

Natural Diet of an Australian Freshwater Pipefish, *Hippichthys heptagonus* Bleeker, 1849

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BACKGROUND AND AIMS

Globally, the family Syngnathidae (seahorses and pipefish) is recognized as being both under threat from anthropogenic pressures and poorly studied. Of the 47 species are listed on the IUCN 2006 Red List, 33 are currently listed as 'data deficient', with inadequate information available to make an assessment of their risk of extinction. Within Australia, the country with the world's widest diversity of syngnathids (>25%), this has prompted environmental assessments of marine species, but no work on the four species that inhabit freshwater. This represents a sizable gap in our knowledge of Australian syngnathids and is of particular concern for three reasons. Firstly, the life-history characteristic of syngnathids (high parental investment-low fecundity, low abundance-low migration, and little to no pelagic dispersal of larvae) increases their vulnerability to natural and anthropogenic pressures (Dulvey et al., 2004; Foster and Vincent, 2004). Secondly, the coastal rivers and wetlands which they inhabit are the most highly modified ecosystems in Australia (Blaber, 2002; Saunders et al., 2002). Thirdly, studies from countries other than Australia (e.g. the Philippines), have shown that the commercial harvesting of syngnathids (for traditional Chinese medicine, as curios, for aquaria, and as by-catch of other fisheries) can have long lasting deleterious effects on local populations (Vincent, 1996). Both federal and state governments now strictly controls the harvest and export of all Australian syngnathids, however much of their ecology is still a mystery. What is known comes from studies of marine syngnathids, which are characterized by their small size, cryptic nature, and close association with complex benthos (Foster and Vincent, 2004). These studies have shown that syngnathids are ambush predators, almost exclusively carnivorous, with specialized diets linked to specific species morphology (e.g. snout length) (Kendrick and Hyndes, 2005). In an effort to better describe the ecology of Australia's freshwater syngnathids, a similar diet analysis of one freshwater species *Hippichthys heptagonus* was carried out. The aim of this project was to investigate the trophic ecology of *H. heptagonus*. This included determining its natural diet, exploring the observed variations between individuals/genders/age-classes, and the extent to which *H. heptagonus* resembled the more thoroughly studied marine Syngnathidae. The results presented are the first such investigation of this species and are a step towards a clearer understanding *H. heptagonus*, its role in the larger Mulgrave River food web, as well as the ecology of the other Australian freshwater syngnathids.



MATERIALS AND METHODS

Specimens for this study were collected from the Mulgrave River, north Queensland, a sixth order stream that experiences a predictable pattern of annual rainfall characteristic of many tropical rivers, with discharge reflecting the distinct wet (October-April) and dry (May-September) seasons. Pipefish were captured on fortnightly field trips, from the end of the wet season (April), and through the dry season (August) of 2005. As previous studies have noted syngnathids to be primarily visual feeders, with guts fullest during the day, sampling occurred between the hours of 800 and 1800 hours (Mosk, 2004; Kendrick and Hyndes, 2005). Pipefish were collected with dip nets and all individuals encountered were preserved within two minutes of capture to prevent further digestion of gut contents. Fish were dissected, prey items were identified to the lowest taxonomic level possible, enumerated, and their contribution to the total volume estimated (Hyslop, 1980).

Due to the exploratory nature of this study, a variety of indices were compared including both component and compound indices. Numerical percentage (%N), percentage of occurrence (%O), percentage by volume (%V), the Index of Relative Importance (IRI), and percent Index of Relative Importance (%IRI) were all considered in the analysis (Cortes, 1997). Following their calculation, the diet data (N, V, and O) were intuitively pooled according to taxonomic and ecological affiliations, and each index was recalculated. Taxonomic groupings roughly resembled those established by Pusey et al. (2004). Prey items were also classified as either planktonic (P) or demersal (D) based on the ecological groupings of other studies. Additionally, a Prey Size Ratio was constructed for each category (Liao et al., 2001). Prey categories with a more positive mean value were designated as larger, and fish with more positive mean PSR that were characterized as consuming larger prey. Mann-Whitney's U test was used to compare the diet between genders and maturity classes. Mature fish were identified by the condition of their gonads, or in males by the developmental state of their pouch. Possible ontogenetic and allometric variations in diet were examined using regression analysis. A percent principal component analysis (%PCA) was used to examine the contribution of individual pipefish to the larger trend in percent volume of prey, and to determine if any subtle groupings existed between genders or maturity classes. The resulting graph shows each of the major prey types linked to an arrow, where the length of the arrow is proportional to the relative volume of that particular prey type, as well as superimposed %PCA results for the individual pipefish.

To establish whether the sample size was sufficient to precisely describe the diet of *H. heptagonus*, a cumulative prey curve was constructed. This method plots the number of new prey species as a function of the number of guts analyzed. For this Brillouin's index was used as described in Hoffman (1979). To precisely determine its shape and the variation around it the order of samples was randomized 30 times and the curve calculated for each randomization.

RESULTS

A total of 22 pipefish were collected ranging in standard length from 11 to 109 mm (mean \pm 1 SE, 74.8 \pm 4.3 mm). The mean gut fullness ranged from 0.2 to 0.9 (0.53 \pm 0.02), and 3 individuals were excluded from the analysis having a gut fullness < 0.3. Copepods were by far the dominant prey item, being the most numerous (58.1 \pm 7.1%), frequently occurring (100%), as well as the most important in terms of volume (40.7 \pm 6.5%). Although ephemeropteran and dipteran larvae occurred less commonly, (4.6 \pm 1.6% and 9.6 \pm 1.8%), they contributed significantly to the total mean volume of prey (15.8 \pm 4.0% and 11.5 \pm 3.1%).

Table 1. Dietary information for *Hippichthys heptagonus* (n=18 from the Mulgrave River, north Queensland). Prey families are summed into larger taxonomic groups as in (Pusey et al. 2004). N, abundance of each prey group identified; O, frequency of occurrence of each prey group; V, the volume of each prey group; IRI, Index of Relative Importance of each prey group. Values for O' and %O are the observed nonempty values, which correspond to a particular food type, not the sum of its components. Values for IRI' and %IRI were calculated using nonempty %O'. Prey are noted as P for planktonic or D for demersal.

Prey Type	Type	N	%N	%V	%O	%O'	IRI	IRI'	%IRI	%IRI'
Microcrustaceans		826	65.9	7.1	272.5	47.4	6.5	35	184.2	100.0
Copepoda	P	778	59.1	7.1	249.5	40.7	6.5	19	100.0	98.1
Cyclopoida	P	48	3.7	0.2	1.0	0.2	0.1	1	0.5	0.5
Cladocera	P	48	3.7	0.2	1.0	0.2	0.1	1	0.5	0.5
Ostracoda	D	1	0.1	0.1	0.1	0.1	0.1	1	0.5	0.5
Aquatic Insects		132	10.3	2.7	126.5	20.5	3.0	36	184.2	100.0
Ephemeroptera	D	43	3.3	1.6	64.8	15.8	4.0	13	68.4	33.9
Diptera	D	83	6.4	1.8	55.3	11.5	3.1	17	89.5	44.3
Trichoptera	D	5	0.4	0.3	8.3	1.3	0.2	3	1.6	0.8
Collembola	D	1	0.1	0.1	0.3	0.0	0.1	1	0.5	0.5
Unsegmented worms		19	1.5	20.8	1.5	1.9	4	21.1	40	19.3
Nemertea	D	17	1.3	1.5	11.3	1.2	0.3	15	76.8	38.0
Platyhelminthes	D	1	0.1	0.1	0.3	0.4	0.1	1	0.5	0.5
Unidentified		12	1.1	0.4	16.0	2.3	1.2	7	36.8	18.2

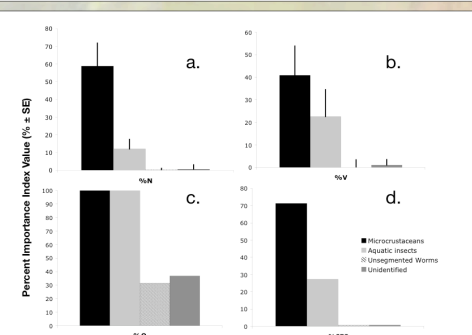


Figure 1. Differences in the importance of prey by type as indicated by four importance indices for *Hippichthys heptagonus* from north Queensland. Prey items are grouped into taxonomic categories as described in the methods section. (a) Percent abundance, (b) percent volume, (c) percent occurrence, and (d) percent IRI.

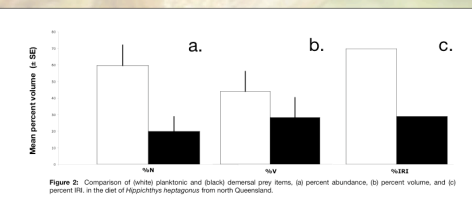


Figure 2. Comparison of planktonic and demersal prey items. (a) Percent abundance, (b) percent volume, and (c) percent IRI, in the diet of *Hippichthys heptagonus* from north Queensland.

In the pooled ecological categories planktonic prey constituted the largest portion numerically (76.2 \pm 5.4%), whereas demersal prey were less common (33.6 \pm 7.0%). Planktonic prey were also more important volumetrically (60.9 \pm 6.5%) than demersal (39.1 \pm 6.5%) (Fig. 2). In all three analyses (raw diet data, grouped taxonomic data, and grouped ecological data) there were no significant differences between genders, in mean percent volume or abundance (Mann-Whitney's U: $P > 0.15$ in all cases). However, adult fish consumed a higher mean percent volume of copepod prey than juveniles (Mann-Whitney's U: $P < 0.01$).

Qualitatively, percent PCA showed no distinct groupings between either genders or levels of maturity. However, it did again distinguish the prey items of importance in the diet of *H. heptagonus*. The first two factors comprised 87% of the total variation (69.7% and 17.3%) (Fig. 3). The cumulative diversity of the stomach contents reached an asymptote at k=10 indicating that the sample of 19 fish was likely large enough for an accurate picture of the diet of this species (Fig. 4).

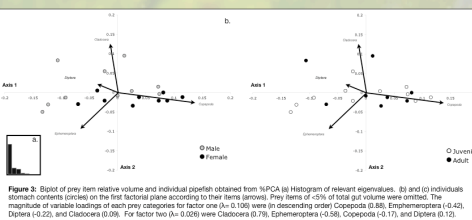


Figure 3. Biplot of prey item relative volume and individual pipefish obtained from %PCA. (a) Histogram of relevant eigenvalues. (b) and (c) individuals stomach contents (circles) on the first factorial plane according to their items groups. Prey items of 25% of total gut volume were omitted. The magnitude of variable loadings of each prey category for factor 1 (0.108) were (in descending order) Copepoda (0.88), Ephemeroptera (-0.42), Diptera (-0.22), and Cladocera (0.08). For factor two (0.020) were Cladocera (0.76), Ephemeroptera (-0.58), Copepoda (-0.17), and Diptera (0.12).

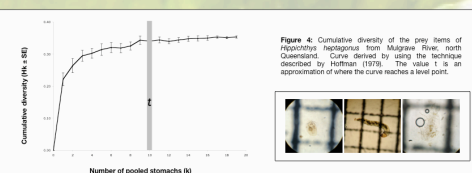


Figure 4. Cumulative diversity of the prey items of *Hippichthys heptagonus* from Mulgrave River, north Queensland. Curve derived by using the technique described by Hoffman (1979). The value 1 is an approximation of where the curve reaches a level point.

DISCUSSION

H. heptagonus collected from the Mulgrave River, north Queensland, were found to prey predominantly on microcrustaceans, both calanoid and cyclopoid copepods, as well as chydorid cladocerans. Each component prey index measured provided specific insight into the feeding habits of a *H. heptagonus*. The numerical abundance of prey items conferred information regarding feeding behavior, the measured volume of different prey types reflected their nutritional value within the diet of *H. heptagonus*, and the occurrence of prey in the diets of pipefish represented population-wide feeding habits. The prevalence of planktonic crustaceans in all of these measures was primarily a manifestation of their size/method of predation, and given the similarity of these findings to published diets of analogous marine species (Kendrick and Hyndes, 2005), it is likely that other aspects of their feeding behavior are also comparable. Additionally, the predominance of planktonic prey in the diet of *H. heptagonus* indicates that their distribution may be limited to the lower reaches of freshwater systems by the availability of these prey. The similarity in diet between maturity classes is likely due to the homogeneity in size between the two maturity classes. Within the larger Mulgrave River food web these fish are among a number of other planktivores (Pusey et al., 2004), however further study is necessary to determine the degree to which their diets overlap. These results also suggest that other freshwater syngnathids may have similar diets as well.

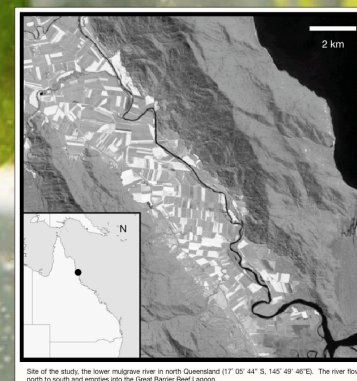


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Site of the study, the lower Mulgrave River in north Queensland (17° 05' 44" S, 145° 49' 40" E). The river flows north to south and empties into the Great Barrier Reef Lagoon.