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Fish gut content analysis by thermochemolysis with tetramethylammonium hydroxide (TMAH) and gas chromatography–mass spectrometry (GC–MS)

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Abstract

Gut content analyses of fish are typically conducted using methods based on visual identification. These can lead to inconsistent results because of their subjective nature, especially when sample sizes are limited or food items are not resolvable. New approaches are required to increase the accuracy of gut content analysis. We investigate whether thermochemolysis with TMAH and GC–MS detection can qualitatively and quantitatively analyse the gut contents of two Monacanthid fishes fed seagrass, epiflora and epifauna under controlled conditions. The three food items could be readily differentiated when analysed individually, seagrass could be distinguished from epiphytes (epifauna + epiflora) in the gut, and quantitative data could be obtained using marker compounds unique to a food item. Thermochemolysis with TMAH and GC–MS represents a new technique for the gut content analysis of fish which can complement traditional techniques and be applied to samples that are very small (0.5–2 mg) and contain difficult to separate items.

Keywords: Gut content analysis; TMAH; Thermochemolysis; GC–MS; Fish

1. Introduction

To determine the diet of particular fish species fish ecologists need to analyse their gut contents. A wide variety of methods can be used such as measuring the weight or volume of a particular food type (gravimetric and volumetric methods, respectively) and counting individual food items (occurrence method, numerical method, various subjective methods such as the points method) [1,2]. These methods require a visual identification of the prey items and not all methods are equally useful for all food types. Numerical counts, for example, are not suitable where plants are among the principal food components because plants, unlike most animal prey, are not consumed as individual items [2]. Application of different methods can lead to very different ideas about a species' diet and even the same method applied by different authors can lead to inconsistent results. Furthermore, no one method of stomach analysis gives a complete picture of dietary importance [2].

In recent dietary and food web studies conventional gut content analysis methods have been complemented and replaced by stable isotope and, to a lesser extent, lipid analysis. However, while these techniques are useful for providing information on a species long-term assimilated diet they lack the taxonomic resolution of prey achievable with gut content analysis [3–7]. Moreover, since gut content analysis provides data on all foodstuffs ingested by a fish and not just the nutritionally important items, it is an irreplaceable tool for determining the impact of fish feeding on their environment (e.g. the impact of grazers on marine plants). In an ongoing study we are investigating the impact of two omnivorous fish species on *Posidonia australis* seagrass meadows in NSW, Australia.

Meuschenia freycineti and *Meuschenia trachylepis* belong to the Monacanthid family Monacanthidae (Leatherjackets)

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which are dominant in Eastern Australian seagrass (*P. australis*) habitats [8]. Juveniles and subadults of the *Meuschenia* species inhabit estuarine seagrass meadows where they are omnivorous canopy browsers. *M. trachylepis* and *M. freycineti* have very similar gut proportions and, like all Monacanthids, lack a true stomach [8–10]. Results of dietary analyses for both species vary widely between studies. Results for *M. freycineti* range from a diet dominated by animal material (60% animal versus 33% plant material) to one heavily dominated by algae and seagrass (76% plant versus 23% animal diet). For *M. trachylepis* results are just as diverse, ranging from a diet rich in animal material (60% animal versus 33% plant matter) to diets with an strong emphasis on plant material (76% plant versus 24% animal) [8,9,11,12]. These differences within studies are at least partly caused by different methods for estimating gut contents.

Thermochemolysis with tetramethylammonium hydroxide (TMAH) is an analytical technique first introduced for the characterisation of synthetic phenolic polymers [13]. It has subsequently been used to asses the molecular composition, degradation state and taxonomic source of bio- and geo-macromolecules in natural materials such as lignin, cellulose, cutin, suberin and humic substances [14–18]. Upon heating with TMAH, macromolecules are chemolytically hydrolysed and methylated yielding low molecular weight compounds that are amenable to separation and detection by gas chromatography–mass spectrometry (GC–MS). The advantage of this technique over conventional pyrolysis is that significantly more structural units are preserved. Lignin produces methyl derivatives of syringyl (S), guaiacyl (G) and *p*-hydroxy phenol (P) type compounds resulting from cleavage of β–O–4 bonds, the relative amounts of which can be used to distinguish between angiosperm, gymnosperm and non-woody vascular tissue. Cellulose produces permethylated saccharinic acids and methoxybenzenes such as the 1,2,4 isomer. Aliphatic biopolymers found in cutan, cutins, cuticles and suberins produce methyl derivatives of fatty acids, hydroxyfatty acids, α,ω-alkanedoic acids, 1,3,5-trihydroxybenzene and 2,4,6-trihydroxytoluene. The distributions of which are also source specific and, in the study of cutan, revealed new structural characteristics [16]. In the analysis of humic substances (HS), TMAH thermochemolysis to preserves structural units such as benzenecarboxylic acids that are degraded under conventional pyrolytic conditions which results in structural characterisations that are more representative and allows inputs to be more readily identified. Saturated and mono-unsaturated (but not polyunsaturated) lipids are effectively transesterified and fatty acids have been profiled in vegetable oils, animal fats and humic substances [19]. The reaction is rapid (30 min) and is performed in sealed glass tubes with the products collected in solvent, concentrated and analysed directly. Products can be quantitated using internal and external standards [20] and the technique can be readily implemented in laboratories having GC–MS capabilities. The technique is therefore very useful for the analysis of natural esters and macromolecules.

We investigated the applicability of thermochemolysis with TMAH for the determination of diet of two Monacanthidae fishes fed seagrass (*P. australis*), epifauna and epiflora under controlled conditions. Gut contents and food sources were analysed both qualitatively and quantitatively. Our aims were to determine: (1) whether or not different food sources can be distinguished alone and in the gut; (2) if the proportion of seagrass in the gut can be established; (3) if any changes through the gut can be observed.

2. Materials and methods

2.1. Fish, dietary sources and visual gut content analysis

Two fishes of 160 mm total length (one *M. freycineti*, one *M. trachylepis*) were caught using a seine net (20 m long, mesh size 16 mm) in a *P. australis* meadow in Careel Bay, Pittwater, NSW, in November 2003. We used two different species because we were unable to catch two specimens of the same species of comparable size. However, due to the similarities in feeding and digestion between the two species (Wressnig, unpublished data) the use of these two different species was considered justified. The fishes were allowed to acclimatise to the laboratory environment in two large holding tanks equipped with a filter (EHEIM classic 2213 and two air stones) for 2 weeks. They were fed *P. australis* blades from the second day onwards. Once a week they were fed brine shrimp as a protein supplement.

After the acclimatisation period the fishes were transferred in to two smaller experimental tanks (no filters, one air stone). There they were fed bare seagrass blades (*M. freycineti*) and seagrass blades covered with epiphytes (*M. trachylepis*) for 4 days. To obtain bare seagrass blades we scraped epiphytes off blades using a glass microscope slide on the day they were fed to the fish. Fishes were then starved for 24 h before they were fed the experimental blades (bare and epiphyte-covered, respectively) on which they were allowed to feed for 1 h. Subsequently the fishes were anaesthetised in clove oil before being frozen until further analysis.

Prior to dissecting the specimens were defrosted at room temperature. The body cavity was opened using scissors and the intestine carefully removed, taking out as much of the intestine as possible, from the oesophageal bulb to the end of the rectum. Since Monacanthids lack a true stomach we used the foregut (10% of overall gut length) for the analysis of gut contents. The foreguts were cut open using scissors and contents carefully removed with forceps (*M. trachylepis* (FG1), *M. freycineti* (FG2)). The foreguts were then rinsed with RO-water to ensure complete removal of all contents. To assess changes in the gut contents through the gut, the hind gut (rectum) from *M. freycineti* was collected (HG2). The samples were then freeze-dried over night and stored in airtight containers in the freezer until analysis.

Seagrass was collected from a *P. australis* meadow in Port Hacking (Little Turriel Bay). This site was chosen because the seagrass is easily accessible and epiphyte growth is rich. Shoots were broken off at the base and stored in plastic bags containing seawater for the transport back to the laboratory. There the shoots were transferred into five small tanks (20.5 cm × 19.5 cm ×