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Effects of sampling and preservation on apparent feeding by chaetognaths

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ABSTRACT: Chaetognaths are abundant predators of marine zooplankton. Their feeding typically has been quantified from gut contents of net-collected specimens. We evaluated the effects of mesh size and sampling duration on the gut contents of chaetognaths. Samples were compared from 333 µm and 64 µm mesh nets towed for 2 min and preserved immediately (controls), or towed for 5 min and preserved after 0, 5, 15, or 35 min. The 333 µm mesh net undersampled small chaetognaths, resulting in different chaetognath species and length distributions and different prey compositions than those obtained from the 64 µm mesh net. Prey loss from gut contents was substantial, with as much as 50% of prey lost in tows of greater than 2 min duration. Cod-end feeding, as indicated by prey in the foregut, undigested prey, and non-prey items, was much less than prey loss. Actual chaetognath predation effects may be more than double estimates made using net tow durations greater than 2 min.

KEY WORDS: Chaetognaths · Predation · Zooplankton sampling methods · Cod-end feeding · Gut evacuation · Shrinkage · *Sagitta*

INTRODUCTION

Chaetognaths are extremely abundant in marine systems, often second only to copepods in number (Feigenbaum & Maris 1984). Copepods are the main prey of these carnivores (reviewed in Feigenbaum & Maris 1984, Alvarino 1985, Feigenbaum 1991). Recent studies have used modifications of Bajkov's (1935) method to estimate natural feeding rates of chaetognaths from the amount of food in the guts of preserved specimens. This field-oriented approach is particularly appropriate for chaetognaths because most species are difficult to collect in good condition and to maintain in the laboratory (Feigenbaum & Maris 1984). The accuracy of this approach depends on how well net-collected gut content samples represent *in situ* feeding. Bias may result if (1) samples misrepresent length or species distributions of chaetognaths and prey, (2) prey are consumed during collection (cod-end feeding), and (3) gut contents are lost during sampling and preservation.

Chaetognaths are notorious cod-end feeders (Feigenbaum & Maris 1984); in plankton samples they are often found grasping inapproprate prey, such as large medusae or salps, or even their own tails (Feigenbaum & Maris 1984). Sullivan (1980) found that consumption by *Sagitta elegans* collected in a 183 µm mesh net was 10 to 50% greater than by specimens collected in a 333 µm mesh net, which retained fewer prey. Methods to reduce errors due to cod-end feeding include minimizing tow duration (Szyper 1978, Øresland 1987, Kimmerer 1984), reducing prey available in the net (Newbury 1978, Feigenbaum 1979, Sullivan 1980), and excluding prey in the foregut (Alvarez-Cadena 1992, Gibbons 1992) and undigested prey from gut content analyses (Pearre 1974, Feigenbaum 1979, Sullivan 1980, Canino & Grant 1985, Alvarez-Cadena 1993, Øresland 1995).

Although the possibility of prey loss during sampling has received little attention, some researchers have speculated that chaetognaths may evacuate their gut contents during sampling due to stress-induced regurgitation or defecation (e.g. Feigenbaum 1977, Øresland 1995). In the only previous direct test of prey loss, Szyper (1978) compared samples preserved immedi-

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ately after towing with duplicate samples preserved 20 to 30 min later, and found that the percentage of chaetognaths with food decreased with time. Preservation per se may cause chaetognaths to evacuate their guts, but this cannot be distinguished from towing effects under normal sampling procedures. Sullivan (1980) reported that she found no evidence of prey loss upon preservation.

The objective of the present study was to evaluate the effects of sampling and preservation on apparent feeding by chaetognaths. The research was done in conjunction with another study (Baier & Purcell 1997 this issue) in which our goal was to determine the importance of chaetognaths as predators and competitors of larval fish.

METHODS

Two series of samples were collected at each of 3 stations (33° 37' N, 77° 02' W; 34° 02' N, 77° 27' W; 34° 28' N, 76° 58' W) in the South Atlantic Bight, off the coast of North Carolina, USA, in February 1993. Mesh sizes, towing speeds, and tow durations were chosen to correspond to the sampling program of Baier & Purcell (1997), which used a multiple-opening-closing net (MOCNESS) fitted with 333 µm mesh nets and 64 µm mesh insets. Samples for the present study were collected in conical plankton nets, each fitted with a flowmeter and 1 l hard cod-end with mesh windows. At each station, one series of tows was made with a 0.75 m diameter net with 333 µm mesh, and one series with a 0.5 m net with 64 µm mesh. Each series consisted of 5 surface tows, made at a speed of approximately 1.5 knots. A 'control' sample for each series was preserved in 5% formalaldehyde within 2 min after the tow began. The remaining samples were collected consecutively in 5 min tows. The 5 min samples were transferred undiluted to 1 l containers, and each tow from a series received one of 4 treatments: preserved immediately, or held for 5, 15, or 35 min at ambient water temperature (15.5 to 18.0°C) in a water bath before preserving. These holding times correspond to durations in the cod-ends with the MOCNESS nets closed.

Subsamples were taken using a Folsom Plankton Splitter as necessary to obtain 100 to 300 chaetognaths from each sample. Chaetognaths were identified to species, and their standard lengths (distance from the front of the head to the end of the tail, excluding fin) measured with a CUE-2 video image analysis program. The position of prey in the gut and the degree of digestion were recorded as in Feigenbaum (1979). Organisms protruding from the mouths of chaetognaths were identified and measured, but were not counted as gut contents. It usually was necessary to excise the gut contents using insect pins to determine the prey types and sizes. Prey size was measured as the prosome length of copepods, standard length of chaetognaths and larval fish, and total length of all other prey types.

All statistical analyses were made using Statistical Analysis Systems (SAS) software (SAS Institute Inc., Cary, NC, USA). Differences due to mesh size in chaetognath densities, and length and species distributions were examined using Analysis of Variance (ANOVA). Only the 2 min control tows were compared in these tests. The effects of sampling duration in different mesh sizes on the number of prey per chaetognath (NPC) were tested using ANOVAs. Least square means were compared using the Bonferroni approach to determine if there were differences, and the significance and direction of effects (Tarone 1990). Measures of variance were reported as ± 1 standard error.

RESULTS

Effects on size and species distributions of chaetognaths and prey

Estimated densities, and length and species distributions of chaetognaths were affected by net mesh size. The mean density of all chaetognaths, estimated from the 2 min 64 µm mesh samples pooled, was 13.5 ± 13.0 m^{-3}. Total chaetognath densities calculated from the 333 µm mesh samples averaged 8.2 ± 8.9 m^{-3}. The difference probably resulted from small chaetognaths passing through the coarser mesh; chaetognaths smaller than 5 mm constituted nearly 50% of the specimens collected in the 64 µm mesh net, but only 15% in the 333 µm mesh samples (Fig. 1A). Chaetognath species composition also differed significantly between mesh sizes in the 2 min samples ($p < 0.001$; Fig. 1B). There was a greater percentage of *Sagitta enflata*, one of the largest chaetognaths, in the 333 µm mesh samples compared with the 64 µm mesh samples, which had a higher percentage of unidentified juvenile chaetognaths (<5 mm).

Probably as a result of the differences in chaetognath size and species distributions, total NPCs and prey compositions differed between meshes in the 2 min samples (Fig. 2A, B). There were significantly more copepods, larvaceans, and 'other' prey in the larger chaetognaths collected by the 333 µm mesh net ($p < 0.05$). The smaller chaetognaths that predominated in the 64 µm mesh samples ate more copepod nauplii and tintinnids. Only the 15 to 20 mm size category showed evidence of increased feeding in the 64 µm mesh net as compared with the 333 µm mesh.

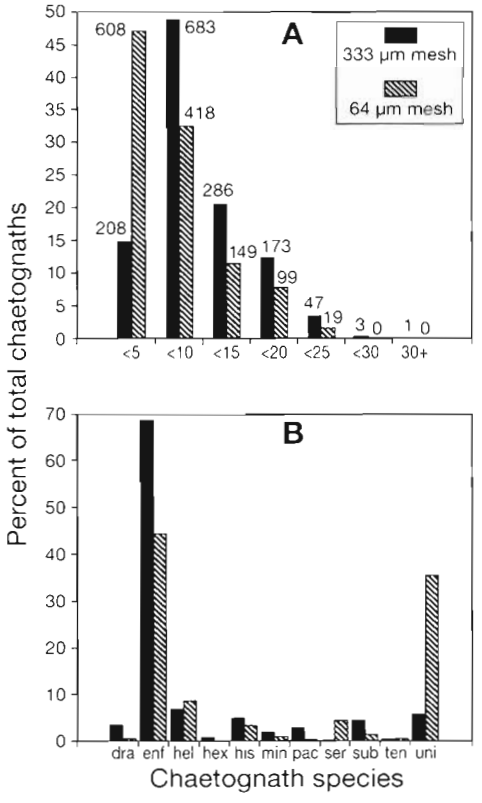

Fig. 1. (A) Comparison of length frequency distributions of chaetognaths (all species) from 2 min tows collected in 333 µm and 64 µm mesh nets. (B) Chaetognath species compositions from 2 min tows with 333 µm and 64 µm mesh nets. Numbers of chaetognaths examined are above each bar. dra = *Pterosagitta draco*, enf = *Sagitta enflata*, hel = *Sagitta helenae*, hex = *Sagitta hexaptera*, his = *Sagitta hispida*, min = *Sagitta minima*, pac = *Krohnitta pacifica*, ser = *Serratosagitta serratodentata*, sub = *Krohnitta subtilis*, ten = *Sagitta tenuis*, and uni = unidentified

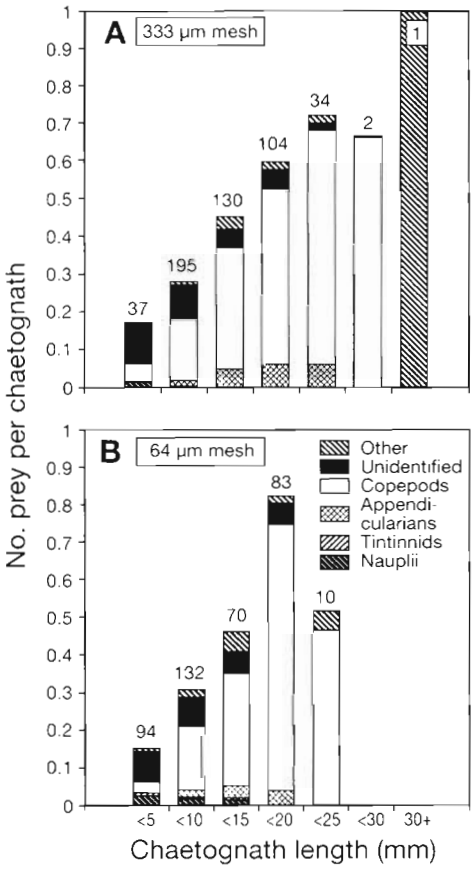

Fig. 2. NPC of prey taxa, by chaetognath length group, in (A) 333 µm, and (B) 64 µm mesh net samples collected in 2 min tows. Numbers of prey examined are above each bar. Numbers of chaetognaths shown in Fig. 1A

Changes in chaetognath gut contents during sampling

Cod-end feeding. Cod-end feeding, as indicated by prey in the foregut, undigested prey, and non-prey items in chaetognath guts, differed among species and was affected by mesh size. In the 2 min samples, *Sagitta enflata* had much larger percentages (12% and 26% in the 333 and 64 µm mesh nets, respectively; Fig. 3) of prey in the foregut than *Sagitta helenae* (0% in both nets). For *S. enflata*, the maximum percentage of prey in the foregut (34%) occurred later (at 10 min) in the 333 µm mesh net than in the 64 µm mesh net (32% at 5 min) (Fig. 3). Non-prey items, including algal clumps and sand grains, were found infrequently in chaetognath guts from the 333 µm mesh net, most likely because the large mesh did not retain such debris. The numbers of non-prey items in chaetognath guts changed erratically with duration in the 64 µm mesh samples, accounting for 0 to 12% of total prey.

Prey loss. The NPC was markedly lower in samples kept in the cod-end from 5 to 40 min compared with samples from 2 min tows (Fig. 4). The amount of prey lost depended on the mesh size and chaetognath species (Table 1). In the 333 µm mesh samples, the NPC

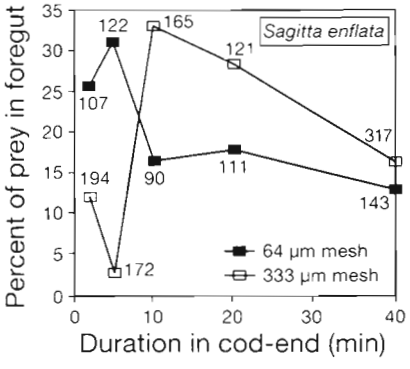

Fig. 3. Effects of duration in the cod-end on the percentages of total prey in the foregut of *Sagitta enflata* collected with 333 µm and 64 µm mesh nets. Total numbers of prey examined are shown by each point

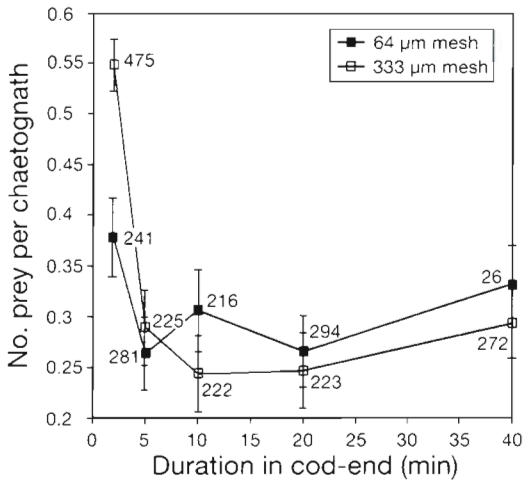

Fig. 4. Mean number of prey per chaetognath ± 1 SE in relation to duration in the cod-end (time from beginning the tow to preservation) for all species combined from samples collected in 333 µm and 64 µm mesh nets. Numbers of chaetognaths examined are beside each mean

was reduced by about 50% in 5 min tows as compared with 2 min tows ($p < 0.01$; Table 1, Fig. 4). There were no significant differences among the 5 min tows for any holding time. In the 64 µm mesh net, there were no significant differences among any sampling durations.

DISCUSSION

The methods of sampling and analysis have varied substantially among studies that quantified feeding by chaetognaths (Table 2). Chaetognaths frequently have been studied from samples taken for other purposes, and therefore tow durations and mesh sizes often have been inappropriate. Criteria used to distinguish prey consumed in the cod-end from 'natural' prey also have varied among studies.

Understanding how sampling procedures affect chaetognaths is critical both for interpreting previous work and for improving future studies. Prior to our study, the only information available on the effects of sampling on chaetognath gut contents was provided by the observations of Szyper (1978) and Sullivan (1980). Unfortunately, actual sampling conditions could not be perfectly simulated in our study. Chaetognaths could be subject to more stress in a net being towed than in the stationary cod-ends used in our experiments. Also, the control tows, especially those made with the 64 µm mesh net, still subjected the chaetognaths to agitation and crowding. Therefore, we believe that our estimates of sampling effects are conservative.

Although researchers have expressed much concern about feeding by chaetognaths during collection in nets, the most notable effect observed in this study was a significant loss of prey. The sudden decrease (50%) in the NPC between 2 min and 5 min samples from the 333 µm mesh net indicates that prey loss was due more to stress-induced gut evacuation than to continued digestion of prey. Szyper (1978) found that *Sagitta enflata* with prey decreased by as much as 80% in samples held for 20 to 30 min. In the 2 studies in which *S. enflata* were collected in tows of <2 min (Szyper 1978, Kimmerer 1984), the NPCs were much higher than in any other studies (Table 2).

Although the 333 µm mesh undersampled small chaetognath populations, it may have represented *in situ* feeding better than the 64 µm mesh samples. Chaetognaths may evacuate their gut contents after a relatively short duration in the 64 µm mesh nets, because the cod-end quickly becomes crowded with small organisms. The 333 µm mesh net filtered 135.8 ± 14.6 m^3 in 5 min, compared with 60.2 ± 13.1 in 2 min. The 64 µm mesh net apparently clogged within 2 min, filtering no more water in 5 min (24.6 ± 5.8 m^3) than in 2 min (29.8 ± 29.0). Cod-end feeding also may begin sooner in response to increased prey concentrations in the 64 µm mesh than in the 333 µm mesh net. This may explain the relatively high proportions of prey in the foregut seen in the 2 and 5 min samples from the 64 µm mesh net. In the 333 µm mesh net, the sudden increase in the proportion of prey in the foregut at 10 min suggests a lag period before the cod-end became crowded enough to provoke chaetognaths to feed.

It is clear that both prey loss and cod-end feeding occur; prey loss appears

Table 1 Results of analysis of variance to test for effects of duration in the cod-end (2 vs 5, 10, 20, and 40 min) on the number of prey per chaetognath (NPC). N = the number of chaetognaths examined. F = calculated F-statistic. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

Chaetognath species	All samples N	All samples F	64 µm mesh N	64 µm mesh F	333 µm mesh N	333 µm mesh F
All species	2710	18.02**	1293	1.70	1417	20.02**
Sagitta enflata	1542	8.20***	573	1.54	969	14.35***
Unidentified	541	9.59***	458	6.89***	82	3.42
S. helenae	209	1.53	112	1.08	97	0.76
Serratosagitta serratodentata	121	4.09**	58	1.83	63	2.82*
S. hispida	112	0.92	43	0.35	69	1.53
Pterosagitta draco	56	2.55	8	0.38	48	2.92*
S. minima	54	1.51	13	0.37	41	1.93
Krohnitta sp.	53	1.97	23	2.17	30	0.68
S. hexaptera	27	4.19*	0	–	27	4.19*
S. tenuis	12	0.95	6	0.54	6	0.44

Table 2. Summary from the literature of sampling methods and estimates of chaetognath feeding (only studies giving tow duration are included). Total duration is from the beginning of the tow to preservation. NPC: number of prey per chaetognath; FR: feeding rate in number of prey chaetognath^{-1} d^{-1} MOC indicates that Multiple-Opening-Closing nets were used and sampling duration varied with each net but was not reported. N: net modified to exclude prey; F: prey in the foregut excluded from the analysis; D: prey in the early stages of digestion excluded from the analysis. All samples were preserved in formalin

Species	Temp. (°C)	Mesh (µm)	Duration (min) Tow	Total	Modifications	NPC	FR	Source
Sagitta enflata	–	330	–	<1	F	1.09–1.18	7.3–11.6	Kimmerer (1984)
	24–26	330	–	<2	–	1.04	7.4	Szyper (1978)
	–	1620	–	15–30	NFD	0.30	2.2	Feigenbaum (1979)
	18	333	–	<2	F	0.54	–	This study
	18	333	–	≥5	F	0.26	–	This study
S. elegans	4–8	333/183	10	–	D	0.04–0.41	–	Sullivan (1980)
	6	450	10–15	–	N	0.06–0.28	0.2–1.0	Øresland (1987)
	–	335	15	–	ND	0.09–0.54	0.8–3.6	Alvarez-Cadena (1993)
	-1	375[a]	1	–	–	0.00–0.76	0.3–1.1	Falkenhaug (1991)
S. setosa	6	450	10–15	–	N	0.29–0.25	2.3	Øresland (1987)
	–	333	15	–	D	0.14–0.38	1.4–5.4	Alvarez-Cadena (1992)
Eukrohnia hamata	–	300	25–50	–	–	0.10–0.36	0.3–1.1	Øresland (1990)
	4–8	333/183	10	–	D	0.10	–	Sullivan (1980)
	-2–1	110/130/333	–	3–25	FD	0.8–0.12	0.3–0.5	Øresland (1995)
Pterosagitta draco	12–25	202/183	–	30	N	0.10–0.12	1.0	Newbury (1978)
S. friderici	–	200	–	MOC	–	0.08	0.5	Stuart & Verheye (1991)
Serratosagitta serratodentata	–	200	–	MOC	F	0.0–1.0	–	Gibbons (1992)
S. tenuis	21–25	333/202	5–10	–	D	0.09–0.45	3.1–8.7	Canino & Grant (1985)

[a]Samples collected with a plankton pump

to occur initially and cod-end feeding may continue throughout the tow. These problems probably are most effectively minimized by sampling chaetognaths in very brief (<2 min), gentle tows. Sullivan's (1980) method of quantifying small chaetognaths from a fine mesh inset and analysing gut contents of chaetognaths from a coarser mesh net may be the best compromise to deal with the respective shortcomings of fine and coarse mesh nets.

When these measures are not possible, some adjustments based on the results of our study may be applicable. In samples towed longer than 5 min with a 333 µm mesh net, NPC decreased by about 50%. The decrease in NPC was not significant for the 64 µm mesh net samples, but perhaps that was because the NPC had begun to decrease within the 2 min duration of the control tows. Therefore, these adjustments may be appropriate for fine mesh as well as large mesh.

Our results suggest that excluding prey in the foregut from gut content analysis was appropriate for the species and temperatures involved in this study; however, this measure is probably effective only in combination with very short tows. Szyper (1978) noted that the percent of prey in the foregut was a poor indicator of cod-end feeding, due to the rapid transfer (0.5 to 2 min) of food to the rear gut of *Sagitta enflata* at temperatures of 24 to 26°C. Reeve et al. (1975) also observed rapid movement of prey to the rear gut in *Sagitta hispida*.

Factors such as temperature and species differences may be important in determining how much and how quickly cod-end feeding and prey loss occur. Therefore, the effects seen in this study would not apply to all species or in all environments. Also, other sources of error, such as experimentally determined digestion rates, may introduce substantial error into feeding rate calculations. The results of our study clearly showed that apparent feeding of chaetognaths is affected by the sampling methods used. Prey loss during sampling, which had received little attention previously, was of greater magnitude than cod-end feeding. The importance of chaetognaths as predators in marine food webs may have been underestimated by half in most studies due to the net sampling methods employed.

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