmiavittigera bellicose*), and Libellulidae (*Rhyothemis* sp.) (Hanboonsong, 2010). In other regions of the world, the most common edible odonata species are *Lestespraemorsus* (Lestidae), *Gomphuscuneatus*, and *Crocothemisservilia* (Libellulidae) (Feng *et al.*, 2001). Protein, fat, amino acids, and trace elements are present in these larvae. Protein, fat, and amino acid levels are on average 58.92%, 25.37%, and 46.03%, respectively. The average content of the eight different types of amino acids required by the human body is 16.41%, or 35.69%, of the total amount of amino acids. Potassium, zinc, calcium, and iron concentrations are 2,960 mg.kg⁻¹, 125.4 mg.kg⁻¹, 2,7616.9 mg.kg⁻¹, and 796.2 mg.kg⁻¹, respectively. Consequently, one of the most nutritious edible insect resources is the dragonfly larva (Feng *et al.*, 2001).

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Microplastics (MPs) are an issue of concern as they can be found in the larvae that are consumed by humans. This is problematic because if these MPs enter the food chain, they have the potential to negatively impact both human health and the environment (Erren *et al.*, 2013; Muhdhar *et al.*, 2021; Kallenbach *et al.*, 2022). Microplastics are all small plastic particles with range size 1 μm to 5 mm (Hale *et al.*, 2022). These may be degradation products originating from primary MPs (manufactured for addition to certain products) or secondary MPs (derived by physical, chemical, and/or biological degradation from larger plastics) and can be readily dispersed in the natural environment (Wang *et al.*, 2018). A variety of aquatic animals can either ingest microplastics from bottom sediments, suspended particulate material from the water column, or even ingest organisms of lower trophic levels containing these particles (Chaukura *et al.*, 2021). Monitoring of these contaminants can provide useful information on the degree of contamination in aquatic ecosystems. Thus, the aim of the study was to confirm the proximate composition of aquatic dragonfly larvae, *Anax* sp., including quantifying the microplastic accumulation in the gastrointestinal tract of the odonatan *Anax* sp.

2. Materials and Methods

2.1. Sample collection and preparation for nutrition analysis

In November 2021, an aquatic dip net was used to collect aquatic insects from the rice plots' margins. The location of the rice plot was in Nakhon Pathom Province, in the central part of Thailand (N14° 0' 31.964", E99° 58' 53.3838"). The collected odonata specimens were placed in white trays for sorting and screening (Figure 1a). The samples were transferred to containers for identification in the laboratory. Odonata larvae were identified using taxonomic keys (Dudgeon, 1999; Yule and Sen, 2004) under a stereomicroscope (Olympus SZ51). Approximately 1,555 mostly final instar larvae, *Anax* sp., were rinsed, sun-dried for one day, and kept until further biochemical analysis. The descriptive detail of the final instars is the long, spread-apart wing buds. The larvae of *Anax* sp. (Figure 1b), approximately 60 individuals, were preserved in vials properly containing 80% ethanol for microplastic analysis.



Figure 1. a) Photograph of *Anax* sp. (Odonata: Aeshnidae) (red arrow) with other aquatic insect organisms, and b) *Anax* sp. (Odonata: Aeshnidae) characteristic.

2.2. Nutrition analysis

Due to limited sample sizes, this study was unable to conduct triplicate analyses for proximate composition. The sampling technique used was composite sampling (pooling the sample). For the biochemical analysis, 1,555 *Anax* sp. larvae were used. The proximate composition was analyzed based on the contents of moisture (AOAC method 925.45), protein (AOAC method 991.20), fat (AOAC method 2003.05), and ash (AOAC method 923.03). Briefly, the protein content (N X 6.25) was determined by the AOAC Kjeldahl method. The fat content was determined by the Soxhlet extraction technique. Total carbohydrate was determined by calculating the percent remaining after all the other components had been measured: %carbohydrates = 100 - (%moisture + %protein + %lipid + %ash). The energy value per 100 g was calculated by multiplying the grams of fat by 9.0 (Sullivan and Carpenter, 1993). The total energy (Kcal/100 g dry matter) was estimated according to FAO (2003). The fatty acid profile was performed using gas chromatograph with a flame ionization detector (FID) (7890-B, Agilent Technologies) and modified Compendium of Methodology for Food Analysis methods (2003). Each parameter of the biochemical analysis was determined only once due to the limited number of specimens.

2.3. Microplastics extraction and identification

A total of 60 dragonfly larvae, *Anax* sp., were rinsed three times with distilled water to get rid of a variety of contaminants. Then, respective larval lengths and weights were determined by calipers (accuracy 0.05 mm) and analytical balance readings (accuracy 0.0001 g). Ten specimens (six replicates) were pooled to measure microplastics in the gastrointestinal tract (GT). The GT of *Anax* sp. was removed from the individuals' guts using metal forceps and weighed using an analytical scale (accuracy 0.0001 g). Then they were placed in separate 30 mL Erlenmeyer flasks with aluminium foil immediately covering the flasks. Prior to microplastics examination, the GT was dried at 40°C for 4 hours in a drying cabinet. Each flask was then filled with 10 mL of a 30% hydrogen peroxide (H_2O_2) solution, immediately covered in parafilm, and placed in an ES-20 environmental shaker incubator, then incubated for 7 days at 150 rpm. After tissue breakdown, the microplastic particles were separated from the remaining matrix using density floatation with a 1.6 g/mL potassium formate (HCOOK) solution. The particles were vacuum-pumped onto 0.45 μm pore size and 47 mm diameter membrane filters. The filters were placed in glass Petri dishes with covers and dried for two days at 50 °C in a drying chamber. To identify MP particles based on their physical properties, each filter paper was visually examined, and images were taken using a stereomicroscope (Leica EZ4E). With the help of an attenuated total reflection-Fourier transform infrared spectrometer (ATR-FTIR), the selected particles were examined to confirm the types of polymers. The spectral range was 4,000 to 500 cm^{-1} , with a 32 cm^{-1} spectral resolution and 32 co-scans for each measurement. The spectra of polymers were matched against a commercial spectral library (Bruker ATR-FTIR Complete Library), with a quality index ≥ 0.7 being accepted (Woodall *et al.*, 2014).

As methods to prevent contamination, special gloves (nitrile), glass, and metal equipment were used. When not in use, all the glass and metal were immediately covered with aluminium foil after being cleaned three times with deionized water. Parallel to the process used for the samples, a procedural blank without tissues was run. The process was completed as quickly as possible.

2.4. Data analysis

The nutritional value was recorded. The abundance, types, and colors of MPs were counted. The data was represented as mean \pm standard deviation (SD).

3. Results and Discussion

The proximate composition is shown in Table 1. The larvae of Odonata, *Anax* sp. were high in protein content (65.7 %). However, the data on the protein content of *Anax* sp. were lower than those for *Sympetrum* sp. (Odonata: Libellulidae) reported by Narzari and Sarmah (2017). However, aquatic insects like Odonata tend to be excellent sources of protein, 40–65% (Williams and Williams, 2017). The fat content in *Anax* sp. was 4.88%. The macronutrient (protein and fat) composition in these edible insects reflects their gross energy value, which is also influenced by other factors, e.g., sex (Kulma *et al.*, 2019), life stages (Ghosh *et al.*, 2021), and diet (Oonincx and Finke, 2021). The total energy content was 360.52 Kcal/100 g and was similar to that found in the great diving beetle (*Dytiscus marginalis*) (Choudhury *et al.*, 2020). However, the obtained energy values for *Anax* sp. are comparably lower than the 431 Kcal/100g reported for the Odonata (Dragonflies and Damselflies), the main order of edible insects commonly consumed worldwide (Ordóñez-Araque *et al.*, 2022). The energy levels of edible insects depend mainly on their fat content. Kouřimská and Adámková (2016) state that because larvae have more lipid stored in their bodies than adults, they are often higher in energy. In contrast, insect species rich in protein contain fewer calories.

Table 1. Proximate compositions and fatty acid compositions of dragonfly larvae, *Anax* sp., g/ 100g dry weight

Proximate composition	
Moisture	10.81
Ash	5.16
Fat	4.88
Protein	65.70
Total carbohydrate	13.45
Total energy, (Kcal/ 100 g)	360.52
Energy from fat, (Kcal/ 100 g)	43.92

Table 2 presents the fatty acid composition of *Anax* sp. Palmitic acid (1.01 g/100 g) was the main saturated fatty acid found in the fatty acids. Oleic acid made up the majority of monounsaturated fatty acids (MUFA), contributing 1.08 g/100 g. Alpha-Linolenic acid (C18:3n3) was the most abundant n-3 polyunsaturated fatty acid (PUFA), while linoleic acid (C18:2n6c) and arachidonic acid (C20:4n6) were the two most abundant n-6 PUFA. These findings were similar to those reported previously regarding common edible dragonfly larvae in Yunnan and Guizhou Provinces, China (Jiang *et al.*, 2017). Long-chain

PUFAs, particularly omega-3 and omega-6 fats such as alpha-linolenic acid and linolenic acid, present in aquatic insects, which are similar to freshwater fish (Zhao *et al.*, 2021). Aquatic insects typically consume small aquatic organisms and algae, which are sources of long-chain PUFAs, or they synthesize PUFAs by expressing the enzymes delta-5 and delta-6 desaturases (D5D and D6D) (Sprecher, 2000).

Table 2. Fatty acid composition of dragonfly larvae, *Anax* sp., g/ 100g dry weight

Fatty acid profile	
Saturated fatty acid (SFA)	
Lauric acid (C12:0)	0.02
Myristic acid (C14:0)	0.05
Pentadecanoic acid (C15:0)	0.04
Palmitic acid (C16:0)	1.01
Heptadecanoic acid (C17:0)	0.13
Stearic acid (C18:0)	0.66
Arachidic acid (C20:0)	0.08
Behenic acid (C22:0)	0.06
Total SFA	2.05
Unsaturated fatty acid	
Palmitoleic acid (C16:1)	0.17
cis-10-Heptadecenoic acid (C17:1n10)	0.06
cis-9-Oleic acid (C18:1n9c)	1.08
Total MUFA	1.31
Linoleic acid (C18:2n6c)	0.53
gamma-Linolenic acid (C18:3n6)	0.02
alpha-Linolenic acid (C18:3n3)	0.28
cis-11,14-Eicosadienoic acid (C20:2)	0.03
cis-8,11,14-Eicosatrienoic acid (C20:3n6)	0.02
Arachidonic acid (C20:4n6)	0.21
Eicosapentaenoic acid (C20:5n3)	0.20
Total PUFA	1.29

Note: MUFA (monounsaturated fatty acids); PUFA (polyunsaturated fatty acids).

In the assessment of microplastic contamination, odonate larvae, *Anax* sp., with comparable weights were used for analysis. The average wet weight of the gastrointestinal tract was 0.1082 \pm 0.0492 g (range 0.0395-0.3056 g). *Anax* sp. had a mean body weight of 0.8046 \pm 0.3736 g (range 0.4112-1.9151 g) and a body length of 40.35 \pm 5.92 mm (range 32.40-55.10 mm). There was no MP contamination in the procedure blank samples. The findings demonstrated that all samples had microplastic particles in their gastrointestinal tracts. Microplastics were discovered in a variety of shapes in the organisms, including fibers, fragments, films, and spheres (Figure 2). Six replicates of pooled samples had microplastics, and the total numbers were 694 items (81-208 items). The average abundance of microplastics in the gastrointestinal tract was 11.57 \pm 4.32 items/GT individual (wet weight) (Table 3). The majority of the particles (87.18%, 605 items) were small (<100 μ m), while 5.48%

(38 items), 3.46% (24 items), and 3.89% (27 items) were in the 200-250 μm , 250-500 μm , and >500 μm size ranges, respectively (Figure 3). Most abundant were fragments (85.59%), followed by fiber (7.78%), sphere (4.90%), and film (1.73%) (Figure 4). The contamination of microplastics in the gastrointestinal tract of *Anax* sp. revealed a wide range of colors. The dominant color was violet (70.89%), followed by orange (12.54%), pink (9.95%), blue (4.76%), transparent colorless (0.86%), brown (0.58%), green (0.43%), and multicolored (0.29%) (Figure 5). As shown in Figure 6, the Fourier transform infrared (FT-IR) spectra revealed that polyethylene, cellulose, polyamide (nylon), polypropylene, and polyethylene terephthalate were the polymers found in the GT of *Anax* sp. Three kinds of polymers, i.e. polyethylene terephthalate, polyethylene, and polypropylene, are similar to those previously reported in freshwater insects (Akindele *et al.*, 2020; Maneechan and Prommi, 2022), while cellulose is similar to that reported by Bertoli *et al.* (2022). Biological organisms are receptors for microplastics and are exposed to microplastics through the air, water, and food they consume (Kallenbach *et al.*, 2022). Odonate larvae like *Anax* sp. are predators (Dudgeon, 1999). They encounter and consume a variety of prey types, with the consequence that the diet is broad. It is possible to state that they might absorb microplastics that are attached to prey with ease or might mistake MP particles for prey and consume MPs (Windsor *et al.*, 2019). This finding is sensitive because MP pollutants can be passed on to other predators like fish, birds, and humans. Because it is impossible to remove insects' intestines before eating the insects, indigestible particles, such as microplastics, enter the trophic chain (Panebianco *et al.*, 2019).

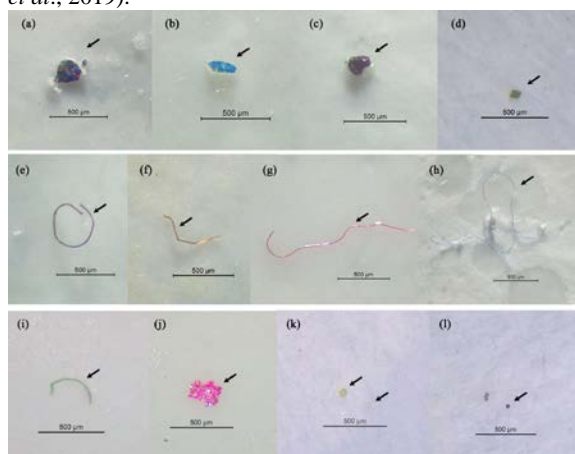


Figure 2. Examples of MPs in biological samples comprise different shapes, colors, and sizes. The arrows indicate fragments (a-d), fibers (e-i), film (j) and spheres (k-l).

Table 3. Basic data and microplastic contamination in *Anax* sp.

Species	Body weight (g)	Body length (mm)	Gastrointestinal tract (GT) wet weight (g)	MP items	
				Total MPs	Average MPs/GT individual
<i>Anax</i> sp.	0.8046±0.3736	40.35±5.92	0.1082±0.0492	694	11.57±4.32
(n = 60)	(0.4112-1.9151)	(32.40-55.10)	(0.0395-0.3056)		

Average \pm standard deviation. Minimum and maximum values in parentheses

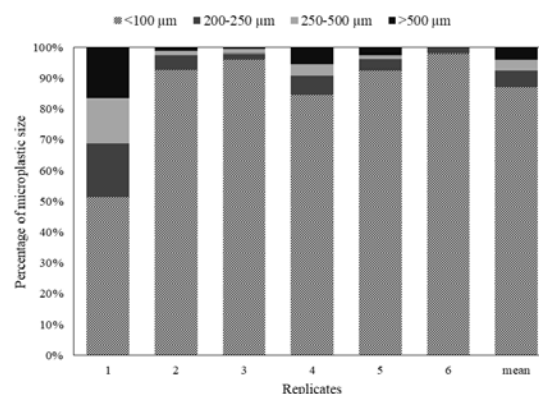


Figure 3. Microplastic size distribution (six replicates)

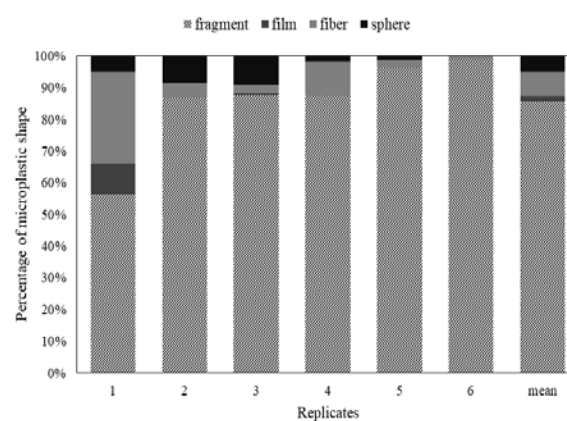


Figure 4. Percent of different microplastic shapes (fiber, sphere, film, and fragment) found in *Anax* sp.

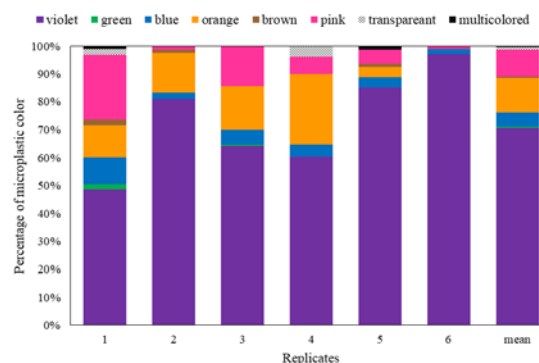


Figure 5. Percent of MPs by colors

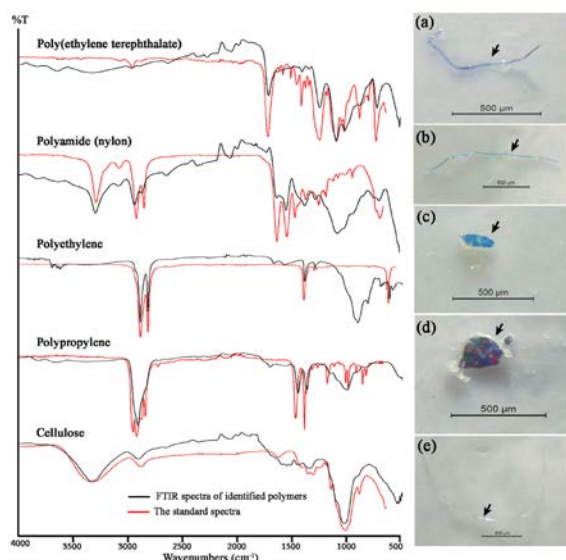


Figure 6. Transmittance spectra of observed microplastics and comparable standard spectra from the spectral library. The black arrows in the photographs indicate a) polyethylene terephthalate, b) polyamide (nylon), c) polyethylene, d) polypropylene, and e) cellulose.

4. Conclusion

In conclusion, odonata larvae (*Anax* sp.) are a good source of protein and fatty acids. The long-chain polyunsaturated fatty acid profile shows an abundance of linoleic acid, alpha-linolenic acid, and eicosapentaenoic acid. Although the odonata larvae were found to be a good source of nutrition, especially protein, a high possibility of microplastic contamination exists, as the current study revealed, and therefore the ingestion of contaminated insects could have some negative consequences on human health. As a result, there are concerns about the potential negative effects of eating these insects. Furthermore, because MPs are a relatively new kind of pollutant with complex and unique properties, more research into their possible effects on edible insects and their vertebrate consumers, such as fish, is strongly recommended.

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