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A Limited Investigation into the Relationship of Diet to the Habitat Preferences of Juvenile Flathead Sole

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Phase I:

A Limited Investigation into the Relationship of Diet to the Habitat Preferences of Juvenile Flathead Sole

by

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PROJECT ORGANIZATION

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EXECUTIVE SUMMARY

This pilot study examined the diet of 80 juvenile flathead sole collected in Kachemak Bay, Alaska over a range of seasons, depths, and substrates. Samples were gathered prior to the present project during a study of seasonal juvenile flatfish habitat (Abookire 1997, Norcross et al. 1998) which defined the preferred range of depth and substrate for flathead sole and rock sole, the most abundant flatfishes in Kachemak Bay. Diets were examined based on season of capture (winter, spring, or summer), depth preference (< 40 m: shallower than preferred; 40_80 m: preferred; > 80 m: deeper than preferred), substrate preference (< 50% mud: larger than preferred; > 50% mud: preferred), and fish size. Ten fish with stomach contents (predators) were examined from winter (regardless of depth or substrate), 10 predators from spring (regardless of depth or substrate), and 60 predators from summer collections (10 within each of 6 substrate/depth combinations). Diets were described based on the numbers of individuals consumed of each prey taxon, and were separately described based on prey biomass. Where a single prey taxon accounted for ≥10% of the diet either by number of individuals consumed or by biomass, that taxon was considered to be important to the diet based on numerical or biomass criteria. Where a prey taxon accounted for ≥10% of the diet both by numbers of individuals and biomass, we were reasonably sure that the prey taxon was substantially important in the diet of the fish caught in that parameter. Statistical comparisons among the use of a prey taxon on any one parameter, e.g., use of Polychaeta during winter, spring, and summer, were performed by applying a logistic model to presence/absence of prey. The significance level was set to _ = 0.05.

The 65 prey taxa consumed by juvenile flathead sole in this study were divided into 13 general taxonomic groups for analysis: Foraminiferida, Polychaeta, Bivalvia, Crustacea (unidentified), Ostracoda, Copepoda, Euphausiacea, shrimps, crabs, Mysidacea, Cumacea, Isopoda, and Gammaridea. The subphylum Crustacea provided the greatest variety of prey taxa and the largest count of individual prey consumed.

Flathead sole collected during summer usually had stomach contents (N = 60 predators of 79 fish examined), indicating they fed more often than fish collected during spring (N = 10 predators of 21 fish examined) or winter (N = 10 predators of 40 fish examined). No prey taxon accounted for ≥10% of both numbers of individuals and biomass consumed in all seasons. Copepods were important numerically in each season, but provided very little biomass in any season. Based on both numerical and biomass criteria, mysids, and amphipods were important in the winter diet, polychaetes were important in the spring diet, and mysids were important in the summer diet. Sample size was not sufficient to determine whether seasonal diets were significantly different based on prey presence/absence.

Euphausids were important in all depth ranges when using both count and biomass criteria. Additionally, based on these criteria, gammarid amphipods were important in depths of < 40 m, mysids were important in depths of 40_80 m, and both shrimps and mysids were important in depths of > 80 m. Mysids were consumed equally at all depth strata (_^{2}=0.15, p=0.926), and shrimps were consumed by more predators where depth > 80 m (_^{2}=8.36, p=0.015).

Based on numerical and biomass criteria, “shrimps” was an important taxon in both less preferred (< 50% mud) and preferred (> 50% mud) substrates, euphausids were important in < 50% mud, and mysids were important in > 50% mud. The logistic model detected no significant difference in number of predators consuming polychaetes, mysids, gammarid amphipods, euphausids, and shrimps over the two substrates. The number of predators consuming Copepoda was greater on substrate $< 50\%$ mud than on the preferred substrate (_$^{2} = 3.75$, $p = 0.053$).

No prey taxon was important to all sizes of flathead sole based on both prey counts and biomass. The taxa Mysidae and Copepoda were important based on prey counts and biomass to small fish (28_51 mm total length); Mysidae and Gammaridea were important to medium fish (52_77 mm); and Euphausiacea and shrimps were important to large fish (78_165 mm). Copepoda (_$^{2} = 7.53$, $p = 0.023$) and shrimps (_$^{2} = 7.32$, $p = 0.026$) were consumed with different frequency among fish of different sizes. The number of small, medium, and large predators consuming Mysidacea, Gammaridae, and Polychaeta were not significantly different.

Though this small sample size ($N = 80$ predators) was insufficient to determine if flathead sole occur in preferred habitats due to the prey associated with those habitats, this research indicated that certain prey taxa were consumed within limited ranges of depth and substrate. In particular, shrimps were consumed at depths > 80 m and by flathead sole ≥52 mm. Copepods are consumed on substrates $< 50\%$ mud and by flathead sole ≤77 mm. Mysids were consumed equally at all depths and on both substrates. Polychaetes and gammarid amphipods were consumed equally on both substrates and by all sizes of flathead sole.

ABSTRACT

The stomach contents of 80 juvenile flathead sole collected in Kachemak Bay, Alaska, were examined based on season of capture, depth, and substrate preference, and fish size. Diets over the various parameters were described based on counts of individuals eaten of each prey taxon and biomass of each prey taxon. Statistical comparisons among the use of a prey taxon on any one parameter, e.g., use of Polychaeta in during winter, spring, and summer, were performed by applying a logistic model to presence/absence of prey.

The sample size was extremely small for a diet study, yet provided sufficient statistical evidence to indicate that flathead sole diets vary among the seasons, habitats, and fish sizes examined. More flathead sole fed during summer (76%) than spring (48%) or winter (25%). Mysids were consumed equally over all depths, including shallower than preferred (< 40 m), preferred (40 80 m), and deeper than preferred (> 80 m) (2 = 0.15, p = 0.926). Shrimps were consumed by more predators where depth > 80 m than in shallower water (2 = 8.36, p = 0.015). No significant differences were detected between the number of predators consuming polychaetes, mysids, gammarid amphipods, euphausids, and shrimps over preferred (> 50% mud) and less preferred (< 50% mud) substrates. The number of predators consuming Copepoda was fewer on substrate < 50% mud than on the preferred substrate (2 = 3.75, p = 0.053). No significant differences were detected among number of small (28 51 mm), medium (53 77 mm), and large (78 165 mm) predators consuming polychaetes, mysids, and gammarid amphipods. Two significant differences in prey use were detected for fish of different size. Copepods were eaten by small and medium flathead sole more than by large fish (2 = 7.53, p = 0.023), and shrimps (2 = 7.32, p = 0.026) were eaten by medium and large fish more often than by small fish.

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1.0 INTRODUCTION

The results of six years of research in southcentral Alaska clearly demonstrate that many species of juvenile flatfishes prefer specific habitats defined by depth and substrate (e.g., Moles and Norcross 1995, Norcross et al. 1995, 1997, 1999). Definition of habitat preference and related research efforts have made a significant contribution towards determining essential habitat for juvenile flatfishes in Alaskan coastal waters (Norcross et al. In review), but the mechanisms for their apparent habitat preferences have not been identified. This research examined one mechanism (Gibson 1994) that has been largely neglected in Alaskan waters, i.e., the evaluation of diet associated with specific habitats of juvenile flatfishes.

Preferred nursery habitats of juvenile flathead sole and rock sole in Kachemak Bay were established during the research project (Abookire 1997, Abookire and Norcross 1998, Norcross et al. 1998) which collected the fish examined during the present study. In that project, seasonal (winter, spring, and summer) surveys of juvenile groundfishes were conducted in Kachemak Bay from 1994 to 1996, over depths 10–150 m, and over all trawlable substrates (mud, sand, gravel). The preferred year-round nursery habitats of flathead sole were based on species abundance, depth, and substrate, and are defined as regions 40 80 m in depth with substrates containing > 50% mud. Preferred spring and summer habitat of rock sole is 10 30 m in depth on sand. In winter, rock sole move offshore and are found in depths to 150 m. Flathead sole and rock sole have very separate habitat requirements in terms of depth and substrate, with species overlap limited to 7–19% of sites examined in the spring and summer, but rising to 51% of those same sites in the winter (Abookire and Norcross 1998).

1.1 Objectives

This research was proposed as a two-phase project with the overall objective to investigate the feeding differences of flathead sole and rock sole in Kachemak Bay relative to seasonality in distribution of fish; distribution of fish in relation to environmental characteristics; size of fish; and co-occurrence of species. A subsample study based on the diets of 80 flathead sole (Phase I) was proposed to be expanded through analysis of additional flathead sole and rock sole (Phase II). Only Phase I of this research was funded, thus this report summarizes the effort to meet Phase I objectives together with descriptions of the flathead sole diet relative to season, depth, substrate, and fish size. Five objectives were proposed for the Phase I subsample study:

1) to investigate the feeding differences of a subsample of 80 flathead sole seasonally and at physically distinct sites in Kachemak Bay,

2) to use a balanced subsample design to determine whether season (winter, spring, and summer), depth preference (shallower than preferred, preferred, deeper than preferred), or substrate preference (preferred grain size and larger than preferred) had a statistically significant effect on diet of predators,

3) to determine the power of statistical analyses,

4) to estimate the sample size necessary to bring the power of the statistical tests to an appropriate level, and

5) to analyze the diets of 80 flathead sole by fish size.

2.0 METHODS

2.1 Collection and subsampling

Fishes and benthos were collected during winter (February 1996), spring (May 1995 and May 1996), and summer (September 1994, August 1995, and August 1996) (Abookire 1997). Fishes were captured via 3.05 m plumb staff beam trawl, with 7 mm mesh and 4 mm codend mesh. Flathead sole of total length (TL) < 200 mm were packaged and frozen by species, station, and cruise, with up to 10 fish of various sizes frozen together from each site. Only flathead sole were examined. Depth used in analyses was the average depth over the tow, as read from the vessel fathometer. A 0.06 m3 ponar sediment grab was deployed once along the tow track, and its contents were subsampled and preserved for analysis of sediment grain size and taxonomy of benthos. In the laboratory, sediment was wet-sieved (Folk 1980) to determine weight proportions of gravel, sand, and mud (Sheppard 1973). Benthos was preserved in an initial solution of buffered 10% formalin, and preserved within one month in a final solution of 50% isopropyl alcohol containing rose bengal biological stain.

Collections of flathead sole were divided into categories based on season, depth, and substrate (Figure 1). During laboratory analysis, stations were randomly selected within the habitat category under consideration, and within each station, up to 10 flathead sole were randomly selected and stomach contents were examined without regard to fish size. One hundred and forty fish were examined to attain a balanced subsample design of 80 fish containing prey (Figure 1). Hereafter those 80 fish are termed ‘predators.’ Ten predators were examined from the winter collection (regardless of depth or substrate), 10 predators were examined from spring collections (regardless of depth or substrate), and 60 predators were examined from summer collections (10 predators within each of 6 substrate/depth combinations).

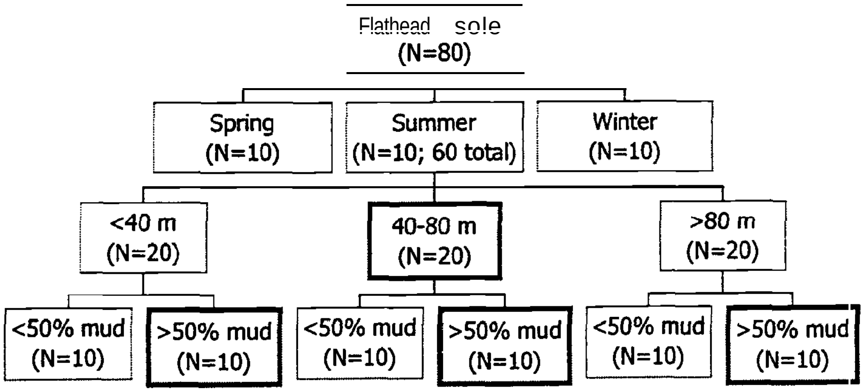


Figure 1. Subsample design (N = number of predators examined). Bold borders indicate preferred depth and substrate strata.

2.2 Laboratory analysis of fish

Fish were defrosted gradually to maintain condition of stomach contents. Each fish was examined for evidence of regurgitation. Fish which had regurgitated would have been omitted from further analysis, but no evidence of regurgitation was found. Fish total length (mm) and weight (mg) were measured before the stomach was excised at the esophagus and pyloric caecum. Stomach fullness was recorded and prey taxa within the stomachs were identified to the lowest practical taxon, counted, preserved in 50% isopropyl alcohol, and weighed (0.1 mg). For each prey taxon, whole individuals and heads were counted; if no heads were present, all fragments that could be assigned to a taxon received a count of one individual regardless of the number of fragments.

Identification of prey to a specific taxonomic level was expected to be necessary to Phase II determinations of dietary overlap between predator groups, and thus prey was identified to the lowest taxonomic level practical. Two factors made identification to species difficult. Many of the prey were juveniles with very small appendages that could not be seen with a dissecting microscope. Juvenile prey could be allocated into general taxonomic groups (e.g. class, family), but further examination was beyond the scope of this project's funding and time allocation. Prey were often in an advanced state of digestion, with much of the soft tissue already digested. Crustaceans could be identified based on chitinous exoskeletons, but identification of those samples was much more difficult than when the prey was whole and in good shape.

Because biomass is a preferred quantification of diet, weights of prey taxa were measured. However, in many cases, the measured prey weights poorly represented the amount of soft tissue (food) provided to the predator. Weights of whole prey may overestimate the food value of animals with heavy shells (e.g., foraminiferans, bivalves). Many feeding studies suggest caution when considering the proportional biomass of these prey taxa. Biomass of shelled prey may also be overestimated if those prey have a longer residence time in the stomach than does soft tissue. An error we view with greater concern is that the state of prey digestion was not consistent among predators; some samples appeared to be relatively undigested, but many were in an advanced state of digestion.

We considered developing length/weight regressions for prey taxa to minimize the error of using inappropriate weights. Development of length/weight relationships would be less appropriate for some prey taxa than others. Since biomass was used as an index of food, and 'food' biomass available from shelled animals was already grossly exaggerated by using weights of entire animals, we concluded it would be inappropriate to develop length/weight regressions to estimate pre-digestive weights for animals such as mollusks and foraminiferans. We also determined it would be inappropriate to develop length/weight regressions for polychaetes, as the polychaetes eaten were generally not whole but were relatively undigested and thus closely approximated food biomass.

We considered developing length/weight regressions of the small crustaceans which comprised the majority of the prey consumed by flathead sole. Crustaceans have a chitinous exoskeleton, and would not be expected to shrink appreciably in the stomach, although that is sometimes the case for prey eaten by larger animals. We hypothesized that length weight regressions could be created for crustacean taxa based on existing benthic, epibenthic and pelagic zooplankton collections at IMS/SFOS/UAF. We estimated that 10 20 specimens in each prey taxon, of similar size to those consumed by the predators, should be sufficient for length/weight regression. Our best source for length and weight data on the prey species consumed by flathead sole was the benthos samples collected concurrently with the fishes of this project. A subsample of 11 (of a total of approximately 200) benthos samples was sorted to use in length/weight regressions of prey taxa. Six crustaceans of taxa consumed by flathead sole were found in the 11 samples. We concluded that processing these benthos samples would likely provide insufficient data for length/weight regression and would take more time than allowed by the limited time-frame of this project.

After investigating alternative sources of biomass data, we concluded the best available source of prey taxa weights were those measured in this project for prey > 0.1 mg. If a prey taxon weight was < 0.1 mg, we used average weights of taxa from Prince William Sound, Alaska (Chris Stark, IMS/SFOS, pers. comm). We did not use measured weights from this project for prey < 0.1 mg because significant error can be associated with blotting dry and weighing one or a few very small, lightweight animals (e.g., copepods). We did not use average weights to represent the weight of larger prey taxa because the imprecision of using an average biomass increases with the size of the individual prey. For example, using an average weight for all Pandalid shrimps would be inappropriate since shrimp weight may change by a factor of 100 depending on size of the animal.

For initial statistical analyses (described below), prey taxa were considered separately at the most specific taxonomic level available and at a more general level (e.g., class, or order). Descriptive analyses and subsequent statistical analyses considered prey only at the general level in order to have sufficient counts of predators consuming a prey taxon to allow statistical analysis.

2.3 Descriptive analyses—Prey biomass and counts

Counts of individuals and proportional prey biomass consumed by flathead sole were plotted over the seasons, depth, substrate, and fish-size parameters. We defined a prey taxon important if it accounted for $> 10\%$ of the diet by either counts of individuals or prey biomass consumed. Proportional counts of individual prey represent the predator’s efforts and success at capturing and consuming the taxon. The biomass plots help visualize approximate food contribution of various taxa, but due to problems with the prey biomass data, they are of limited quantitative value and must be interpreted with caution. If a prey taxon figured substantially in both count and biomass plots, we were confident it did contribute substantially to the predator’s diet.

2.4 Initial statistical analyses—Prey biomass

We initially performed the following analyses based on prey biomass, as specified in the proposed objectives. Data transformations and statistical tests used Statistica software (StatSoft 1995), with the exception of power analyses, which used SigmaStat software (Jandal Scientific 1994). Square-root and natural log transformed biomass data were used to produce Bray-Curtis dissimilarity matrices. Each Bray-Curtis dissimilarity matrix was processed via non-metric multidimensional scaling (NMDS) to produce two-dimensional axes used as variables for further analysis. The transformation of biomass data via square-root transformations was compared with $\ln + 1$ transformations to determine which transformation resulted in lower stress of NMDS results. The square root transformed data provided only slightly better results using NMDS.

Preliminary analyses showed that the statistical tests originally proposed (NMDS, principal component analysis (PCA), ANOVA, and t-test) were inappropriate. For reasons explained previously, the accuracy and precision of the prey biomass data upon which these tests were based were questionable. Each flathead sole consumed only one or a few prey taxa, yielding data matrices of predator by prey which were comprised of only a few non-zero data points per predator. The data matrix of predator by prey contained so few non-zero data (i.e., the data were sparse) that calculation of an ecologically meaningful similarity matrix was not possible and NMDS results were not meaningful. Principal components analysis was not applicable to these data because PCA requires a data set to contain more columns (variables) than rows (cases). Generally fewer prey species were identified (variables) than predators examined (cases), and therefore PCA could not be used. The sparseness of this data set also precluded comparisons between habitat categories based on means (e.g., ANOVA) or ranks (e.g., nonparametric ANOVA) for different prey categories.

2.5 Statistical analyses—Presence/absence of prey

Following the initial unsatisfactory statistical analysis, data were converted to presence/absence format and logistic models were applied. The presence/absence data consisted of an indicator variable defined as 1 if a prey taxon was present and 0 otherwise. These indicator variables were tabulated for each prey taxon and the number of times a prey was present or absent in fish stomachs was calculated. The logistic model was applied using the CATMOD procedure in SAS (SAS Institute 1989, Stokes et al. 1995). As applied here, the results from CATMOD were analogous to performing an ANOVA comparison using linear regression with dummy variables with the effects coding (e.g., a -1, 1 coding vs. a 0,1 coding). For all comparisons, a saturated model was used and thus, no log-likelihood statistics were available. The significance of effects was determined from maximum likelihood analysis of variance tables from CATMOD that used Wald's statistic to test the null hypothesis of no effect. Under the null hypothesis, the Wald's statistics is distributed as a chi-square variable. The 5% level of significance was used for all analyses but effects significant at lower levels (0.05 < 0.10 and 0.10 < 0.20) were noted. The presence of zero counts was problematic in these analyses as maximum likelihood could not be estimated for effects with zero counts. Thus, a small number, 0.05, was added to the zero counts (Agresti 1990) if the overall count for the prey taxon was ^{3}5. The resulting comparisons may not be reliable because of the low counts (4) for many prey items consumed, and are assumed to be approximate. For this report, the general guideline for 2 statistics of a value of 5 for each cell of a data table was used to discuss the reliability of the statistical significance determined. Statistical tests are tabulated for the main effects only. The logistic model was not used for prey categories with overall counts < 5.

Power analyses were to be done to determine sample size necessary to achieve statistically valid results using biomass data. The power analyses proposed for this project are standard for parametric tests but not for the 2 tests the data required. Power analyses for these 2 tests require simulations based on choosing hypothetical values for individual cell probabilities (Agresti 1990). A widely used guideline for valid 2 tests is that each cell should have a value of at least 5. Many comparisons did not meet the criterion of > 5 counts, therefore we did not feel that using the observed data to choose the hypothetical values on which to base power calculations was reasonable or valid. Simulations to approximate the power of these tests would have been more appropriate if the criterion of counts > 5 had been met as we would have had more confidence in the relative frequencies of prey in stomachs.

3.0 RESULTS

The stomach contents of 140 flathead sole < 200 mm total length were examined to attain a balanced sample design of 80 predators (Figure 1, Table 1). These 80 predators were from 19 sites in Kachemak Bay (Figure 2). Seventy-six percent of the fish examined from summer collections had fed recently, compared with 48% during spring and 25% during winter (Table 2). Fishes collected during summer were usually predators, regardless of depth or substrate of capture (71–87% of fish examined from summer collections had fed recently; Table 2).

Depth	Substrate	Winter		Spring		Summer	
(m)	(% mud)	Empty	Predators	Empty	Predators	Empty	Predators
0–40	0–50	0	2	0	0	3	**10**
0–40	50–100	0	0	0	2	0	**10**
40–80	0–50	0	2	2	2	3	**10**
40–80	50–100	25	5	4	4	5	**10**
> 80	0–50	0	1	0	0	1	**10**
> 80	50–100	5	0	5	2	7	**10**
Total (all parameters)		30	**10**	11	**10**	19	**60**

Table 1. Number of flathead sole examined within each season, substrate, and depth parameter. "Empty" refers to fish with 0% stomach fullness. "Predators" refers to fish with > 0% stomach fullness.

Parameter	N fish examined	Predators
Winter	40	25%
Spring	21	48%
Summer	79	76%
Depth < 40 m	23	87%
Depth 40 80 m	28	71%
Depth > 80 m	28	71%
Substrate < 50% mud	37	81%
Substrate > 50 %	42	71%
Total (all parameters)	140	57%

Table 2. Percentage of flathead sole examined which were predators (i.e., feeding) within each season, and within depth and substrate strata during summer.

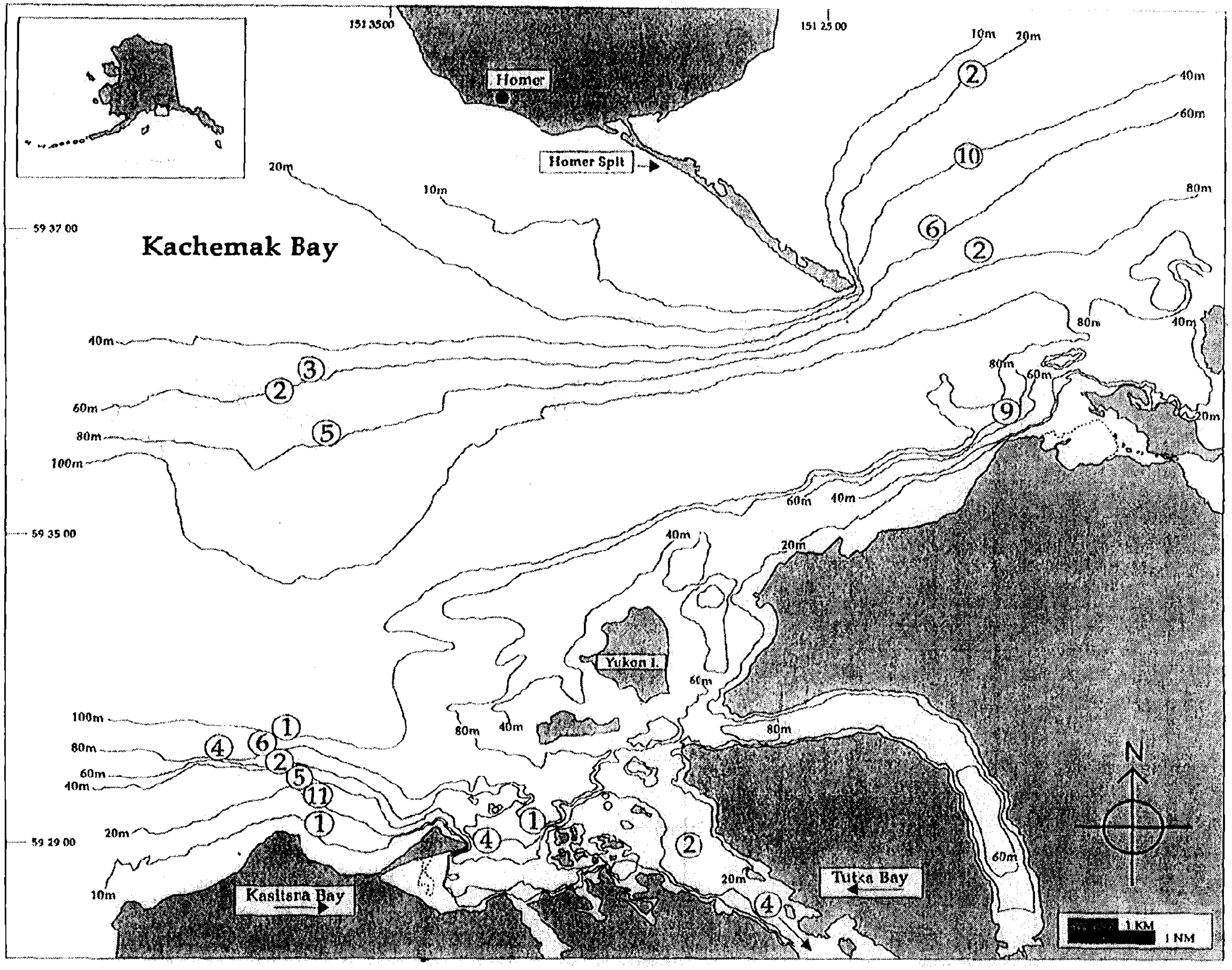


Figure 2. Count of flathead sole predators examined from each collection site in Kachemak Bay (N = 80 predators).

Table 3. Counts of prey individuals within the stomachs of all flathead sole with stomach contents (N = 80 fish). This table summarizes the most specific prey taxonomy available. For each prey taxon, whole individuals and heads were counted; * all fragments within a single stomach which could be assigned to a taxon received a count of one individual regardless of the number of fragments. Taxa in bold font were used for analyses of general groupings. Dashes (-) mean standard deviations could not be calculated because either a single individual was found or because the fragment count for each predator was constrained to 1. Standard deviation = 0.00 means that each predator consuming the prey taxon ate the same number of individuals of that taxon.

General prey grouping	Taxonomic level of identification	Sum	Mean	Standard deviation
Phylum Sarcomastigophora, Class Granuloreticulosa, Order Foraminiferida				
	(Foraminiferans)	26	5.2 ±	4.02
Phylum Annelida, Class Polychaeta				
	(Polychaetes)	89	4.9 ±	15.08
	Aphroditoidea	1	1.0	
	Polychaeta (unidentified)	3	1.0 ±	0.00
	* Polychaeta fragments	6	1.0	
	Polychaeta larvae	74	24.7 ±	35.10
	Polynoidae	4	1.0 ±	0.00
	Sternapsis scutata	1	1.0	
Phylum Mollusca, Class Bivalvia				
	(Bivalves)	17	1.2 ±	0.58
	* Bivalve siphon (fragments)	7	1.0	
	Bivalvia (unidentified)	3	1.0 ±	0.00
	Yoldia sp.	6	2.0 ±	1.00
	Yoldiella sp.	1	1.0	-
Subphylum Crustacea (unidentified)		5	1.3 ±	0.50
	* Crustacea fragments	3	1.0	
	Malacostraca juveniles	2	2.0	
Subphylum Crustacea, Class Ostracoda				
	(Ostracods)	2	2.0	-
Subphylum Crustacea, Class Copepoda, Orders Calanoida and Harpacticoida				
	(Copepods)	108	4.3 ±	6.79
	Aetididae	6	1.5 ±	1.00
	Cryptoniscidae	1	1.0	-
	Harpacticoida (unidentified)	7	2.3 ±	2.31
	Harpacticus sp.	3	3.0	-
	Harpacticus uniremous	1	1.0	-
	Laophontidae	1	1.0	
	Metridia pacifica	39	13.0 ±	18.25
	Oncaea sp.	1	1.0	
	Tisbe sp.	2	1.0 ±	0.00
	Zaus sp.	47	5.9 ±	3.18
Subphylum Crustacea, Class Malacostraca, Order Euphausiacea				
	(Euphausids)	68	4.5 ±	7.32
	Euphausia pacifica	1	1.0	
	Thysanoessa inermis	2	1.0 ±	0.00
	Thysanoessa longipes	1	1.0	
	Thysanoessa raschi	9	3.0 ±	2.65
	Thysanoessa spp.	3	1.0 ±	0.00
	Thysanoessa spinifera	52	10.4 ±	10.83

Table 3. Continued.

General prey grouping	Taxonomic level of identification	Sum	Mean	Standard deviation
Subphylum Crustacea, Class Malacostraca, Order Decapoda, Infraorder Caridea				
	(Shrimps)	45	1.5 ±	0.97
	Crangon alaskensis	2	1.0 ±	0.00
	Crangonidae zoea	3	1.0 ±	0.00
	Eualus pusiolus	1	1.0	-
	Eualus spp.	6	1.2 ±	0.45
	Heptacarpus spp.	3	1.0 ±	0.00
	Hippolytidae zoea	14	2.0 ±	1.41
	Pandaiidae zoea	15	1.9 ±	1.13
	Pandalus jordani	1	1.0	-
Subphylum Crustacea, Class Malacostraca, Order Decapoda, Infraorders Anomura and Brachyura				
	(Crabs)	8	1.0 ±	0.00
	Chionecetes *c.f. bairdi*	1	1.0	
	Hyas **sp.**	2	1.0 ±	0.00
	Majidae	1	1.0	
	Pagurus sp.	3	1.0 ±	0.00
	Pugettia richii	1	1.0	
Subphylum Crustacea, Class Malacostraca, Order Mysidacea				
	(Mysids)	64	2.4 ±	2.53
	Acanthomysis c.f. ***Paracanthomysis stelleri***	2	1.0 ±	0.00
	Acanthomysis spp. juvenile	4	1.0 ±	0.00
	Alienacanthomysis macropis	1	1.0	-
	Mysidae (unidentified)	2	1.0 ±	0.00
	Mysidae juveniles (unidentified)	55	3.1 ±	2.88
Subphylum Crustacea, Class Malacostraca, Order Cumacea				
	(Cumaceans)	3	1.0 ±	0.00
	Eudorella sp.	1	1.0	
	Leptostylus sp.	1	1.0	
	Leucon nasica	1	1.0	
Subphylum Crustacea, Class Malacostraca, Order Isopoda				
	(Isopods)	17	5.7 ±	7.23
	Munna sp.	1	1.0	
	Sphaeromatidae	16	8.0 ±	8.49
Subphylum Crustacea, Class Malacostraca, Order Amphipoda, Suborder Gammaridea				
	(Gammarid amphipod)	45	1.3 ±	0.60
	Ampelisca c. f. ***macrocephala***	3	1.0 ±	0.00
	Ampelisca spp.	4	1.3 ±	0.58
	Ampeliscidae	5	2.5 ±	2.12
	Amphipoda fragment	1	1.0	
	Ampithoe sp.	1	1.0	
	Bathymedon sp.	1	1.0	
	Gammaridea (Amphipoda)	6	1.0 ±	0.00
	Monoculodes schnerderi	8	1.1 ±	0.38
	Monoculodes spp.	7	1.8 ±	0.50
	Oedicerotidae	2	1.0 ±	0.00
	Protomediae spp.	2	1.0 ±	0.00
	Rhacotropis oculata	5	1.3 ±	0.50
Total (all taxa)		497	2.6 ±	5.91

Not all prey were identifiable to species: 46.5% were identified to genus or species, 26.4% to family, and 28.2% to order, class, or subphylum. Each predator consumed a mean of 2.0 _ 1.15 prey taxa per predator when considered at the specific taxonomic level, and 2.4 _ 1.67 prey taxa per predator considered at an aggregated level (i.e., order, class, or subphylum; Table 3). Flathead sole preyed on the taxonomic groups Foraminiferida, Polychaeta, Bivalvia, and Crustacea (Table 3). Sixty-five prey taxa were partitioned into 13 general groups for further analysis (Table 3). The greatest variety of taxa (N = 41 taxa) and the largest count of individual prey (N = 250 individuals) was from the Class Malacostraca of the Sub-phylum Crustacea. In order of decreasing counts of individual animals of each prey taxon consumed, flathead sole in Kachemak Bay fed on copepods, polychaetes, euphausids, mysids, shrimps, gammarid amphipods, foraminiferans, bivalves, isopods, crabs, cumaceans, unidentified crustaceans, and ostracods. Individual predators took relatively large numbers of foraminiferans, polychaetes, copepods, euphausids, and isopods (mean number of animals of a single taxon taken by a single predator was 4.3_5.7; Table 3). In order of decreasing biomass of each prey taxon, predators consumed euphausids, shrimps, bivalves, amphipods, mysids, polychaetes, crabs, copepods, unidentified crustaceans, cumaceans, foraminiferans, isopods, and ostracods.

Foraminiferans and all crustacean taxa were nearly always consumed whole, while bivalves were consumed both whole and as siphon fragments. Very small (juvenile) polychaetes were consumed whole, but often only the head or tail end was consumed of larger polychaetes.

3.1 Diet examined by season

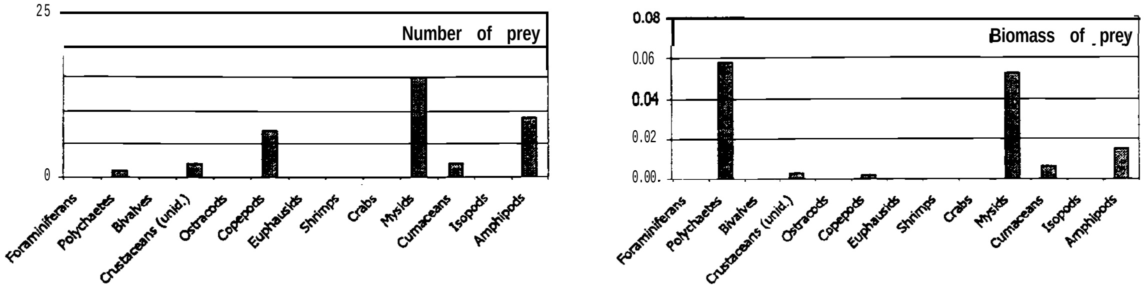
No prey taxon was important (accounted for > 10% of total) for both numerical and biomass criteria in all seasons (Figure 3). Copepoda was important numerically in each season, but was not important in any season in terms of biomass.

Based on numerical and biomass criteria, mysids, and amphipods were important to the flathead sole's winter diet (Figure 3a). During winter, flathead sole mainly ate individuals of Mysidacea (42%), Gammaridea (25%), and Copepoda (19%). Biomass of winter prey was composed primarily of polychaetes (42%), mysids (38%), and gammarid amphipods (11%). Although several copepods were eaten, this taxon accounted for only a small part of the winter prey biomass. A single polychaete was large enough to make Polychaeta the heaviest taxon consumed during winter.

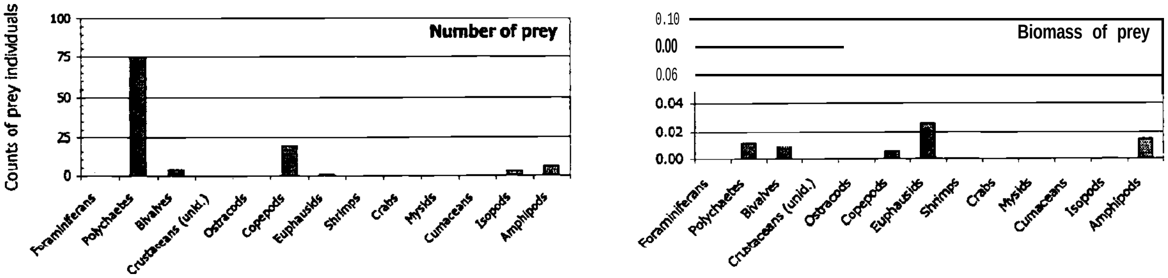
Polychaeta was important to the spring diet of flathead sole based on both numerical and biomass criteria (Figure 3b). Predators captured during spring consumed larger numbers of polychaetes (69%) and copepods (18%) than of other prey taxa. A display of prey biomass indicates although relatively large numbers of polychaetes and copepods were eaten during spring, individual prey of those taxa were very small and lightweight. Many of the polychaetes were juveniles. The single euphausid eaten during spring accounted for 38% of the entire prey biomass of that season. Six amphipods accounted for 21% of spring prey biomass. Seventy-five polychaetes accounted for 18% of spring prey biomass, and four bivalves accounted for 14% of spring prey biomass.

Mysids were important to the summer flathead sole diet on the basis of both numerical and weight criteria (Figure 3c). Based on numbers of prey individuals consumed, the 10 predators examined from summer collections consumed mainly mysids (33%), copepods (26%), and

3a. Diet of flathead sole collected during winter (N=10 predators)



3b. Diet of flathead sole collected during spring (N=10 predators)



3c. Diet of flathead sole collected during summer (N=10 predators)

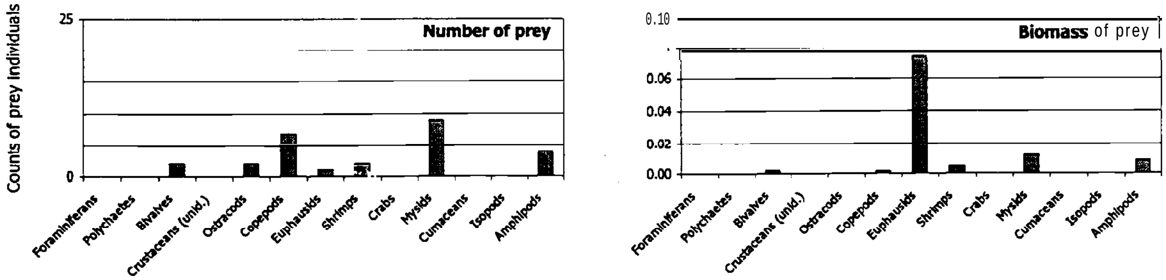


Figure 3. Counts and biomass of prey taxa consumed by flathead sole during winter (a), spring (b), and summer (c), regardless of fish size, depth, or substrate of capture.

amphipods (15%). Biomass of summer prey was dominated by a single euphausid (72%) and nine mysids (11%).

The 30 predators randomly selected for seasonal diet examination had consumed prey from 11 (Table 4) of the 13 general prey taxa eaten by the 80 fish examined in the entire study (Table 3). The predators selected for seasonal analysis had not consumed foraminiferans or crabs. The sparseness of these data created difficulty in making statistically-based conclusions on seasonal dietary differences (Table 4). Only Copepoda and Gammaridae had enough numbers in each cell to allow calculation of statistical significance without addition of constant values, and no difference was determined for these two prey taxa. However, as these cells all contained < 5, we could not place as much confidence in these results as if the sample size were larger. All other prey taxa had zero values for the taxon during at least one season. Where, after the addition of a small constant to those zero values, a test could be performed, none of the data compared were significantly different. Using the small sample size of 10 predators for each of three seasons, it was not possible to determine if these zeros were valid, i.e., whether the prey taxon was actually not eaten in certain seasons, or if it was absent in this study simply as artifact of the small sample size. For example, Mysidacea had filled cells in summer and winter and a zero value in spring; this may or may not indicate mysids are not consumed in spring.

General prey grouping	Winter	Spring	Summer	Total	$\_^{2}$	p
	N = 10	N = 10	N = 10	N = 30		
Polychaeta	1	4	0*	5	3.05	0.217
Bivalvia	0*	4	2	6	1.92	0.383
Crustacea (unidentified)	2	0	0	2	Test not performed	
Ostracoda	0	0	1	1	Test not performed	
Copepoda	4	4	1	9	1.05	0.591
Mysidacea	7	0*	5	12	2.41	0.300
Cumacea	2	0	0	2	Test not performed	
Gammaridea (Amphipoda)	4	4	3	11	0.29	0.867
Isopoda	0	1	0	1	Test not performed	
Euphausiacea	0	1	1	2	Test not performed	
Shrimps	0	0	2	2	Test not performed	

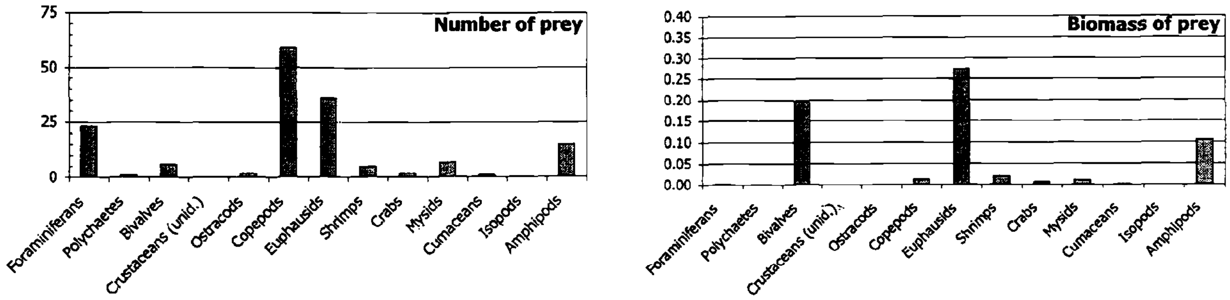
* 0.05 was added to this value.

Table 4. Number of predators consuming each prey group by season. N = number of predators examined, disregarding depth, substrate, and size of predator. Total = number of predators that consumed individual prey taxa across seasons.

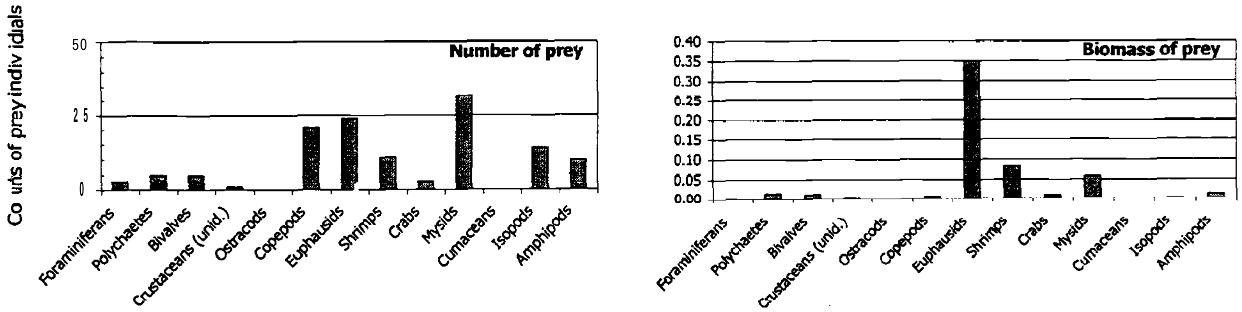
3.2 Diet examined by depth preference

Euphausiacea was the only taxon which amounted to ^{3}10% of the prey by numbers and biomass within all depth ranges examined (Figure 4). Euphausids appeared to be more

4a. Diets of **flathead** sole collected <40 m depth (N=20 predators).



4b. Diets of **flathead** sole collected in 40-80 m depth (N=20 predators).



4c. Diets of **flathead sole collected** >80 m depth (N-20 **predators**).

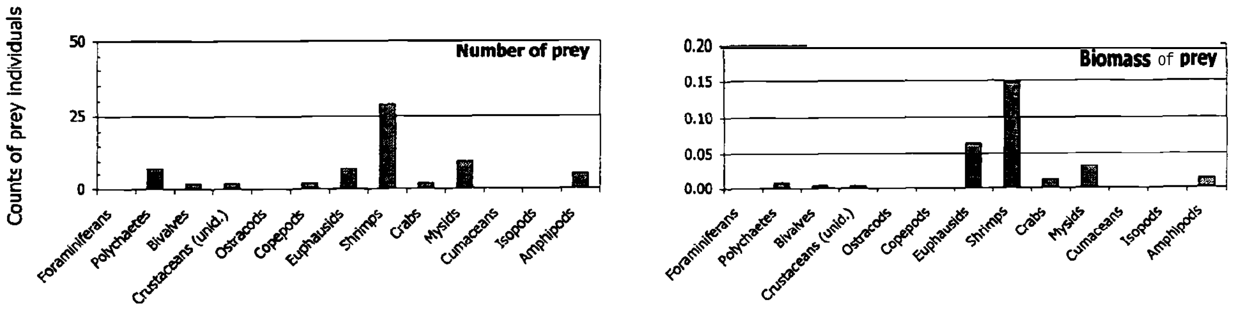


Figure 4. Counts and biomass of prey taxa consumed by flathead sole during summer, over all substrates, in <40 m (a), the preferred depth range of 40-80 m (b), and >80 m (c).

important in shallower than preferred (< 40 m) and preferred (40 80 m) depth ranges than in water deeper than flathead sole prefer (> 80 m).

Euphausids and gammarid amphipods were important in flathead sole diets in the shallowest depth strata using both count and biomass criteria (Figure 4a). Counts of prey taken at < 40 m depth were primarily copepods (38%), euphausids (23%), foraminiferans (15%), and amphipods (10%). Prey biomass in the shallow depth range was dominated by euphausids (43%), bivalves (31%), and amphipods (17%).

Euphausids and mysids were important to the diet within flathead sole's preferred depth range (40 80 m) using both count and biomass criteria (Figure 4b). Fish in this depth range ate individuals of Mysidae (25%), Euphausiacea (19%), Copepoda (16%), and Isopoda (11%). Prey biomass was dominated by euphausids (64%) as in the shallower depth stratum, in addition to shrimps (15%) and mysids (11%), which were not important in shallower depths.

Based on both count and biomass criteria, shrimps, euphausids, and mysids were important to fish caught deeper than 80 m (Figure 4c). Flathead sole caught in this depth range consumed individuals of shrimps (44%), mysids (15%), euphausids (11%), and polychaetes (11%). In terms of biomass, the diet of predators captured deeper than 80 m was mainly composed of shrimps (52%), euphausids (22%), and mysids (11%).

General prey grouping	< 40 m	40–80 m	> 80 m	Total	2	p	
	N = 20	N = 20	N = 20	N = 60			
Foraminiferida	4	1	0*	5	2.67	0.263	
Polychaeta	1	5	7	13	4.26	0.119	
Bivalvia	3	4	2	9	0.76	0.683	
Crustacea (unidentified)	0	1	1	2	Test not performed		
Ostracoda	1	0	0	1	Test not performed		
Copepoda	3	5	2	10	1.6	0.448	
Crab	1	3	2	6	1.03	0.596	
Mysidacea	6	7	6	19	0.15	0.926	#
Cumacea	1	0	0	1	Test not performed		
Gammaridea (Amphipoda)	11	6	4	21	5.4	0.067	
Isopoda	0	1	0	1	Test not performed		
Euphausiacea	3	4	4	11	0.22	0.895	
Shrimps	5	5	13	23	8.36	0.015	#, S

* 0.05 was added to this value.

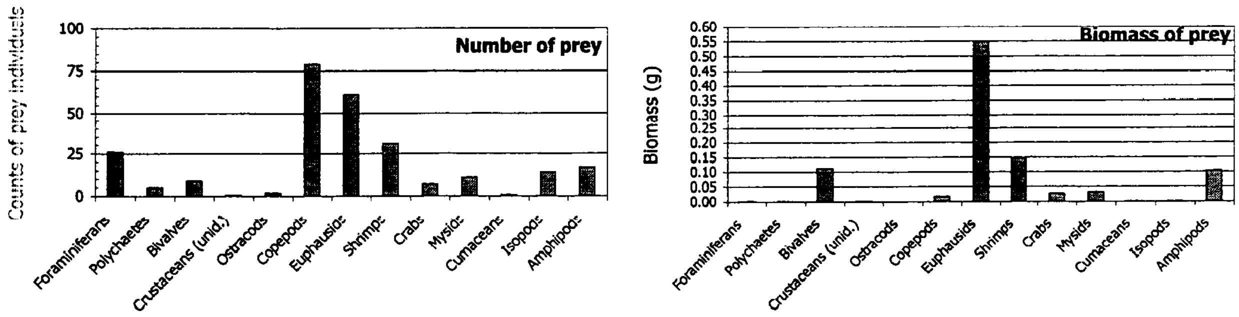
Table 5. Number of predators consuming each prey taxon by depth range. N = number of predators examined, disregarding season, substrate, and size of predator. Total = number of predators that consumed individual prey taxa across depth ranges. Prey taxa are marked where they have the minimum number of data (#), or where they are significantly different over the parameter examined (S).

Unlike the seasonal data (Table 4), there are fewer zero values in the depth analysis (Table 5), thus allowing for greater confidence in the statistical comparisons of eight prey taxa. Of these comparisons, only two (Mysidacea, _^{2} = 0.15, p = 0.926, and shrimps, _^{2} = 8.36, p = 0.015) satisfy the criteria of five or more observations per cell. Based on these results, we conclude that mysids were consumed equally at all depth strata, but shrimps were consumed by more predators where depth was > 80 m. At a lower significance level (_ = 0.10), gammarid amphipods were consumed by more predators where depth < 40 m. Fewer predators may consume polychaetes where depth was < 40 m. The _^{2} value (4.26) for polychaetes is marginally significant (p = 0.119), but counts of predators consuming bivalves, copepods, crabs, and euphausids were not significantly different between depths. There were no significant differences among depth strata for Foraminiferida, Bivalvia, Copepoda, crabs or Euphausiacea.

3.3 Diet examined by substrate preference

The “shrimps” category was the only taxon which amounted to ^{3}10% of the prey by numbers and biomass within both substrate ranges (Figure 5). It was approximately equally important in both preferred (> 50% mud) and less preferred (< 50% mud) substrates, both numerically and by weight.

5a. Diets of **flathead** sole collected on substrate **<50%** mud **(N=30** predators).



5b. Diet5 of **flathead** sole collected on substrate **>50%** mud **(N=30** predators).

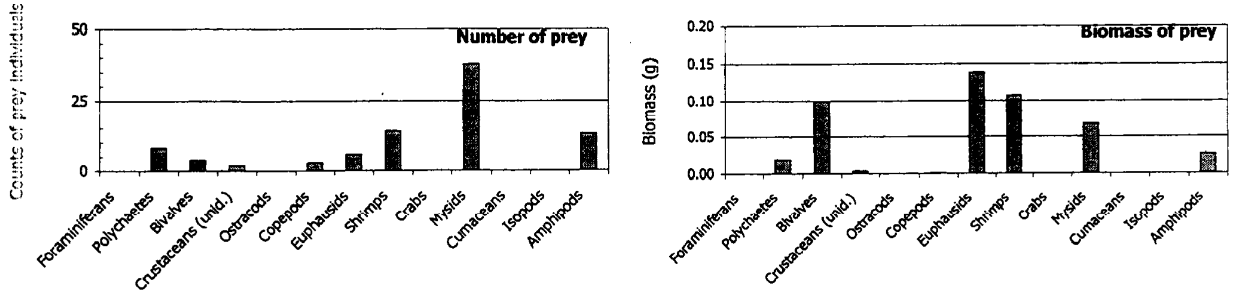


Figure 5. Counts and biomass of prey **taxa** consumed by **flathead** sole during summer, over all depths, on substrate **<50%** mud (a) and the preferred substrate of **>50%** mud (b).

In substrate of $<50\%$ mud, euphausids and shrimps were important based on both numerical and biomass criteria (Figure 5a). Flathead sole caught on this substrate consumed individuals of Copepoda (30%), Euphausiacea (23%), shrimps (12%), and Foraminiferida (10%). Euphausids provided the largest biomass (55%), followed by shrimps (15%), bivalves (11%), and amphipods (10%).

Mysids and shrimps were important in the diet both numerically and by weight on flathead sole's preferred substrate of $>50\%$ mud (Figure 5b). Counts of prey taken on this substrate were primarily of mysids (43%), shrimps (16%), and gammarid amphipods (43%). Important prey biomass included euphausids (30%), shrimps (23%), bivalves (21%), and mysids (15%).

Comparison of diets by substrate was based on a larger number of samples (Table 6) and thus has fewer zero cells ($N=5$) than season (Table 4: $N=14$) or depth (Table 5: $N=8$) comparisons. Of the statistical comparisons, only Copepoda was significant ($2=3.75$, $p=0.053$). Shrimps had an adequate number of observations per cell and yielded marginal results ($2=1.74$, $p=0.187$). The number of predators consuming the other taxa were not significantly different over the two substrate categories.

General prey grouping	$<50\%$ $N=30$	$>50\%$ $N=30$	Total $N=60$	X^2	P	
Foraminiferida	5	0*	5	1.1	0.288	
Polychaeta	5	8	13	0.8	0.351	#
Bivalvia	6	3	9	1.1	0.286	
Crustacea	1	1	2	Test not performed		
Ostracoda	1	0	1	Test not performed		
Copepoda	8	2	10	3.7	0.053	S
Crab	6	0*	6	1.2	0.265	
Mysidacea	8	11	19	0.6	0.407	#
Cumacea	1	0	1	Test not performed		
Gammaridea	12	9	21	0.6	0.418	#
Isopoda	1	0	1	Test not performed		
Euphausiacea	5	6	11	0.1	0.739	#
Shrimps	14	9	23	1.7	0.187	#

* 0.05 was added to this value.

Table 6. Number of predators consuming each prey taxon by substrate. N = number of predators examined, disregarding season, depth, and size of predator. Total = number of predators that consumed individual prey taxa across substrates. Prey taxa are marked where they have the minimum number of data (#), or where they are significantly different over the parameter examined (S).

3.4 Diet examined by fish size

Fish examined were limited to juvenile flathead sole < 200 mm TL. The actual size range of the 80 predators used to analyze diet by season, depth or substrate was 28 165 mm TL. Because subsamples were based on environmental parameters without regard to fish size, we tested for bias. No significant differences were observed in predator size by season, depth, or substrate (|t| 1.248, p 0.211; Table 7). For analysis of diet by fish size, predators were divided into three approximately equal groups of 26 27 fish (Figure 6). These groups of small (28–51 mm), medium (52–77 mm), and large (78–165 mm) flathead sole approximated the lengths of age-0, age-1, and age- > 2 flathead sole reported by Abookire (1997).

No prey taxon was important to each size range of predators based on both numerical and biomass criteria (Figure 7). No taxon was important to all sizes of fish based on counts of prey consumed. “Shrimps” was the only taxon important to all sizes of fish based on prey biomass.

Mysids and copepods were important in the diet of small flathead sole by both counts of individuals and weight criteria (Figure 7a). Small flathead sole consumed more individuals of copepods (50%) and mysids (11%) than other prey taxa. Mysids composed the largest prey biomass eaten by small predators (38%), followed by copepods (16%), shrimps (15%), and amphipods (11%).

	Predator mean length ± StDev (mm)		t-value	df	p
Season	Winter 54.4 + 20.01	Spring 65.6 + 18.57	-1.298	18	0.211
	Winter 54.4 + 20.01	Summer 60.3 + 32.28	-0.491	18	0.629
	Spring 65.6 + 18.57	Summer 60.3 + 32.28	0.450	18	0.658
Depth	< 40 m 75.3 + 33.14	40–80 m 66.35 + 29.93	0.896	38	0.376
	< 40 m 75.3 + 33.14	> 80 m 75.9 + 32.40	-0.053	38	0.958
	40–80 m 66.35 + 29.93	> 80 m 75.85 + 32.40	-0.963	38	0.342
Substrate	< 50% mud 72.2 + 28.91	> 50% mud 72.8 + 34.61	-0.065	58	0.949

Table 7. Mean length of predators within each stratum.

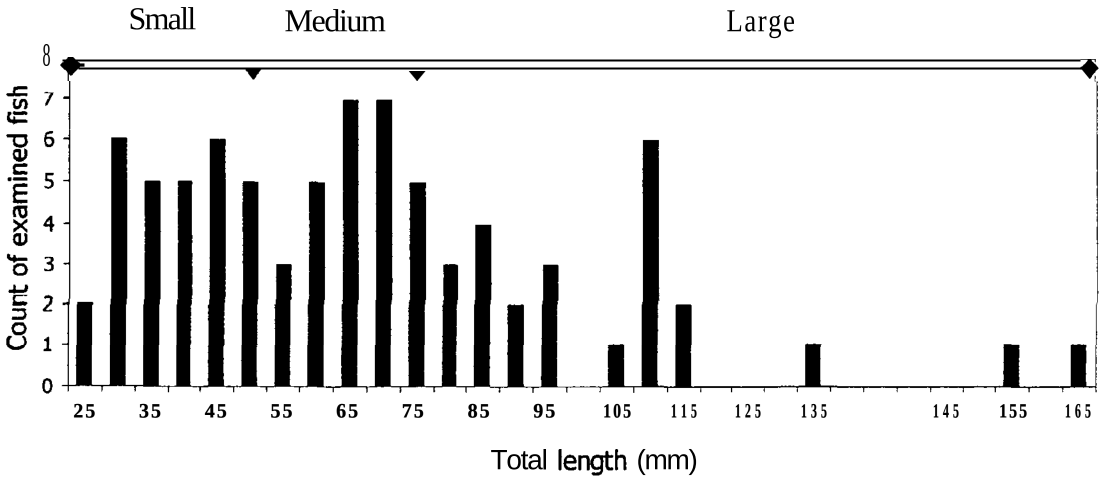


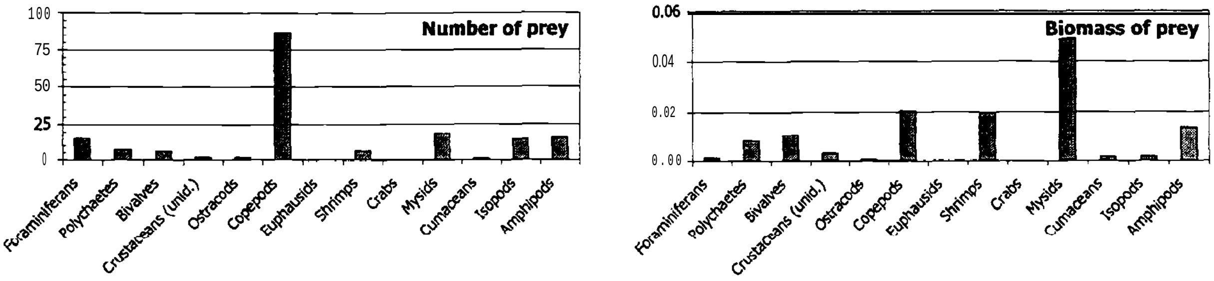
Figure 6. Length frequency histogram of flathead sole predators (stomach fullness > 0%) examined. Numbers on x-axis are the first number of a 5 mm length increment.

General prey grouping	Small	Medium	Large	Total	X^2	p	
	N = 27	N = 27	N = 26	N = 80			
Foraminiferida	4	0*	1	5	2.50	0.287	
Polychaeta	7	5	6	18	0.43	0.807	#
Bivalvia	5	2	6	13	2.33	0.311	
Crustacea (unidentified)	2	1	1	4	Test not performed		
Ostracoda	1	0	0	1	Test not performed		
Copepoda	11	6	1	18	7.53	0.023	S
Crab	0*	2	4	6	1.72	0.423	
Mysidacea	9	12	5	26	3.69	0.158	#
Cumacea	1	2	0	3	Test not performed		
Gammaridea (Amphipoda)	9	12	8	29	1.21	0.546	#
Isopoda	1	1	0	2	Test not performed		
Euphausiacea	0*	4	8	12	3.16	0.206	
Shrimps	2	10	11	23	7.32	0.026	S

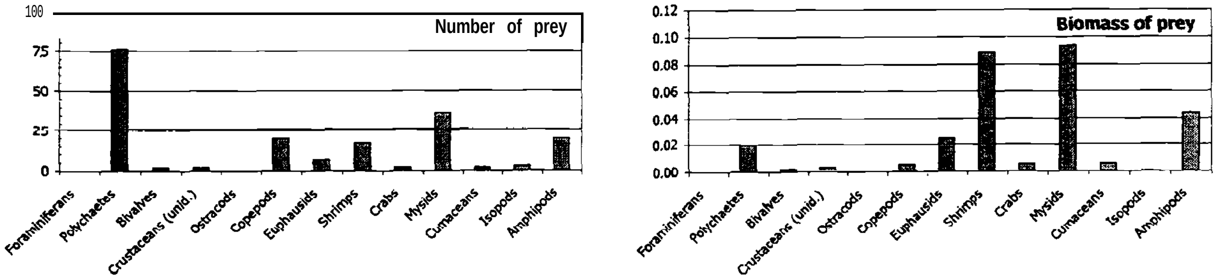
* 0.05 was added to this value.

Table 8. Number of predators consuming each prey taxon by fish size. N = number of predators examined, disregarding season, depth, and substrate. Total = number of predators that consumed individual prey taxa across fish size ranges. Prey taxa are marked where they have the minimum number of data (#), or where they are significantly different over the parameter examined (S).

7a. Small predators (28-51 mm, N=27 fish)



7b. Medium predators (52-77 mm, **N=27** fish)



71::. Large predators (78-165 mm, **N=26** fish)

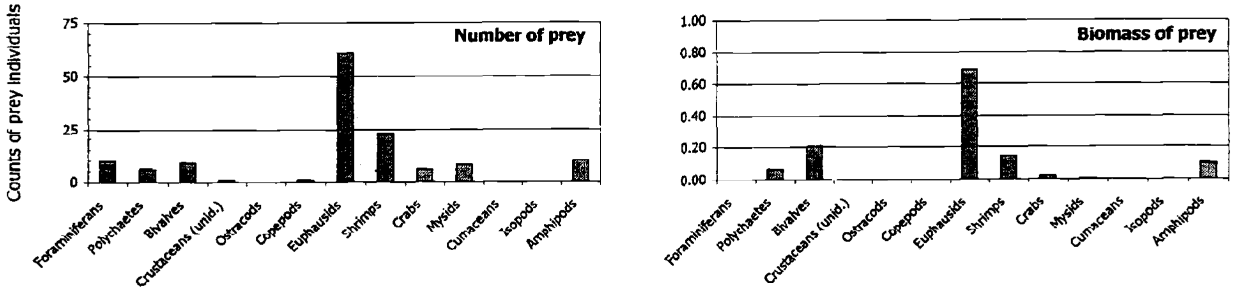


Figure 7. Counts and biomass of prey taxa consumed by small (a), medium (b) and large (c) predators.

Mysids and amphipods were important in the diet of medium-sized flathead sole by both count and weight criteria (Figure 7b). Medium flathead sole ate individuals of Polychaeta (40%), Mysidacea (20%), Copepoda (11%), and Gammaridea (11%). Prey biomass of medium flathead sole was mainly Mysidacea (32%), shrimps (30%), and Gammaridea (15%). A large proportion of the polychaetes eaten by medium flathead sole were the tiny juvenile polychaetes eaten during spring (Figure 3).

Euphausids and shrimps were important in the diet of large flathead sole in both numbers and biomass (Figure 7c). Large flathead sole consumed numerically more euphausids (46%) and shrimps (16%) than other prey taxa. Euphausids composed the dominant prey biomass of large predators (55%), followed by bivalves (17%) and shrimps (12%).

The comparison of diet by size of fish contained seven zero values: unfortunately the distribution of these zero values across prey taxa allowed only five reliable _^{2} calculations (Table 8). Two prey taxa (Copepoda and shrimps) were consumed with significantly different frequency (_ = 0.05) among fish of different sizes. Consumption of Mysidacea was marginally significantly different among different sizes of fish (_^{2} = 3.69, p = 0.158). The number of small, medium, and large predators consuming the other taxa were not significantly different.

4.0 DISCUSSION

Valuable descriptions of feeding versus habitat were gathered from the present research, but the sample size (N = 80 predators) was very small for a diet study. Statistical evidence from the few data collected indicate that flathead sole diets vary among the habitats examined. The logistic model uses Wald's statistic, distributed as a 2 variable, to assess goodness of fit of the data to the model of no differences among habitat categories (i.e., no effect). An accepted guideline for the 2 statistic is that most cells of the data matrix should have a count of 5 or more. Additionally, the numerous zero counts of predators consuming a prey taxon within habitat boundaries were problematic and required the addition of a small constant (0.05) to the zero values. Extrapolation of these data suggests that at least 40 but fewer than 100 predators within each habitat category would be necessary to achieve counts of > 5 in each important prey taxon. Our confidence in the apparent significant differences observed in this study (e.g., Copepoda by substrate) would be much higher if the data fit this guideline.

We emphasize that the tables reporting number of predators within a certain parameter consuming a specific prey taxon (Tables 4 6, Table 8) each report the results of testing a different hypothesis, that diet varied with season, depth, substrate, or fish size. For example, a significant difference in diet by depth, using a sample size of 20 fish per category, does not indicate that significant differences in another parameter would be detected using that sample size. A number of logistic model comparisons of prey could not be made due to zero values. Some of these prey taxa were consumed in low frequencies in all habitats (e.g., Ostracoda and Isopoda), and may represent less preferred prey. In other cases, such as that of Polychaeta in the seasonal comparison, a larger sample size would probably result in sufficient data in all cells to achieve the minimum of 5 counts per cell.

Though analyses based on this small sample size (N = 80 predators) were insufficient to determine if flathead sole occur in preferred habitats due to the prey associated with those habitats, they did indicate that certain prey taxa were consumed within limited ranges of depth and substrate. In particular, shrimps were consumed at depths > 80 m and by flathead sole ^{3}52 mm. Copepods are consumed on substrates < 50% mud and by flathead sole £77 mm. Mysids were consumed equally at all depths and on both substrates. Polychaetes and gammarid amphipods were consumed equally on both substrates and by all sizes of flathead sole.

This project provided limited yet valuable baseline descriptions of juvenile flathead sole diet over a range of substrates and depths in Kachemak Bay analogous to those provided for several species of juvenile flatfishes collected near Kodiak Island, Alaska (Holladay and Norcross 1995a, 1995b). Near Kodiak Island as in Kachemak Bay, mysids, shrimps, amphipods, and bivalves were important prey consumed by flathead sole. Additionally, copepods, euphausids, and polychaetes were important prey in some habitats in Kachemak Bay but not near Kodiak.

Results from Kodiak Island, as from the present study, indicated that different sizes of flathead sole consumed different prey (Holladay and Norcross 1995b). In order of importance, the prey of age-0 (small) flathead sole near Kodiak Island were primarily gammarid amphipods and bivalves, and the prey of age-1 (medium large) flathead sole were mysids, shrimps, and gammarid amphipods. In the present study, mysids and copepods, but not amphipods or bivalves, were important to small flathead sole, based on numbers and biomass of prey. Similar to the Kodiak region, mysids, shrimps, and amphipods were important to the medium and large flathead sole examined from Kachemak Bay, although in Kachemak Bay, euphausids were also important.

Analysis of seasonal diet has not been published for the size range of flathead sole we examined. This study determined that juvenile flathead sole feed year-round but are more likely to have fed recently during the summer. Unfortunately, the number of predators examined in the seasonal portion of this study was too small to determine whether seasonal diets of flathead sole are significantly different. The distribution and abundance of juvenile flathead sole in Kachemak Bay have been thoroughly examined with respect to season, depth, and substrate (Abookire 1997, Abookire and Norcross 1998, Norcross et al. 1998). This project has provided preliminary information on flathead sole diet in Kachemak Bay. In doing so, this project has assisted the planning of a more intensive study which could help explain the variation in the abundance and distribution of juvenile flathead sole that is not accounted for by season or physical parameters.

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