FISH FEEDING IN HAMILTON HARBOUR
DIET OF PUMPKINSEED SUNFISH (Lepomis gibbosus) AND BROWN BULLHEAD (Amerius nebulosus) IN THE LITTORAL ZONE OF HAMILTON HARBOUR

by

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Abstract

Benthic macroinvertebrates, pumpkinseed sunfish (*Lepomis gibbosus*) and brown bullhead (*Ameirus nebulosus*) were sampled at three sites in western Hamilton Harbour and Cootes Paradise in early June 1995. The diversity, abundance, similarity and variability of the substrate benthic community and the gut contents of the fish were analyzed. The gut contents of both species of fish reflected the diversity, similarity and variability of the site at which they were caught. Mean gut fullness, analyzed using prey abundance and volume, shows no significant difference between sites, suggesting that the fish were eating similar amounts of prey at all three sites. Both pumpkinseed sunfish and brown bullhead fed selectively on certain size classes and benthic taxa, including several taxonomic groups previously unreported for these species.

Differences in benthic community structure at the three sites and the resultant differences in fish diet have important implications for the remediation of Hamilton Harbour. As water quality improves and the macrophyte cover increases, benthic diversity and abundance will increase. These improvements will increase the area of the littoral zone and the quality of the rearing and feeding environments for the recovering warmwater fisheries in Hamilton Harbour.
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INTRODUCTION

In 1987, the federal and provincial governments listed Areas of Concern for the Great Lakes under the Great Lakes Water Quality Agreement (The Remedial Action Plan for Hamilton Harbour 1991). As one of the Areas of Concern, a Remedial Action Plan was developed for the restoration of Hamilton Harbour. The plan includes a strategy to restore and manage the littoral zone habitat in the Harbour and in Cootes Paradise marsh (Fish and Wildlife Habitat Restoration in Hamilton Harbour and Cootes Paradise 1992, Hamilton Harbour Remedial Action Plan Team 1992).

Part of the strategy to improve the water clarity in Cootes Paradise includes the construction of a carp exclusion barrier between the marsh and the harbour. The stakeholders assume that excluding carp will result in a decrease in the turbidity of the marsh waters and an increase in the abundance of emergent and submergent vegetation necessary for fish spawning and refuge (Hamilton Harbour Remedial Action Plan Team 1992). These improvements will provide more heterogeneous habitat for benthic invertebrates and may, in fact, increase benthic biomass and diversity (Gaufin 1973, Voigts 1976, Gregg and Rose 1985, Cyr and Downing 1988, Beckett et al. 1992), which in turn will provide a more varied diet for benthivorous fish (Fox 1994, Gerking 1994). Moreover, many fish species require vegetated habitat for spawning and refuge (Mittelbach 1988). This important habitat is currently scarce in
Hamilton Harbour and is a factor limiting the distribution and abundance of many fish species (Holmes and Whillans 1984, Leslie and Timmins 1992).

Pumpkinseed sunfish (*Lepomis gibbosus*), one of the most abundant littoral fish species in Hamilton Harbour (Holmes and Whillans 1984), are mainly visual predators (Keast and Webb 1966). Recent studies indicate that they may be also able to detect prey motion in turbid water through receptors in the lateral line (N. Collins pers. comm.). Studies of the ontogenetic niche shifts of pumpkinseed indicate that young-of-the-year fish live in nearshore macrophyte beds in the summer, feeding on chironomids, isopods and ostracods, and move into deeper water in October, switching to a diet of chironomids, cladocera and Ephemeroptera (Keast 1978, Laughlin and Werner 1980, Hanson and Qadri 1984, Mittelbach 1984, Mittelbach 1988, Osenberg et al. 1988, Osenberg et al. 1992, Fox 1994). Year I-VII pumpkinseed live nearshore in the summer, feeding mainly on chironomids, while increased size and jaw strength allows year VI+ to consume gastropods (Keast 1978, Laughlin and Werner 1980, Mittelbach 1984, Osenberg and Mittelbach 1989, Mittelbach et al. 1992, Osenberg et al. 1992, Pierce et al. 1993). Evidence from laboratory studies of pumpkinseed metabolism (Evans 1984) and field studies at Lake Opinicon suggest that there is virtually no overwinter feeding in pumpkinseed sunfish (Keast 1978, Danylchuk and Fox 1994).

Brown bullhead, a species of moderate abundance that once thrived in Hamilton Harbour (Holmes and Whillans 1984), are usually
found in shallow warm water with abundant macrophytes (Scott and Crossman 1973, Klarberg and Benson 1975). They are highly tolerant of low oxygen levels and high concentrations of organic pollution (Scott and Crossman 1973, Klarberg and Benson 1975). The brown bullhead has chemotactic barbels that favour bottom feeding and feeding in a low-light environment (Keast and Welsh 1968, Keen 1982).

Keast (1985b) found that bullhead are generalist feeders at the prey order level, opportunistically feeding on amphipods and isopods, and under-utilizing oligochaetes and Trichoptera. Evidence from West Virginia also indicates preference of chironomids over oligochaetes, but it also suggests that bullhead are selective feeders (Klarberg and Benson 1975). Like pumpkinseed, bullhead undergo diet switching through ontogeny (Keast 1985b). Year 0 fish feed initially on Cladocera and switch to chironomids, amphipods and Ephemeroptera nymphs as the grow. Year I-III bullhead feed mainly on chironomids, but in late summer, they feed opportunistically on amphipods, isopods and Cladocera (Keast 1985b). Year IV-VII bullhead show diet variation over the season, with initially high chironomid consumption dropping off in late summer to early fall, with a coincident increase in feeding on gastropods, isopods, fish and ostracods (Keast 1985b).

Changes in the macrophyte density in Cootes Paradise, the goal of the RAP, will induce changes in the benthic community structure. Many authors (Boyle et al. 1984, Krieger 1984, Resh and Jackson 1993) advocate the use of more than one index to investigate community structure. Indices of diversity and similarity are considered effective in
the study of benthic fauna at different sites, as well as in the gut contents of fish (Haedrich 1975). Similarity can be used as a calculation of overlap in composition and abundance (Haedrich 1975). The use of diversity indices to assess water quality (presumably, high diversity indicates better water quality) is advantageous because it yields a single number that can be used to compare sites or dates. It should be noted, however, that species may be removed due to a decrease in water quality and may be replaced by a more pollution tolerant species with no resultant change in the diversity index (Boyle et al. 1984).

Variation in fish diet can be due to many factors, including spatial variation in food availability or fish behavior, habitat in which fish are feeding and differences in individual fish, all of which have implications for this study (Smagula and Adelman 1981, Holbrook and Schmitt 1992, Jobling and Baardvik 1994, Bridcut and Giller 1995). Similarity indices and coefficients of variation have been used to study variation between individual fish (Smagula and Adelman 1981, Jobling and Baardvik 1994, Bridcut and Giller 1995). The use of indices of prey selection can be used to further investigate relationships between habitat fauna and gut contents. Selectivity is said to take place when the relative frequency of a prey item in the diet is different from the relative frequency of that item in the environment (Chesson 1978, Johnson 1980, Chesson 1983). Selectivity is an important factor in the study of fish feeding because it determines the relative energy intake, which has implications for growth, reproductive success, and survival (Mittelbach 1988, Osenberg and Mittelbach 1989). Although the Manly-Chesson
selectivity index used in this study provides valuable information about diet choice, there are some problems inherent in the index (Johnson 1980). Selectivity indices can only indicate the relative preference or avoidance of a prey item, rather than absolute preference (Chesson 1978, Johnson 1980). For selectivity indices, the probability of prey encounter is assumed to be directly proportional to relative density of prey in environment, not taking into account refuges, prey switching (Chesson 1978) or prey avoidance behavior. It also assumes that encounters with the food items which do not result in consumption do not affect the consumer's subsequent behavior, for example, it assumes that fish would not learn to avoid eating a poisonous prey item. Despite these problems, the benefits outweigh the drawbacks and selectivity indices are an accepted method of characterizing diet (Mittelbach 1988, Osenberg and Mittelbach 1989, Schael et al. 1991, Bremigan and Stein 1994).

Benthic invertebrates offer many advantages for the study of water and habitat quality issues because they occur in large numbers and in a large variety of habitats. The benthic community is composed of a large number of species that represent a range of pollution tolerances and, due to their sedentary nature, they characterize a location and make it possible to study spatial patterns of pollutant effect (Brinkhurst 1974, Moore 1980, Lang 1990, Rosenberg and Resh 1993). Given the importance of benthos to fish, their rich composition and responsiveness to local conditions, one might speculate that changes in the habitat characteristics due to remedial actions will propagate through the food web of Hamilton Harbour. Understanding what may be the nature of
anticipated responses can be addressed by comparing sites with different habitat qualities and their effects on common benthivorous fish. Specifically, this study seeks to gain insight into the feeding habits of pumpkinseed sunfish and brown bullhead at three sites in western Hamilton Harbour. I am interested in whether different attributes of benthic community structure such as benthic density, diversity and composition have a measurable effect on the amount and quality of food consumed by fish. This information is necessary for making linkages between the anticipated expansion of vegetated habitats and its impact on fish populations.
MATERIALS AND METHODS

Study Site

Hamilton Harbour is a highly eutrophic body of water at the western end of Lake Ontario, receiving inputs from Redhill Creek in the southeast, Grindstone Creek in the northwest and Spencer Creek, via Cootes Paradise, in the west (The RAP for Hamilton Harbour 1991). The Harbour also receives inflow of municipal treated waste water and overflow from storm sewers (Leslie and Timmins 1992). These factors, as well as lake morphometry and hydrology, create a range of habitats varying in quality as potential food sources for fish.

Sampling took place at three areas: Cootes Paradise (Princess Point), and two sites in Hamilton Harbour (one at the outlet of Grindstone Creek and the other at Harbourfront Park: Fig. 1). These sites were chosen because it was hypothesized that due to varying water quality and habitat parameters, they would show a gradient of benthic species density and diversity. Harbourfront Park, the site in the Harbour proper, is a marina in the southwestern end of the bay. This area is characterized by high submerged macrophyte densities (covering > 60% of the sampling area; pers. obser.), good water clarity (average Secchi transparency of 1.3 m; Charlton 1992) and a heterogeneous substrate composed of rocks, cobble, sand and silt (pers. observ.). Grindstone Creek drains the north central portion of the Harbour watershed (The
Remedial Action Plan for Hamilton Harbour 1991). The site at the outlet of Grindstone Creek is in a pool called Sunfish Pond, with low water clarity (pers. observ.), a band of emergent macrophytes (*Typha* sp.) along the shore (composing <30% of the sampling area; pers. obser.), and a substrate composed of sand, silt and detritus (Holmes and Whillans 1984). Sampling in Cootes Paradise took place at Princess Point, in the southeastern part of the wetland. The sampling area was mainly open water with *Typha* sp. distributed in a narrow band along the shore, covering less than 15% of the area sampled (pers. obser.). Cootes Paradise, a highly eutrophic and degraded wetland, is characterized by very low water clarity with mean Secchi disc transparencies of 20-30 cm (Painter et al. 1989). The substrate is homogeneous composed of fine silt mixed with detritus (Simser 1982, Holmes and Whillans 1984).
Figure 1: Study area showing the western end of Lake Ontario, Hamilton Harbour and Cootes Paradise. The three sampling areas for both benthos and fish are indicated by (●).
Fish Sampling and Gut Content Analysis

The fish were caught using a 15 m x 2 m seine, with a stretched mesh size of 2.5 cm. Seining took place between 8 am and 12 pm from May 31, 1995 to June 16, 1995, the morning sampling time a period of overlap for gut fullness for the two species (Keast and Welsh 1968, Johnson and Dropkin 1993). While still in the field, standard length (SL) and mouth gape were measured and the stomach contents were removed from the fish and preserved in 70% ethanol. Since only one brown bullhead was caught at Harbourfront Park, this species was omitted from the analysis at this site.

Once returned to the lab, the prey items were sorted from the gut contents, without washing on a 250 μm mesh net. Benthic invertebrates in the guts were identified to as high a taxonomic resolution as possible. Invertebrates for both gut contents and substrate were identified using the following keys: Brinkhurst (1986) Clarke (1981), Merritt and Cummins (1984) Oliver and Roussel (1983), Stimpson et al. (1982) and Thorpe and Covich (1991). The gut contents were analyzed using both numerical and gravimetric methods. The number of items per prey species in each gut was counted and the biomass of the prey items was estimated using length-weight regressions developed by Smock (1980). Prey abundance is important in characterizing diets and the measurement of prey volume may have some relationship to caloric value (Hynes 1950, Windell and Bowen 1978, Hyslop 1980, Wallace 1981).

The analysis of gut contents included species identification as well as measurement of abundance, calculation of Shannon-Weiner
diversity, ANOVA (using the software package Statistica from Statsoft), and percent similarity (Gauch 1986, Sokal and Rohlf 1981). The Manly-Chesson index was used in the estimation and analysis of feeding preference (Manly 1974, Chesson 1978, Chesson 1983) and power analysis was performed on the fish gape and standard length (Krebs 1989, Sokal and Rohlf 1981).

Appendix I lists the taxa and abundances consumed by the fish.

**Benthos Sampling**

Benthic invertebrates were sampled with a core sampler (surface area= 7.2 cm$^2$) to a depth of approximately 10-20 cm on the day following fish seining for each site. Fifteen sites were sampled in each area, 3 replicate cores per site, except for the Harbourfront Park site. Since certain regions of the substrate at Harbourfront were composed of boulders, the core sampler could not be used. An Ekman or ponar grab sampler would also have been ineffective. Therefore, only 10 sites were sampled at Harbourfront, with 3 replicates per site. All samples were washed in the field on 250 μm mesh netting and, once returned to the lab, preserved in 70% ethanol. In total, 8309 benthic invertebrates were hand-sorted from the sediment samples with the aid of a dissecting microscope and identified to the highest taxonomic resolution possible. Abundance and diversity were calculated for investigation of community structure at each site. The sampled taxa and their abundances are listed in Appendix II. Biomass was calculated using the length-weight regressions developed by Smock (1980).
Indices and Analysis

Percent similarity (PS) (Gauch 1986) was used to calculate similarity, rather than Euclidean distance, Squared Euclidean distance, or Manhattan distance, since this was found to be the only index that was not effected by the large number of zeros in the data set.

\[
PS = 200 \sum \min(A_{ij}, A_{ik}) / \sum (A_{ij} + A_{ik})
\]

Where \( A_{ij} \) and \( A_{ik} \) are the abundances of species \( i \) in samples \( j \) and \( k \).

Mantel's test (Mantel 1967, Schnell 1985) was used to investigate the within-site versus between-site similarity for pumpkinseed and bullhead diet. This test was chosen in addition to a traditional ANOVA, since it calculates statistical significance correcting for sample interdependencies (Schnell 1985).

The Manly-Chesson selectivity index (Manly 1974, Chesson 1978, Chesson 1983) was used to calculate the fish feeding selectivity for benthic taxa:

\[
a = (r_i/n_i) / \sum (r_j/n_j), \quad i=1, \ldots, m
\]

Where \( r_i \) is the proportion of food type \( i \) in the diet, and \( n_i \) is the proportion of food type \( i \) in the substrate.
Diversity for substrate and diet benthic taxa was calculated using the Shannon-Weiner diversity index:

\[ H' = \sum p_i \log p_i \]

where \( p_i \) is the proportion of prey item \( i \)

All other statistics (ANOVA, chi-square, standard deviation) were analyzed using the software package Statistica from Statsoft.
RESULTS

Substrate Characteristics and Benthic Diversity

Harbourfront Park had both the highest average abundance and richness (R) of benthic invertebrates at 189,130 individuals per m$^2$, R=41, followed by Grindstone at 56,271 ind./m$^2$, R=33, and Cootes Paradise at 15,271 ind./m$^2$, R=24. Of the 44 taxonomic groups found in the littoral zone sediment, 14 genera of chironomids and 7 genera of oligochaetes were identified. The data were analyzed at two levels of taxonomic resolution; high resolution, with the Chironominae and Oligochaeta split into their respective genera, and low resolution, with these genera grouped together. The diversity of the benthic community was found to be significantly different at and between each of the three sites, with Harbourfront having the highest diversity, followed by Grindstone and Cootes (ANOVA: p<.0001 for both low and high resolution data for the all three sites and for each pairwise site comparison; Fig. 2).

The mean diversities of pumpkinseed diet using high resolution data were not significantly different between the three sites (ANOVA: p=0.23, Fig 3a). At low taxonomic resolution (ANOVA: p=0.2; Fig. 3b), the diversity of the pumpkinseed diet at the three sites follows the same trend as was found for benthic diversity. Fish caught at Harbourfront had the most diverse fauna in their gut contents, followed by fish at the
Grindstone and the Cootes sites. When the between-site comparisons were calculated, it was found that the difference between Cootes and Harbourfront (p<.0119) may have been causing the significant result, since between site comparisons between Cootes and Grindstone and Grindstone and Harbourfront did not show significant differences.

Neither the high or low resolution data for bullhead gut contents produced significant results, although a trend similar to that noted for the pumpkinseed is detectable (Fig. 4). Bullhead diet diversity, using both methods, is lower for fish caught at the Cootes Paradise site than for fish caught at Grindstone.
Figure 2: The mean benthos diversity found in the substrate at the three sites for both high (a) and low (b) resolution data. Large boxes and bars represent the standard error and standard deviation, respectively.

Substrate benthos diversity at the three sites
(Chironomids and oligochaetes split into genera)
F(1,116)=66.93; p<.00001

Substrate benthos diversity at the three sites
(Chironomid and oligochaete genera grouped together)
F(1,116)=87.52; p<.00001
Figure 3: The mean diversity of pumpkinseed diet at the three sites for both high (a) and low (b) resolution data. Large boxes and bars represent the standard error and standard deviation, respectively.

Pumpkinseed diet diversity at the three sites (chironomids and oligochaetes split into genera)

\[ F(2, 37) = 1.55; \ p = 0.23 \]

Pumpkinseed diet diversity at the three sites (chironomids and oligochaete genera grouped together)

\[ p = 0.02 \]
Figure 4: The mean diversity of brown bullhead diet at the three sites for both high (a) and low (b) resolution data. Large boxes and bars represent the standard error and standard deviation, respectively.

Bullhead diet diversity at two sites (chironomids and oligochaetes split into genera)

$F(1,31)=1.427; p=0.24$

Bullhead diet diversity at two sites (chironomid and oligochaete genera grouped together)

$F(1,31)=2.462; p=0.13$
Fish Size and Gape

The pumpkinseed sunfish caught at the three sites ranged in size from 5.9 cm to 12.6 cm standard length (SL) with gape sizes ranging from 0.4 to 1.3 cm. (Table 1).

Table 1: The means, standard deviations (SD), and ranges for the standard length (SL: cm) and gape (cm) data for pumpkinseed (a) and bullhead (b) at the three sites.

<table>
<thead>
<tr>
<th>Pumpkinseed Sunfish</th>
<th>Cootes</th>
<th>Grindstone</th>
<th>Harbourfront</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Mean SL</td>
<td>SD</td>
<td>Range</td>
</tr>
<tr>
<td>13</td>
<td>8.79</td>
<td>0.6</td>
<td>8.0-9.9</td>
</tr>
<tr>
<td>9</td>
<td>9.74</td>
<td>2.19</td>
<td>5.9-12.0</td>
</tr>
<tr>
<td>18</td>
<td>10.12</td>
<td>1.79</td>
<td>7.2-12.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Brown Bullhead</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cootes</td>
</tr>
<tr>
<td>N</td>
</tr>
<tr>
<td>25</td>
</tr>
<tr>
<td>8</td>
</tr>
</tbody>
</table>

When all three sites are considered together, the mean SL of fish was not significantly different (ANOVA: p=0.09; Fig. 5a), nor was the mean gape (ANOVA: p=0.06; Fig. 5b). This relationship was consistent for all but one between-site pairing. An ANOVA between the mean standard length of fish at Cootes and Harbourfront show that pumpkinseed caught at Harbourfront were significantly larger than those caught at Cootes (p=0.02). This size difference was not reflected in the gape size of the fish at these two sites. The results of this pairing suggest, however, that
Figure 5: The mean standard length (a) and gape size (b) of pumpkinseed sunfish at the three sites. The large boxes and bars represent the standard error and standard deviation, respectively.
Figure 6: The mean standard length (a) and gape size (b) of brown bullheads at the two sites. The large boxes and bars represent the standard error and standard deviation, respectively.

Mean bullhead SL at the two sites
$F(1,31)=.298; p=0.59$

Mean gape of bullhead at the two sites
$F(1,31)=.3433; p=0.56$
differences among sites might influence other results and thus require cautious interpretation.

The bullhead caught in Cootes and in Grindstone range in size from 7.3 to 21.0 cm SL, with no significant difference between the mean sizes of fish at each site (ANOVA: $p=0.59$; Fig 6a) nor in the mean gape (ANOVA: $p<0.56$; Fig 6b).

Power analysis ($p<.05$, power level=.80) was performed on the standard length and gape of both species to determine the sample size necessary to consistently see a significant difference between the three sites. The necessary sample sizes were substantially larger than those used, to the point of being impractical for bullheads (Table 2).

Table 2: Results of power analysis ($p<.05$, power level=.80) for the standard length and gape.

<table>
<thead>
<tr>
<th>Sample Categories</th>
<th>Sample Size</th>
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<tbody>
<tr>
<td>Pumpkinseed: SL</td>
<td>54</td>
</tr>
<tr>
<td>Pumpkinseed: gape</td>
<td>42</td>
</tr>
<tr>
<td>Bullhead: SL</td>
<td>427</td>
</tr>
<tr>
<td>Bullhead: gape</td>
<td>343</td>
</tr>
</tbody>
</table>

**Diet Characterization**

In Cootes Paradise, pumpkinseed are eating proportionally more chironomid larvae than expected from the availability estimates (Fig 7). Bullheads, and to a lesser extent pumpkinseed, seemed to have consumed a disproportionate number of copepods, although this could have been due to the lack of sieving of gut contents. Neither fish species took advantage of the high proportion of oligochaetes available in the habitat. At the Grindstone Creek site, both species consumed proportionally more
chironomids, amphipods and isopods than expected from availability in the substrate, while neither took advantage of the high proportion of gastropods (Fig 8). At Harbourfront, the pumpkinseed consumed chironomids, water mites, cladocera, amphipods, isopods, and trichoptera larvae in much greater proportions compared to what was available in the substrate (Fig 9). At all three sites, both species of fish mainly consumed chironomid larvae in the 3 to 8 mm range (Fig 10).
Figure 7: A histogram of the proportions of each benthic taxa found in substrate (solid bars), pumpkinseed (striped bars) and bullhead (hatched bars) gut contents at the Cootes Paradise site.
Figure 8: A histogram of the proportions of each benthic taxa found in substrate (solid bars), pumpkinseed (striped bars) and bullhead (hatched bars) gut contents at the Grindstone site.

Proportion of benthic taxa in substrate and fish diets at Grindstone
Figure 9: A histogram of the proportions of each benthic taxa found in substrate (solid bars), pumpkinseed (striped bars) and bullhead (hatched bars) gut contents at the Harbourfront site.
Figure 10: Histograms of the proportions of each chironomid size class found in substrate (solid bars), pumpkinseed (striped bars) and bullhead (hatched bars) gut contents at Cootes Paradise (a), Grindstone Creek (b) and Harbourfront Park (c).

Proportion of chironomid size classes for Cootes substrate and fish diet

Proportion of chironomid size classes for Grindstone substrate and fish diet
Proportion of chironomid size classes for Harbourfront substrate and fish diet
All observations on fish gut contents were significantly different from the expected values found in the substrate for the benthic taxa. (Table 3). For the size classes of chironomids, bullhead and pumpkinseed at all sites and pumpkinseed at Grindstone were significantly different from the expected values.

Table 3: Chi-square tests between the substrate and gut fauna for taxa of invertebrates and size classes of chironomids. The test compared the fauna found in the substrate (expected values) and those in the pumpkinseed and bullhead gut contents (observed values).

<table>
<thead>
<tr>
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<th>Harbourfront</th>
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<td>substrate</td>
<td>substrate</td>
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<td></td>
<td>pumpkinseed</td>
<td>pumpkinseed</td>
<td>pumpkinseed</td>
</tr>
<tr>
<td></td>
<td>bullhead</td>
<td>bullhead</td>
<td>bullhead</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
</tr>
</tbody>
</table>

The Manly-Chesson selectivity indices for the benthic taxa quantifies the relationships seen in the histograms (Figs. 7-9) by calculating whether certain prey items are selected for or against in comparison to availability in the substrate. According to the index, chironomids, copepods, ostracods were selected for at the Cootes site, chironomids, amphipods, isopods, and Corixidae were selected at
Grindstone, and water mites and trichoptera were selected diet items at Harbourfront Park (Table 4).

Table 4: Manly-Chesson selectivity index for the fish at all three sites. Diet items selected for in comparison to substrate availability (marked with a *) have values greater than .07. Values less than .07 indicate the item was selected against, while values equal to .07 indicate random feeding on that item.

Since the substrate samples were sieved through mesh netting, as compared to the unsieved gut contents, microinvertebrates such as cladocerans and copepods may have been lost from the sediment samples. To examine preference relationships without the bias of the possibly underrepresented microinvertebrates, Manly-Chesson selectivity was re-
calculated excluding cladocerans and copepods (Table 5). Similar selectivities were seen, with the inclusion of leeches in bullhead diet at Cootes and Corixidae in pumpkinseed diet at the Grindstone site.

Table 5: Manly-Chesson selectivity index for the fish at all three sites, with copepod counts not included. Diet items selected for in comparison to substrate availability (marked with a *) have values greater than .08. Values less than .08 indicate the item was selected against, while values equal to .08 indicate random feeding on that item.

<table>
<thead>
<tr>
<th></th>
<th>Cootes Pumpkinseed</th>
<th>Cootes Bullhead</th>
<th>Grindstone Pumpkinseed</th>
<th>Grindstone Bullhead</th>
<th>Harbourfront Pumpkinseed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chironomids</td>
<td>*0.775</td>
<td>*0.440</td>
<td>*0.108</td>
<td>0.047</td>
<td>0.019</td>
</tr>
<tr>
<td>Oligochaetes</td>
<td>0.000</td>
<td>0.037</td>
<td>0.000</td>
<td>0.006</td>
<td>0.000</td>
</tr>
<tr>
<td>Ostracoda</td>
<td>*0.225</td>
<td>*0.417</td>
<td>0.008</td>
<td>0.001</td>
<td>0.000</td>
</tr>
<tr>
<td>Water Mite</td>
<td>0.000</td>
<td>0.000</td>
<td>*0.641</td>
<td>*0.709</td>
<td>*0.296</td>
</tr>
<tr>
<td>Amphipoda</td>
<td>0.000</td>
<td>0.000</td>
<td>*0.060</td>
<td>*0.236</td>
<td>0.018</td>
</tr>
<tr>
<td>Isopoda</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.056</td>
</tr>
<tr>
<td>Trichoptera</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>*0.564</td>
</tr>
<tr>
<td>Dugesia</td>
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</tr>
<tr>
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<tr>
<td>Corixidae</td>
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<td>0.000</td>
<td>*0.180</td>
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<tr>
<td>Leech</td>
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<td>*0.104</td>
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<td>0.000</td>
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<tr>
<td>Gastropod</td>
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<td>0.002</td>
<td>0.000</td>
<td>0.001</td>
<td>0.007</td>
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<tr>
<td>Sphaeriidae</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
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</tr>
</tbody>
</table>
**Gut Fullness**

Analysis of gut fullness, investigated using both number of prey items and volume of prey items as a measure of fullness, found no significant difference between the means at the three sites (Fig 11 and 12). No significant difference was found for comparisons between pairs of sites, indicating that the fish had similar gut fullness at the time of sampling.

**Similarity**

The percent similarity between gut and substrate benthic fauna at each site was calculated on proportional data because of the differences in sampling size between an entire site and a fish stomach. For both pumpkinseed (ANOVA: p=0.051) and bullhead (ANOVA: p=0.42), the similarity between benthos in the substrate and in the diet was not significantly different from site to site (Fig 13) when all three sites were analyzed together. A between-group comparison found that the mean similarity between pumpkinseed and the fauna at Cootes and Harbourfront were significantly different (p=0.025), while the Cootes and Grindstone and Harbourfront pairings showed no significant difference. When the mean similarity between individual fish at each site was analyzed, pumpkinseed at the Cootes site had a significantly higher diet similarity, followed by the fish at the Harbourfront and Grindstone sites (ANOVA: p<.0001 Fig 14a). ANOVA's for each pair grouping confirmed this result. Bullhead follow a similar trend, showing high among-individual diet similarity at Cootes, and a
significantly lower similarity for fish at Grindstone (ANOVA: p=0.004; Fig. 14)

The calculation of similarity between individual fish at a site introduces dependence into the matrix. Mantel's test was used to compensate for these interdependences and to determine whether the within-site diet similarities were significantly different from the between-site diet similarities. Pumpkinseed and bullhead diets were found to be more similar within each site than between sites (p<0.05).

Table 6: Between-site diet similarities; results of Mantel tests (t) and matrix correlations (r). All results are significant (p<0.05), with 2000 Monte Carlo simulations calculated.

<table>
<thead>
<tr>
<th>Species</th>
<th>Site Pairing</th>
<th>t</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pumpkinseed</td>
<td>Cootes-Grindstone</td>
<td>8.8121</td>
<td>0.5446</td>
</tr>
<tr>
<td>Pumpkinseed</td>
<td>Cootes-Harbourfront</td>
<td>8.2171</td>
<td>0.355</td>
</tr>
<tr>
<td>Pumpkinseed</td>
<td>Grindstone-Harbourfront</td>
<td>11.3039</td>
<td>0.5577</td>
</tr>
<tr>
<td>Bullhead</td>
<td>Cootes-Grindstone</td>
<td>6.2089</td>
<td>0.3383</td>
</tr>
</tbody>
</table>
Figure 11: The mean gut fullness found for pumpkinseed sunfish at the three sites calculated by number of prey items (a) and by volume (b). Large boxes and bars represent the standard error and standard deviation, respectively.

Mean number of items in pumpkinseed guts
F(2, 3) = 1.899; p = 0.16

Mean volume of items in pumpkinseed guts
F(2, 3) = 1.363; p = 0.27
Figure 12: The mean gut fullness found for brown bullhead at the two sites calculated by number of prey items (a) and by volume (b). Large boxes and bars represent the standard error and standard deviation, respectively.

Mean number of items in bullhead guts
$F(1,31) = 2.484; p = 0.13$

Mean volume of items in bullhead guts
$F(1,31) = 0.0148; p = 0.90$
Figure 13: The mean percent similarity between substrate and diet fauna found for pumpkinseed sunfish (a) and brown bullhead (b). Large boxes and bars represent the standard error and standard deviation, respectively.
Figure 14: The mean percent similarity between individual pumpkinseed (a) and bullhead (b) at each site. Large boxes and bars represent the standard error and standard deviation, respectively.

Mean similarity of pumpkinseed diet at the three sites
F(2,346)=11.486; p<.00001

Mean similarity for bullhead diets at two sites
F(1,393)=8.259; p=0.004
Standard Deviation and Coefficients of Variation

The standard deviation (ANOVA: p<.0001; Fig 15a) and coefficients of variation (ANOVA: p=0.09; Fig 15b) for substrate benthos were significantly different between sites. The mean standard deviations indicate that Harbourfront has the most variable substrate fauna, followed by Grindstone and Cootes Paradise. This is in contrast to the plot of mean coefficient of variation, which indicates that Cootes has the highest level of benthic variation, followed by Grindstone and Harbourfront.

The analysis of the standard deviations calculated for pumpkinseed diet at each site indicates that within-site diet variation is significantly different when all three sites are analyzed together (ANOVA: p=0.02; Fig 16a). All pairs of sites are significantly different from one another, except for Cootes and Harbourfront. The fish sampled from Cootes Paradise show the highest standard deviation of gut fauna, followed by Harbourfront and Grindstone Creek. The coefficients of variation of the pumpkinseed diet for each site were not significantly different (ANOVA: p= 0.09; Fig. 16b).

The standard deviations for bullhead diet for Cootes and Grindstone were not significantly different (ANOVA: p=0.13; Fig. 17a), although the trend is similar to that found for the coefficient of variation (ANOVA p=0.03; Fig 17b). The fish sampled at Cootes Paradise show higher variability in benthic fauna found in the diet than fish caught at Grindstone Creek.
Figure 15: Mean standard deviation (a) and coefficient of variation (b) for the substrate benthic community at each site. Large boxes and bars represent the standard error and standard deviation, respectively.

**Standard deviation of benthos counts at each site**

\[ F(2, 117) = 43.83; p < 0.0001 \]

**Coefficient of Variation of benthos counts at each site**

\[ F(2, 117) = 18.67; p < 0.0001 \]
Figure 16: Mean standard deviation (a) and coefficient of variation (b) for the pumpkinseed sunfish diet at each site. Large boxes and bars represent the standard error and standard deviation, respectively.

Standard deviation of benthos counts in pumpkinseed gut contents

\[ F(2,3) = 4.438; p = 0.02 \]

Coefficient of Variation of the benthos counts in the pumpkinseed gut contents

\[ F(2,37) = 2.55; p = 0.09 \]
Figure 17: Mean standard deviation (a) and coefficient of variation (b) for the brown bullhead diet at each site. Large boxes and bars represent the standard error and standard deviation, respectively.

Standard Deviation of the benthos counts in bullhead gut contents

\[ F(1, 31) = 2.357; p = 0.13 \]

Coefficient of variation of benthos counts in bullhead gut contents

\[ F(2, 31) = 5.23; p = 0.03 \]
DISCUSSION

Diversity

The three sites used in this study show benthic diversities that reflect the differences in habitat quality. Cootes Paradise, characterized by high turbidity, low vegetation cover and homogeneous soft-sediment substrate had low benthic diversity. Grindstone, of intermediate vegetation cover and habitat heterogeneity, had intermediate diversity values, while Harbourfront Park with its relatively low turbidity, higher submergent vegetation cover, and high habitat heterogeneity had the highest benthic diversity. The relationship between habitat heterogeneity and increased diversity is supported by the work of many researchers (e.g.: Gregg and Rose 1985, Barton 1988, Ricklefs and Schluter 1993, Wohl et al. 1995). Increased substrate heterogeneity and macrophyte cover provide benthic invertebrates with greater surface area for attachment, feeding and refuge from predators (Gilinsky 1984, Barton 1988, Gregg and Rose 1985, Mbaizireki et al. 1991, Wohl et al. 1995).

Pumpkinseed and bullheads, at both low and high data resolution, have the lowest diversity of gut fauna at Cootes Paradise (Fig. 3 and 4). This seems to be a reflection of the low diversity of prey available at this site. Both species have a higher diversity of prey items in their gut contents at the more diverse sites. An interesting difference in gut content diversity can be seen by examining the pumpkinseed diets for Grindstone and Harbourfront for the two data resolutions. Analysis
performed on the pumpkinseed gut contents with chironomids and oligochaetes identified to genera shows no significant difference between these two sites. When chironomids and oligochaetes were grouped at the family level, the mean diversities for Grindstone and Harbourfront were significantly different, with the fish at the latter site showing a higher gut faunal diversity. This difference between the two levels of resolution imply that pumpkinseed select general chironomid characteristics such as body shape and behavior, ignoring the taxonomic differences between the separate genera.

**Fish Size and Gape**

Pumpkinseed sunfish and brown bullheads both undergo ontogenetic diet switching throughout development (Keast 1978, Keast 1985b, Osenberg et al. 1988, Osenberg et al. 1992, Fox 1994). It is important to determine that the between-site diet differences are not due to different size structuring of the sub-populations at each site. The overall lack of inter-site significant difference between the standard length and gape of both species of fish (Fig. 5 and 6) shows that there is no overall difference in fish size between the three sites, although the borderline p values demand cautious interpretation. Power analysis indicates that large sample sizes would be needed before a significant difference, if present, would be seen consistently, assuming the data collected in this study is representative of the population. The lack of significant difference in mean length and gape suggests that inter-site differences that were investigated are not due to size differences or the
ontogenetic stage of the fish. The one exception to the lack of inter-site size difference is a significant difference between the mean standard lengths of pumpkinseed at Cootes and Harbourfront. There is no significant difference between the mean gape sizes at these two sites. The range of gape sizes is very small; the inter-individual variation in gape may be too small to show a difference. These two results suggest that fish size may be a factor in any differences in feeding behavior seen for pumpkinseed at Cootes and Harbourfront. The lack of significant gape difference suggests that any differences in feeding behavior of pumpkinseed at these two sites could be due to different stages of development rather than gape limitation (Schael et al. 1991, Gerking 1993, Bremigan and Stein 1994).

**Diet Characterization**

There were high proportions of oligochaetes in the substrate of Cootes and Harbourfront (and to a lesser extent, Grindstone) that were not taken advantage of by foraging fish. Calculation of selectivity indicates that both pumpkinseed and bullhead select against oligochaetes, but it is important to note that the Manly-Chesson index does not take into account the avoidance behavior of prey species. Upon disturbance, oligochaetes retract the body into the substrate. It is likely that in this case, the selectivity index reflects the behavior of the prey rather than that of the predator.

The histograms of benthic taxa in the substrate and gut contents reflect the results found for the calculation of selectivity and
illustrate similar diet choices for these two species between the Hamilton Harbour ecosystem and previous studies examining the diet of these fish. Pumpkinseed in Hamilton Harbour consume chironomid larvae, ostracods, cladocera, isopods, and gastropods, similar to previous studies of diet for this fish species (Keast 1978, Laughlin and Werner 1980, Hanson and Qadri 1984, Mittelbach 1984, Osenberg et al. 1988, Osenberg et al. 1992, Pierce et al. 1993, Fox 1994) while bullhead consume ostracods, cladocera and amphipods (Keast 1985b). These fish also eat prey items in Hamilton Harbour that have not been documented in other studies, such as copepods (for both species) and amphipods, water mites, and trichoptera for pumpkinseed, suggesting that these species feed opportunistically on the available fauna.

The results of the chi-square test yielded highly significant p values for the substrate taxa-fish diet comparisons and for the substrate chironomid size-diet chironomid size comparisons. Both species of fish chose a significantly different diet than would be expected from the taxonomic and size composition of the substrate benthos.

The histograms and chi-square test (p<0.0001 at all sites) show a discrepancy between the size of chironomid larvae available in the substrate and that being consumed by both species of fish. This result may at first seem contrary to previous studies of size-selectivity of sunfish (Werner and Hall 1974, Werner and Hall 1977, Mittelbach 1988) and the extensive work in the area of optimal foraging theory, predicting that fish will choose larger prey, all else being equal (Pyke 1984, Gerking 1994). It is possible that in this system, all else is not equal; larger
into the feeding behavior of pumpkinseed show that this species is able to learn the different evasion capabilities of *Daphnia* and copepods and associate these capabilities with a search image. Pumpkinseed may forgo a larger, more evasive prey item for one that is smaller, but easier to capture in a trade-off between energy gain in calories and energy expenditure in prey capture (Vinyard 1980).

The Manly-Chesson selectivity index, like the chi-square analysis, tests the relationships observed in the histograms of benthic taxa and chironomid size class in the substrate and fish gut contents by comparing the composition found in the diet with that found in the substrate. Comparing the substrate benthos and gut fauna must be done with caution for small microinvertebrates such as cladocerans and copepods. While the sediment samples were sieved through a 250 μm mesh net, the entire, unsieved gut contents were transferred to a petri plate for sorting under the microscope. This may cause microinvertebrates to be under-represented in the substrate. The second selectivity test, excluding microinvertebrates, may remove some of the bias in the data set.

**Gut Fullness**

The lack of significant difference between the mean prey abundance and volume in the gut contents (Fig. 11 and 12) suggests that both species of fish had consumed similar amounts of food at the three sites. Two scenarios seem most likely to explain this result: either food is not limited at any of the three sites, or food is limited at all three sites. With a range in abundance from 15,271 ind./m² at Cootes Paradise to
189,103 ind./m² at Harbourfront Park, it seems unlikely that food would be a limiting factor. It would be more likely, if this were the case, that fish in the habitat with the lowest benthic abundance (i.e. Cootes Paradise) would have the lowest gut fullness. Since no such trend was observed, it is unlikely that food was limiting at any of the three sites, although fish feeding at the Cootes sites may have had to forage longer or over greater distances to achieve the same gut fullness as fish at the other two sites. A way to test whether the fish are food-limited would be to design an in-lab experiment where fish are fed to satiation and compare this abundance/volume value to that found in the field. To determine whether fish are foraging for a longer period of time or over greater distances in Cootes Paradise as compared to the other two sites, an intensive study of the diel feeding habits of these fish in combination with a radio tracking study to determine the spatial scaling of foraging would need to be employed. Both this suggested field study and the laboratory feeding project are undertakings that are beyond the scope of this project.

**Similarity**

Although the fish diet/benthos mean similarity is not significantly different from site to site for either pumpkinseed or bullhead, the similarity of pumpkinseed diet and substrate at the Harbourfront site is significantly higher than at the Cootes site. Overall, there is no difference in overlap between taxa in the diet and those available in the substrate at each site. The significantly higher similarity
seen at the Harbourfront site may be a function of the proportion of substrate taxa that are oligochaetes, a taxa not eaten by pumpkinseed. At the Cootes site, 7 of the 24 benthic taxa present are oligochaetes, as compared to 8 of 41 at Harbourfront. The higher proportion of oligochaetes in the Cootes substrate fauna may be causing this significant result, rather than an actual difference in fish feeding behavior between these two sites.

The mean similarity of pumpkinseed and bullhead diets indicates that fish at the Cootes site have a significantly more similar diet than those at the Grindstone and Harbourfront sites. This reflects the low diversity of the substrate benthic fauna at the Cootes Paradise site. Fish foraging at this site may be forced to have a diet similar to that of the substrate fauna because there is no variety of food items available. At the Grindstone and Harbourfront sites, the higher substrate diversity is reflected in the lower inter-individual similarity seen at these sites.

The analyses suggest that benthic diversity, diet-benthos relationships and similarity of individual fish diets are related. For example, as benthic diversity increases, the diet-substrate similarity and the inter-fish similarity decreases. This three-way relationship can be represented as a hypothetical conceptual model (Fig. 18). This model captures the quantification of the linkage between fish and benthos, a linkage that may be strongly influenced by the heterogeneity in benthos distribution, abundance, and taxonomy.
Figure 18: Three dimensional graph illustrating the relationship between benthos diversity, diet-benthos similarity and among-fish diet similarity.

Standard Deviations and Coefficients of Variation

The plot of mean standard deviation shows a trend of increasing variability in the benthic abundance, with the Cootes site having the lowest values, followed by Grindstone and Harbourfront. Low variation at the Cootes Paradise site implies that it may provide a poor environment for diet choice for these fish. Although the coefficients of
variation seen to show the reverse trend, it must be noted that CV is calculated by dividing the SD by the mean. Since Cootes Paradise has such low benthic abundance and large number of 0-values, the denominator for this calculation would be very low in comparison to that for the Grindstone or Harbourfront sites. The trend for coefficients of variation is reflecting the inverse of the mean abundance.

Both pumpkinseed and bullhead show the highest degree of variability in their diet at the Cootes Paradise site. Since this site has the least diverse substrate fauna and shows the most similarity, the fish may have to eat whatever they encounter. At the other two sites, which show higher diversity, the fish can eat preferred taxa, resulting in lower diet variability.

**Study Limitations**

Although this study provides some interesting information about the linkages between fish diet and benthic invertebrates in Hamilton Harbour, methodological limitations require cautious interpretation of the results. The main limitation of this study is the small sample size for both species of fish. Small sample size reduces the power of the statistical tests and may result in non-significant results. Only three sites were studied for this project, with a single sampling period. Increasing the number of sites and the number of sampling times may provide a more complete picture of the interactions between fish and benthos in Hamilton Harbour and Cootes Paradise.
CONCLUSIONS

The patterns of benthic abundance and diversity for the three sites reflect the habitat heterogeneity and water quality conditions at each site. The turbid, homogeneous Cootes Paradise site exhibited the lowest benthic abundance and diversity while the Harbourfront Park site, with the highest habitat heterogeneity, exhibited the highest benthic diversity. The lack of significant difference between the gut fullness at each site suggests that the fish are eating similar amounts of food at all three sites. Pumpkinseed sunfish and brown bullhead respond to differences in substrate benthos diversity; gut content diversity reflects the benthic diversity found at the site. The differences in substrate between the three sites is also reflected in the analysis of similarity. Fish sampled at Cootes Paradise show a high inter-individual similarity reflecting the low diversity at this site. Fish at the more diverse sites, with more diet choice available, had lower inter-individual similarity.

Pumpkinseed sunfish and brown bullhead are selective feeders, selecting certain taxa and certain size classes of chironomids out of proportion to the substrate availability. The selective consumption of smaller prey items may reflect the increased evasive capabilities of larger prey or other factors such as actual availability of substrate benthos to fish, rather than strictly the abundance of certain classes.

Differences in the habitat, such as varied substratum, macrophyte cover and water quality, have important implications for both
the benthic invertebrates and the fish species that feed on this community. This relationship has important implications for the remediation of Cootes Paradise and the littoral zone of Hamilton Harbour. With water quality improvements and an increase in macrophytes, there will be an increase in the abundance and diversity of benthos. These improvements and the resultant change in community structure will increase the littoral zone area and the quality of the rearing and feeding environment for the recovering warmwater fishery in western Lake Ontario.
REFERENCES


APPENDIX I

Total abundance of each benthic taxa in the fish diets at each site.
<table>
<thead>
<tr>
<th></th>
<th>Cootes Pumpkinseed</th>
<th>Cootes Bullhead</th>
<th>Grindstone Pumpkinseed</th>
<th>Grindstone Bullhead</th>
<th>Harbourfront Pumpkinseed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chironomus</strong></td>
<td>292</td>
<td>42</td>
<td>10</td>
<td>5</td>
<td>27</td>
</tr>
<tr>
<td><strong>Dicrotendipes</strong></td>
<td>23</td>
<td>9</td>
<td>14</td>
<td>2</td>
<td>38</td>
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<td><strong>Glyptotendipes</strong></td>
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<td>1</td>
<td>13</td>
<td>0</td>
<td>3</td>
</tr>
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<td>20</td>
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<td>2</td>
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</tr>
<tr>
<td><strong>Phaenopspectra</strong></td>
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<tr>
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<td>12</td>
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<td>9</td>
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<td><strong>Unknown Chir.</strong></td>
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<td>24</td>
<td>5</td>
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<td>0</td>
</tr>
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<td>0</td>
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<td>0</td>
</tr>
<tr>
<td><strong>L. hoffmeisteri</strong></td>
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APPENDIX II

Abundance of each benthic taxa found in the substrate at each site.
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