Trophic Position of Two Mysid Species (Crustacea: Mysidacea) in an Estuarine Ecosystem in Auckland, New Zealand, Using Stable Isotopic Analysis

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Abstract The trophic position of an organism can be determined by the stable isotope studies of nitrogen and carbon. The main objective of this study was to determine the trophic position of two mysid species, Tenagomysis chiltoni and T. novaezealandiae in the Kakamatua stream ecosystem in Auckland, New Zealand using 13C and 15N isotopes. Samples were collected during two weeks period in late December 2008 and early January 2009 including primary producers, leaf litter, aquatic invertebrates, fish species and sediment samples. Aquatic invertebrates and fish were collected from the stream using a hand net. Litter samples were collected randomly from the stream floor. Samples were sealed in plastic bags, and stored in a freezer until processing. All the samples were oven-dried, then ground to obtain a homogeneous powder. Three replicates of each sample were prepared. Samples were processed by the Waikato Stable Isotope Unit, University of Waikato, Hamilton, New Zealand. A trophic-level effect of 13C and 15N enrichment was clearly observed. Stable isotopic data indicated that T. chiltoni and T. novaezealandiae exhibited variety of feeding habits (feeding on the first and the second trophic levels) and can be considered as omnivores. Among the other invertebrates analysed, mysids seem to be an important invertebrate fauna which are capable of energy transferring towards the higher trophic level both from primary food sources and the secondary consumers as well. Differences in the isotopic composition were observed among same species depending upon the ontogenetic development.

Keywords: nitrogen isotope, carbon isotope format, food web analysis, estuary, New Zealand


1. Introduction

Mysids are small crustaceans which show high abundance in estuarine waters. Consequently, they serve as a major food source for ecologically and commercially important fish species which use estuary as nursery grounds. Thereby, they become an important link in estuarine food chains.

Stable isotope analysis (SIA) can provide a measure of trophic position, which integrates the assimilation of energy flow (or mass) through all the possible trophic pathways leading to an organism [1]. This analysis may not necessarily reflect short term feeding patterns and it provides an effective tool for integrating long-term assimilation of nutrients [2]. Therefore more traditional methods of gut content analysis have several draw backs, notably some organisms digest their prey quickly, making identification difficult, and this method reflects immediate feeding (if the diet is in an undigested state) pattern only [3]. In such cases, SIA can provide a useful alternative tool and give an insight into the feeding relationships between the organisms within a given food web [4,5].

SIA approach has been successfully applied to the study trophic interactions in variety of organisms, including mysids and zooplankton [2,6,7,8,9]. SIA is achieved by using isotopic fractionation of carbon, nitrogen and sulphur. The stable isotopes are different sizes and one is heavier than the other (e.g.: 12C and 13C, 13C is 8.3% heavier than 12C). Many reactions alter the ratio of heavy to light isotope, or “fractionate” stable isotopes, and however, the degree of fractionation is typically quite small [1]. In biological processes, when inorganic carbon is used to make organic compounds, 12C is weakly bonded and reacts more readily than 13C, because of its lighter mass. The level of this isotope effect is expressed as a fractionation factor. Therefore, the isotopic fractionation is the fluctuation in the isotope ratios as a result of natural biochemical processes due to their differences in atomic mass. Most ecological studies express isotopic composition in terms of δ values, which are parts per thousand (‰) differences from a reference standard [1].

The carbon isotopic compositions of the whole body of an animal reflects the isotopic composition of its diet, but the animal is on average enriched in δ13C by about 1 ‰ relative to the diet values. However, the δ15C values of
whole bodies of individuals of a species raised on the same diet may differ by up to 2‰ [10].

The relationship between the $^{13}$C/$^{12}$C ratio of a tissue and the $^{13}$C/$^{12}$C ratio of the diet depends both on the type of tissue and the nature of the diet [10]. Many of the isotopic relationships among the major biochemical fractions, namely the lipid, carbohydrate and protein fractions are qualitatively preserved as diet carbon is incorporated into the animal. However, the difference between the δ$^{13}$C values of a biochemical fraction in an animal and in its diet may be as large as 3‰ [10].

The δ$^{15}$N and δ$^{13}$C contents of animals reflect their diets. The ratio of stable isotopes of nitrogen (δ$^{15}$N) can be used to estimate trophic position because the δ$^{15}$N of a consumer is typically enriched by 3–4‰ relative to its diet [4,10]. Enrichment of δ$^{15}$N through the trophic network is widely recognized among most animals, including invertebrates and vertebrates, leading to a value of $3.4 \pm 1.1$‰ [4,11]. Stable isotope studies on mysid feeding relationships are rare. However, several world-wide studies using stable isotopic analysis of different mysid species have been reported [2,8,12,13,14]. The isotopic data of Paramysis lacustris Czerniavsky, from Curonian lagoon, suggested that diet changes occur during ontogenetic development [12]. The isotopic signatures of the prey of M. relicta in Lake Ontario [2] showed 25% feeding on amphipod parts and 20% on phytoplankton. The remainder of the diet consisted of zooplankton and rotifers. Stable isotope δ$^{13}$C, δ$^{15}$N values of the winter diets of M. mixta and M. relicta determined that they exploit both benthic and pelagic habitats [14]. These facts suggest that isotopic composition of aquatic organisms can provide basic information on their food source and trophic level. In theory, we can thus construct a SI food web (δ$^{15}$N versus δ$^{13}$C) of an ecosystem.

The main objective of this study is to determine the trophic position of two mysid species, Tenagomysis chiltoni and T. novaeezlandiae in an estuarine ecosystem. To realize this goal, isotopic ecological structure was determined in an estuarine ecosystem in Greater Auckland region using $^{13}$C and $^{15}$N isotopes.

2. Methodology

Kakamatua Stream is situated in the west coast of Auckland region (Figure 1) and one of the major waterways on the northern side of the Manukau Harbour, into which it drains. It has most mature and dense riparian vegetation and reed beds along the stream. Samplings were performed at the lower reaches of this stream, which inundated by the tidal action.

The mysid species T. chiltoni and T. novaeezlandiae dominated in this site, sympatrically with the glass shrimp (Palaemon affinis). The most common fish population at this site was Inanga - whitebait (Galaxias maculates). In addition juvenile short finned eel (Anguilla australis), Flat fish (flounder) and Athrinidae sp. (hardy heads) were rarely caught at this site.

Samples were collected in late December 2008 and early January 2009 including primary producers (Ulva sp., filamentous green algae and grass), leaf litter (terrestrial), consumers (aquatic invertebrates: amphipods, isopods, mollusc, decapods, mysids), fish and sediment samples. Samples were sealed in plastic bags, and stored in a freezer (−20°C) until processing. Aquatic invertebrates and fish were collected from the stream by a hand net. Litter samples were collected randomly from the stream floor. Surface sediments were collected by using a core sampler.

All the samples were oven-dried to a constant weight at 40°C, then ground in an Agatha stone mortar and pestle to
obtain a homogeneous powder. For the fishes, only the muscle parts were used for the analysis. Three replicates of each sample were prepared and the whole body of the animal samples was considered. The plant and animal samples of approximately 20 mg and sediment samples of 200 mg were processed by the Waikato Stable Isotope Unit, University of Waikato, Hamilton, New Zealand.

The carbon value ($\delta^{13}C$) was measured to a precision of ±0.1% and samples were referenced to a precalibrated C$_4$ sucrose standard that was cross-referenced to the Pee Dee belemnite standard [27]. The nitrogen value $\delta^{15}N$ was measured to a precision of ±3%, and samples were referenced to an urea standard which was traceable to atmospheric nitrogen [28].

The ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ($^{13}C$, $^{15}N$- heavy isotope; $^{12}C$, $^{14}N$- light isotope) are expressed as relative difference using the following equations.

$$\delta^{13}C = \left(\frac{^{13}C/^{12}C_{sample}}{^{13}C/^{12}C_{standard}} - 1\right) \times 10^3$$

$$\delta^{15}N = \left(\frac{^{15}N/^{14}N_{sample}}{^{15}N/^{14}N_{standard}} - 1\right) \times 10^3$$

Where $\delta^{13}C$ and $\delta^{15}N$ is the isotopic compositions referenced to the standards. $^{13}C/^{12}C_{sample}$ and $^{15}N/^{14}N_{sample}$ is the isotopic composition of the sample. $^{13}C/^{12}C_{standard}$ and $^{15}N/^{14}N_{standard}$ is the isotopic composition of the standard.

The stable isotope values of $\delta^{15}N$ and $\delta^{13}C$ were used to visualise the trophic position of each link (carbon and nitrogen sources) of the Kakamatua Stream ecosystem.

### 3. Results

The $\delta^{13}C$ and $\delta^{15}N$ mean (± SD) stable isotopic values of animals and their diets are given in Figure 2. These values demonstrate a separation between producers and consumers, and the evidence of $\delta^{13}C$ and $\delta^{15}N$ enrichment with increasing trophic level. It is important that these values demonstrate a noticeable separation between allochthonous (A & C-terrestrial) and autochthonous (B-in-stream) samples collected from this ecosystem (Figure 2). Leaf litter (allochthonous) had the most depleted $\delta^{13}C$ values and lower $\delta^{15}N$ values among the samples which were collected from the ecosystem (Table 1). The mineral soils are more enriched with $\delta^{13}C$ and $\delta^{15}N$ than the leaf litter (Table 1).

### Table 1. $\delta^{13}C$ and $\delta^{15}N$ values of Carbon and nitrogen sources collected from Kakamatua stream

<table>
<thead>
<tr>
<th>Carbon and nitrogen sources</th>
<th>$\delta^{15}N$ (‰)</th>
<th>$\delta^{13}C$ (‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulva sp.</td>
<td>8.37 to 9.15</td>
<td>−27.58 to −23.82</td>
</tr>
<tr>
<td>filamentous algae</td>
<td>8.88 to 10.08</td>
<td>−21.45 to −19.94</td>
</tr>
<tr>
<td>Grass</td>
<td>6.84 to 6.98</td>
<td>−13.56 to −13.42</td>
</tr>
<tr>
<td>Detritus 1</td>
<td>−1.6 to 0.59</td>
<td>−31.99 to −31.86</td>
</tr>
<tr>
<td>Detritus 2</td>
<td>1.91 to 3.21</td>
<td>−29.84 to −29.78</td>
</tr>
<tr>
<td>Detritus 3</td>
<td>−0.03 to 0.25</td>
<td>−25.21 to −24.9</td>
</tr>
<tr>
<td>Sediments</td>
<td>6.01 to 7.67</td>
<td>−24.21 to −23.54</td>
</tr>
<tr>
<td>amphipod</td>
<td>9.36 to 11.53</td>
<td>−23.65 to −21.9</td>
</tr>
<tr>
<td>Austridotea anectens (isopod)</td>
<td>8.35 to 9.58</td>
<td>−20.85 to −19.00</td>
</tr>
<tr>
<td>Helice crassa (mud crab)</td>
<td>10.2 to 10.63</td>
<td>−20.13 to −20.82</td>
</tr>
<tr>
<td>Juvenile T. chiltoni</td>
<td>11.38 to 11.49</td>
<td>−23.09 to −23.2</td>
</tr>
<tr>
<td>Adult T. chiltoni</td>
<td>10.61 to 12.5</td>
<td>−21.48 to −20.49</td>
</tr>
<tr>
<td>T. novaezealandiae</td>
<td>10.9 to 12.3</td>
<td>−20.06 to −19.58</td>
</tr>
<tr>
<td>P. curvirostris (shrimp)</td>
<td>9.07 to 9.91</td>
<td>−22.42 to −23.55</td>
</tr>
<tr>
<td>Juvenile G. maculates</td>
<td>11.77 to 12.86</td>
<td>−22.49 to −21.66</td>
</tr>
<tr>
<td>Adult G. maculates</td>
<td>12.9 to 13.6</td>
<td>−20.76 to −19.92</td>
</tr>
<tr>
<td>Juvenile Athrinidae sp.</td>
<td>11.09 to 10.92</td>
<td>−19.5 to −19.43</td>
</tr>
<tr>
<td>Juvenile flounder</td>
<td>13.56 to 13.62</td>
<td>−18 to −17.73</td>
</tr>
<tr>
<td>Anguilla. australis (eel)</td>
<td>14.87 to 15.04</td>
<td>−20.18 to −20.04</td>
</tr>
</tbody>
</table>

Legend: JGA, juvenile Galaxias maculates (4–5 mm); GAL, larger G. maculates (7–8 mm); JCH, juvenile T. chiltoni; ACH, adult T. chiltoni; NOV, T. novaezealandiae; JEE, A. australis; JFLO, juvenile flounder (Flat fish); JATH, juvenile Athrinidae sp; AMP, amphipods; ISO, Austridotea anectens (isopod); GAS, Potamopyrgus antipodarum (mollusc); JMC, juvenile Helice crassa (mud crab); PLS, P. curvirostris (shrimp); GRA, grass; ALG 1, Ulva sp.; ALG II, filamentous algae; SED, sediments; LEL1 – LEL3, leaf litter (originally terrestrial but collected from the stream flow).
The grass which were collected from the boundary of the stream, showed more enriched $^{13}$C values than the aquatic primary producers ($Ulva$ sp., filamentous algae) in-stream. The opposite is true for $^{15}$N values. $Ulva$ sp. and filamentous algae more enriched with $^{15}$N than the terrestrial primary producer, grass (Table 1).

The $^{13}$C values of invertebrates ranged from $-24.22$ to $-20.06\%$. Among the animals collected from the stream, the moluscs gave rather high $^{13}$C values ($^{13}$C $-13.57$ to $-12.29\%$) than all the other samples. The $^{13}$C values of fish ranged from $-22.49$ to $-17.73\%$ (Table 1).

The $^{15}$N value of invertebrates varied from $8.35$ to $12.5\%$. It is increased in the following order: Isopod, palamond shrimp, amphipods, mud crab, $T$. novaezealandiae, adult $T$. chiltoni and juvenile $T$. chiltoni. The increasing order of $^{15}$N values of fish species: $A$. chiltoni, juvenile $G$. maculates, adult $G$. maculates, juvenil founfloder and juvenile $A$. australis (Table 1).

Among these primary consumers $T$. novaezealandiae were more enriched with $^{13}$C and $T$. chiltoni were more enriched with $^{15}$N values. It is significant that $T$. chiltoni adults are more enriched with $^{15}$N values and $^{13}$C values than their juveniles. When analysing the isotope values related with the above hypothesis, the most possible common food links of both mysid species appear as isopods, filamentous algae and amphipods (Table 1).

The higher trophic level (secondary consumers) represented higher $^{15}$N values than other trophic levels, it mainly consist of fish. The juvenile short finned eel ($A$. australis) is the most $^{15}$N enriched link ($^{15}$N $15.04$ to $14.87\%$) and the juvenile flounder is the highest $^{13}$C enriched ($^{13}$C $-18.00$ to $-17.73\%$) link among the fish. Similar to other fish $G$. maculates also indicated higher $^{15}$N values ($^{15}$N $12.9$ to $13.6\%$). Juvenile $G$. maculates show lower $^{15}$N values than their adults. However, the juvenile $A$. chiltoni sp. has significantly lower $^{15}$N values ($^{15}$N $10.92$ to $11.09\%$) than other fish species, which is similar to the values of primary consumers (Table 1).

4. Discussion

The stable isotopic signatures of different food links facilitated the identification of trophic pathways and the trophic position of mysid species in Kakamatua Stream. Overall $^{13}$C measurements ranged from between $-31.6\%$ ($\pm0.62$) for leaf litter and $-12.95\%$ ($\pm0.64$) for molluscs, and $^{15}$N values ranged from between $-0.9\%$ ($\pm1.02$) for leaf litter and $14.96\%$ ($\pm0.09$) for juvenile short finned eel ($A$. australis).

4.1. First Trophic Level

Depending on the hypothesis of differences in $^{15}$N value between the consumer and the diet, a consumer is typically enriched by $3-4\%$ relative to its diet [1,4,11] and thus the primary food link and the primary consumers should have a difference of $^{15}$N values between $3-4\%$. On the argument of the differences in $^{13}$C value between the consumer and the diet, the consumer is enriched up to by $1\%$ and it may be large as $3\%$ relative to the food sources [10].

Therefore, based on isotopic signatures, the first trophic levels mainly supporting the primary consumers are identified as algae and sediments. The $^{15}$N values of leaf litter collected from the stream do not fit into the above hypothesis as potential food source of collected primary consumers. The filamentous green algae and $Ulva$ sp. had more enriched $^{15}$N values than sediments. The filamentous algae had higher enrichment of $^{13}$C than other aquatic primary food sources, is in agreement with $^{13}$C values that have been reported for filamentous green algae of different stream ecosystems in New Zealand [15].

The invertebrates in Kakamatua except mollusc were more dependent on autochthonous (in-stream) carbon sources ($Ulva$ sp., filamentous green algae), and this is similar to the previous results of [16], in which insects from adjacent grassland streams mainly depend on autochthonous material. Therefore the aquatic inputs mainly $Ulva$ sp. and, filamentous green algae sustain the aquatic food web at Kakamatua stream.

The results suggest a dependency of invertebrates on aquatic food sources rather than terrestrial leaf litter. Regardless, the stream ecosystem is not directly supported by the detritus based food chain. Because, the collected leaf litter samples offered depleted $^{13}$C and $^{15}$N values and could not be able to support the invertebrate groups which were collected for the analysis, though different invertebrate groups or different types of leaf litter may lead to the detritus based food chain. However, most of the primary consumers; amphipods, isopods, $H$. crassa, $P$. curvirostris, and $T$. chiltoni feed on sediments, the high nutrient, muddy substrates, which suggests sediments (substrate) may trap nutrients from detritus and make them available to the stream fauna. This is evident from the isotopic values of sediments, and it indicates higher $^{13}$C and $^{15}$N enrichment than the leaf litter. This argument agrees with a study of the trophic interactions within an estuarine food web (northern New Zealand) using fatty acid biomarkers and stable isotopes [17]. Finally it can be suggested that both autochthonous and allochthonous leaf litter-derived nutrients in sediments may be available to invertebrates. This agrees with a New Zealand stable isotopic study [15], which indicated that the food webs of pasture streams are based on both autochthonous and allochthonous material.

4.2. Second Trophic Level

Based on the isotopic signatures of invertebrates molluscs can be categorized as a different trophic group due to their extremely high $^{13}$C values than other invertebrates. Further to that, among the food sources analysed, grass is the possible food source for mollusc which shows higher $^{13}$C values. Therefore, based on the hypothesis of differences in $^{15}$N value and $^{13}$C values (DeNiro & Epstein; 1981; 1978), molluscs and grass (diet) can be considered as an entirely separate trophic relationship.

Depending on the differences of both $^{13}$C and $^{15}$N values, each consumer and their most potential food source/s can be assigned as: $P$. curvirostris (shrimp) - sediments; Amphipods - $Ulva$ sp. and sediments; juvenile $T$. chiltoni - $Ulva$ sp. and sediments; $A$. chiltoni - sediments; juvenile $H$. crassa (mud crab) - sediments; adult $T$. chiltoni - filamentous algae, $Ulva$ sp. sediments, amphipods and isopods; $T$. novaezealandiae - filamentous algae, isopods, and amphipods; molluscs – grass.

Stable isotopic data indicated that $T$. chiltoni and $T$. novaezealandiae exhibited variety of feeding habits and...
can be considered as omnivores. This stable isotopic data is supported by the gut content analysis of *T. chiltoni* in Lake Ellesmere [18], where the ingested foods had both aquatic and terrestrial origin (both from primary and secondary trophic levels).

Depending on the differences of δ\(^{13}\)C and δ\(^{15}\)N values for juvenile *T. chiltoni* and adult *T. chiltoni*, it is apparent that their food preference were also different, juvenile *T. chiltoni* fully depended on herbivorous diet (*Ulva* sp.) and adults are omnivores (filamentous algae, *Ulva* sp., sediments, amphipods and isopods). Similar observations on mycid diet changes during ontogenetic development have been demonstrated by several studies [19,20,21] and the findings here agrees with a stable isotope study of *Paramysis lacustris* [13]. Thus, the significant enrichment of δ \(^{15}\)N values and δ \(^{13}\)C values of *T. chiltoni* with the increasing size is in agreement with the similar observations of *P. lacustris* [13]. Similarly, enrichment of δ \(^{15}\)N values of *T. chiltoni* than *T. novaezealandiae* reflects that larger the size of mysids (even the different species), the enrichment of δ \(^{15}\)N value also higher and larger mysid species probably depends on more varieties of food than smaller.

Among the other invertebrates analysed, mysids are an important intermediate link of this ecosystem and capable of energy transfer towards the higher trophic level both from primary food sources and the secondary consumers as well. Therefore, this study provides the evidence that mysids depend on variety of food items, and also if abundant they can have a substantial effect on energy transfer at all trophic levels.

### 4.3. Third Trophic Level

Differences in the isotopic composition were observed among same species (*G. maculates*) depending upon the ontogenetic development, juvenile galaxid showed comparatively low isotopic compositions (δ\(^{15}\)N δ\(^{13}\)C values) than adult galaxid. Based on the differences of δ\(^{13}\)C and δ\(^{15}\)N values, the potential food sources of juvenile *A. australis* are *T. chiltoni*, *T. novaezealandiae* and juvenile *G. maculates*. Similarly, the likly food sources which closely link with *G. maculates* are *T. chiltoni*, *T. novaezealandiae*, isopods and amphipods while juvenile *G. maculates* link with juvenile *T. chiltoni* and amphipods. The δ\(^{13}\)C and δ\(^{15}\)N values of flat fish closely link with the isotope values of *T. novaezealandiae* and isopods as their potential food sources, as explained earlier, depending upon their lower δ\(^{15}\)N values. *Athriniidae* sp. do not link with any food item collected from the ecosystem.

The juvenile short finned eel has the highest enriched δ\(^{15}\)N values and is the top carnivore. Similar results have been reported from short finned eels in the South Island [22]. The juvenile fishes captured from the stream displayed a variety of δ\(^{13}\)C and δ\(^{15}\)N values, presumably reflecting a wide variety of food sources at different trophic positions [23]. The present study shows that the juvenile short finned eel displayed different trophic positions, as a top carnivore in the fourth trophic level (feeding on the second trophic level) and in the third trophic level (feeding on *T. chiltoni*, *T. novaezealandiae* and juvenile *G. maculates* at second or third trophic levels). Similar observations on the short finned eels which fed on *T. chiltoni* and *G. maculates* in a lake in New Zealand [24] and juveniles of short finned eel contained mysids in their diet as the second most common food [25].

*Galaxius maculates* also exhibits different trophic positions in the third and fourth trophic level. The juvenile flounder is the highest carbon enriched link among the fish and closely link with mysids. It is in agreement with similar stable isotopic between flounder and mysids [26].

The stable isotopic signature of juveniles and adult *G. maculates* showed that they fed at different trophic levels; juveniles feed on juvenile *T. chiltoni* while adults feed on adult *T. chiltoni*. This agrees with the observation of [26], that the juvenile spotted flounder contained a greater number of mysids in their stomachs while in larger fish, decapods and some fishes were more abundant in their stomachs.

### 5. Conclusion

The δ\(^{13}\)C and δ\(^{15}\)N values demonstrate between producers and consumers, and the evidence of δ\(^{13}\)C and δ\(^{15}\)N enrichment with increasing trophic level. It is important that these values demonstrate a noticeable separation between allochthonous (terrestrial) and autochthonous (in-stream) samples collected from this ecosystem.

Leaf litter had the most depleted δ\(^{13}\)C values and lower δ\(^{15}\)N values among the samples which were collected from the ecosystem. Grass which were collected from the boundary of the stream, show more enriched δ\(^{13}\)C values than the aquatic primary producers (*Ulva* sp., filamentous algae) in-stream. The opposite is true for δ\(^{15}\)N value: *Ulva* sp. and filamentous algae more enriched with δ\(^{15}\)N than the terrestrial primary producer, grass. Among the samples collected from the stream, the moluscs gave the highest δ\(^{13}\)C values.

*T. chiltoni* and *T. novaezealandiae* exhibited variety of feeding habits, feeding on the first and the second trophic levels and can be considered as omnivores. Among the other invertebrates analysed, mysids seem to be an important invertebrate fauna which are capable of energy transferring towards the higher trophic level both from primary food sources and the secondary consumers as well. Differences in the isotopic composition were observed among same species depending upon the ontogenetic development.

![Figure 3. Using SIA values, the potential food web for Kakamatua Stream](image)
Using the stable isotopic values of collected food links and their trophic positions, the achievable food web for Kakamatua Stream is illustrated in Figure 3.

Acknowledgement

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References