

Sublethal predation: field measurements of arm tissue loss from the ophiuroid *Microphiopholis gracillima* and immunochemical identification of its predators in North Inlet, South Carolina, USA

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ABSTRACT: A daily rate of sublethal predation of arm tissue of the burrowing amphiuroid brittlestar *Microphiopholis gracillima* (Stimpson) was determined by experimental field manipulation. A concurrent field predator survey documented the ingestion of arm tissue by invertebrate and vertebrate predators. Fish, shrimps and crabs were collected by seining and trawling in Debidue Creek, North Inlet, South Carolina, USA. Gut contents of these predators were examined both visually and immunochemically to determine presence or absence of arm tissue. Of white shrimp guts tested, 88% were positive for *M. gracillima* arm tissue, while 70% of blue crab guts and 39% of brown shrimp guts tested positive. Several fish species and hermit crab species were found to prey on the brittlestar arm tissue less frequently or in lesser quantities. To determine the daily sublethal predation rate, individual brittlestars of known arm length were emplaced subtidally in Debidue Creek, retrieved after either 24 or 72 h, and then had their arm tissue remeasured. Loss of brittlestar arm tissue was statistically measurable over a 24 h period relative to laboratory controls. Brittlestars held for 72 h in the field lost 3 to 4 times more arm tissue than those exposed to predation for only 24 h. An average daily sublethal loss rate of 20 mm arm tissue brittlestar⁻¹ was calculated for the late summer/early fall period. Trophic transfer due to sublethal predation on this individual species of brittlestar in Debidue Creek ranges between 3.3 and 9.7 g AFDW m⁻² yr⁻¹, an amount equivalent to total community macrobenthic secondary production in other systems.

KEY WORDS: Sublethal predation · Ophiuroid · Immunoassay · Secondary production

INTRODUCTION

Predation usually implies death of the prey organism, followed by complete or partial consumption. However, when tissue is lost to browsing predators (termed sublethal predation by Zajac 1985), the prey organism survives the predation event. Furthermore, plants and some invertebrates regenerate this lost tissue which then becomes available to predators at subsequent predation events. Sublethal predation occurs commonly in terrestrial and aquatic communities. Co-

lonial organisms in coral reef communities regularly experience partial predation (Jackson & Palumbi 1979, Harvell 1984, Lasker 1985, Harvell & Suchanek 1987). In marine soft-sediment communities, a variety of infaunal organisms lose tissue to browsing predators, via tail or palp nipping upon polychaetes (e.g. De Vlas 1979a, b, Woodin 1984, Zajac 1985), siphon-nipping of bivalves (e.g. Trevallion 1971, Peterson & Quammen 1982, DeVlas 1985, Zwarts & Wanink 1989, Kammars & Huitema 1994), and arm consumption of brittlestars (Bowmer & Keegan 1983, Duineveld & Van Noort 1986). Sublethal predation has been shown to be a potentially significant source of energy flow in the few studies where it has been examined (DeVlas 1979b, 1985, O'Connor et al. 1986).

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Several studies have documented extensive tissue loss in amphiuroid brittlestars collected from their native environments (Buchanan 1964, Singletary 1980, Bowmer & Keegan 1983, Stancyk et al. 1994, Sköld & Rosenberg 1996). Arm regeneration rates and frequency of scarring in brittlestar arm populations form the basis of existing estimates of energy flow due to sublethal predation on ophiuroids (Duineveld & Van Noort 1986, O'Connor et al. 1986). Although these estimates have been preliminary, they demonstrate that the contribution to secondary production via ophiuroid arm regeneration is ecologically important.

Arm loss in amphiuroid populations has been attributed to unknown predators (Bowmer & Keegan 1983, O'Connor et al. 1986). Although many motile invertebrates and vertebrates may ingest brittlestars or brittlestar arm tissue, most studies have focused on bottom-feeding fishes such as dab, plaice, yellowfin sole, flathead sole, and haddock as brittlestar predators (Feder & Jewett 1981, 1987, Bowmer & Keegan 1983, Mattson 1992, Zamarro 1992). Macerating predators, such as shrimps and crabs, have not been studied as extensively, although they are also known to ingest ophiuroids (Wassenberg & Hill 1987, Feder & Pearson 1988, Hill & Wassenberg 1992). Both penaeids and crabs macerate their prey, rendering visual identification of prey in these predator guts difficult. Therefore, determination as to whether the gut contents of these macerating predators contains brittlestar arm tissue would require an immunochemical approach (sensu Feller et al. 1979, Hunter & Feller 1987).

Microphiopholis gracillima is an infaunal amphiuroid ophiuroid which lives with its small central disc buried 10 to 15 cm below the sediment surface. It undulates its long, slender arms to pull water through mucus-lined burrows for respiration (Woodley 1975). Amphiuroid brittlestars are selective deposit and/or suspension feeders (Buchanan 1964, Clements & Stancyk 1984) and usually have 3 of their 5 arms extended upon the surface for feeding and respiration (authors' pers. obs.). Singletary (1980) reported a density of 56 m⁻² for this species in Biscayne Bay, Florida, USA, and in Debidue Creek, North Inlet, South Carolina, USA, the natural subtidal density of these brittlestars is 35 m⁻² (Stancyk unpubl. data). A 12 mo arm scar survey conducted on brittlestars collected in North Inlet found that 85% of the 2405 *M. gracillima* arms examined had at least 1 regeneration scar. Only 3% of the 675 individual brittlestars examined bore no discernible arm scars (Stancyk et al. 1994). Again, the scarring was attributed to unknown predators.

Existing studies of energy flow resulting from sublethal predation on ophiuroids lack information concerning both identification of the local predators and their grazing rates on the brittlestar arms. After mea-

suring laboratory ingestion rates (Pape-Lindstrom 1994), the goals of the present study were: (1) to learn which local predators graze *Microphiopholis gracillima* arm tissue in North Inlet; (2) to measure field loss rates of brittlestar arm tissue due to grazing by natural predators; (3) to compare loss rates in the field with those due to white shrimp predation in the laboratory; and (4) to determine the relative importance of the sublethal predation in terms of trophic energy flow.

MATERIALS AND METHODS

Individual core emplacement field experiments.

Microphiopholis gracillima were collected during low tide from a shallow, subtidal mudflat in Debidue Creek, North Inlet (37° 20' N, 79° 10' W) and transported by boat to the Baruch Field Laboratory, Georgetown, South Carolina. There, brittlestars were held in an aerated aquarium with a salinity of approximately 30‰ prior to use. Next, brittlestars were anesthetized in a 1:1 mixture of 35‰ MgCl₂ and seawater, and relaxed individuals were placed in a large shallow petri dish, oral side up. Arms of each brittlestar were straightened with forceps and their lengths measured to the nearest mm from the point at which the arm joins the oral frame, to the arm tip. The oral diameter of the brittlestar was measured as described in Singletary (1980), using an ocular micrometer mounted in a dissecting microscope (mag. 24×). If ripe gonads were present, the sex of the individual was also noted.

After being measured, brittlestars were allowed to burrow in individual gray polyvinyl chloride (PVC) cores (inside diameter = 5 cm, length = 9 cm). The cores contained sieved (mesh = 3 mm) mud from the subtidal field collection site and were covered on the bottom with Nitex mesh (mesh = 0.39 mm) and on the top with fiberglass window screen (mesh = 1.1 mm) or Nitex mesh (see Table 1). The cores were held upright in an aerated fiberglass tank (2 × 0.5 × 0.5 m), until the following low tide. This 12 h holding time allowed the brittlestars to recover from anesthetization and become established in their subsurface burrows inside the cores.

Each brittlestar was assigned randomly to 1 of 3 treatments: laboratory control, field control or field treatment. The laboratory control cores were held in an aerated fiberglass tank in the indoor seawater laboratory for the duration of the experiment. The water temperature was maintained at approximately 25°C. The brittlestars in laboratory control cores were given a boat ride to the field study site during deployment of field cores, to mimic as closely as possible the handling stresses of the field-deployed brittlestars.

At low tide the field control and field treatment cores were emplaced in the creek bottom with the core tops flush with the sediment surface, using a modified flower bulb planter to create appropriately sized holes. The cores were arrayed in a square grid ($n = 18$ to 24 for each treatment; see Table 1), with brittlestars in alternating treatment and field control cores spaced about 30 cm apart within the grid. The grid was situated below the low tide line approximately 10 m from the water's edge in a subtidal mudflat where the natural brittlestar population occurs. At absolute low tide the area was covered by about 10 cm of water. The field control cores retained the Nitex top coverings, while the field treatment cores had the Nitex top covering removed, thus allowing predators access to brittlestar arm tips. All cores retained the Nitex bottom covering.

On the 2 experimental dates, 2 arrays of cores were emplaced side by side, with one set of cores being retrieved after 24 h and the other after 72 h (Table 1). After retrieval from the field, the brittlestars were returned to the field laboratory, carefully removed from their cores and arms and oral diameters were measured again. The mud in each core was carefully sieved to recover any arm fragments which the brittlestars may have autotomized. The total amount of arm tissue lost was calculated for each individual brittlestar.

Field predator surveys. Collection of potential predators: Survey collections consisted of seining for flatfishes, crabs and shrimps in the area of Debidue Creek where the brittlestars occur. Field collections were conducted intermittently from 1990 to 1992, with several day and night collections made during the deployment of PVC cores for the arm loss rate experiment in 1992. In all cases, upon capture the predators were immediately frozen on dry ice, returned to the laboratory in Columbia, South Carolina and stored frozen at -70°C until dissection.

Collection of predator gut contents: Potential predators were identified to species level and their foreguts removed. Gut contents of each predator were examined visually under a dissecting microscope and then placed in individual 1.5 ml microcentrifuge tubes. The dissections were performed carefully to avoid contaminating the gut contents with body tissues or tissue from the digestive tract of the predator. The gut contents were homogenized using a glass stirring rod with TES buffer (5 mM N-tris [hydroxymethyl] methyl-2-aminoethane sulfonic acid, 30 mM NaOH and 150 mM NaCl, pH 7.3). (The volume of buffer used was 100 μl for each white shrimp and brown shrimp *Penaeus aztecus* gut content sample, which were usually similar in body size and gut content volume. Buffer was added in a 1:1 gut content weight/TES volume ratio for all other species of predators.) During processing, the predators and their gut contents were kept on ice to

Table 1. Dates, number of replicates (n) and descriptions of field and laboratory treatments performed. top/bottom: type of covering on the top and bottom of individual PVC cores; nc: not covered; ws: fiberglass window screen, mesh size = 1.1 mm; ntx: Nitex, mesh size = 0.39 mm

Date	Covering	Lab control n	Field predation n	Total n
24 h duration				
29 Aug 1992		23	23	46
	top bottom	ws ntx	nc ntx	
25 Sep 1992		18	18	36
	top bottom	ntx ntx	nc ntx	
72 h duration				
29 Aug 1992		25	24	49
	top bottom	ws ntx	nc ntx	
25 Sep 1992		19	23	42
	top bottom	ntx ntx	nc ntx	

avoid further digestion of prey proteins. The predator gut contents were stored at -70°C until later immunoassay.

Immunoassay of predator gut contents: Polyclonal antiserum raised to soluble proteins from the arm tissue of *Microphiopholis gracillima* was used to assay predator gut contents following the micro-Ouchterlony technique (Feller et al. 1979). To test for the presence of brittlestar arm tissue in the gut content samples, the following spatial arrangement of samples on the immunoassay template was employed: the center well contained the anti-brittlestar arm tissue antiserum, wells 1 and 4 contained homologous brittlestar arm tissue antigen and wells 2 and 3 contained solubilized gut contents of 2 individual predators. Formation of 2 or more lines of identity (precipitin bands) between the wells containing predator gut contents and the wells containing the homologous brittlestar arm antigen was scored as 'positive' for the presence of *M. gracillima* arm tissue (Fig. 1). The criterion of at least 2 identity bands was chosen because, of the echinoderm species present in North Inlet, only the brittlestar *Ophiothrix angulata* (which does not occur in the mudflat area where predators were collected) formed more than 2 precipitin bands with the anti-*M. gracillima* antiserum (Pape-Lindstrom 1994).

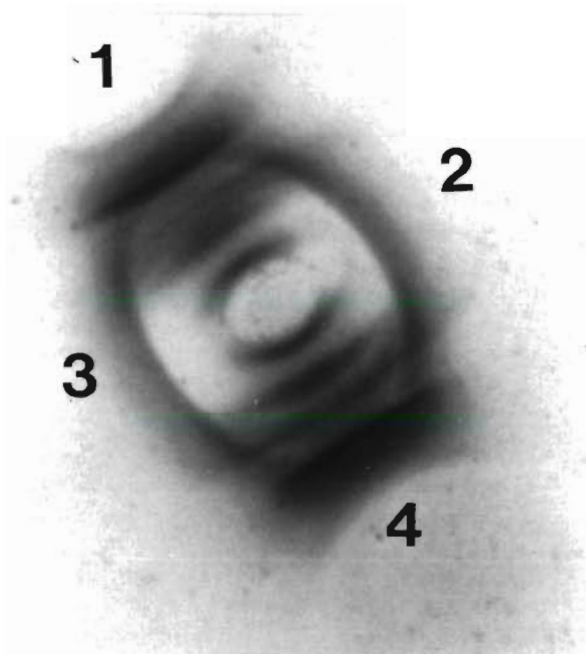


Fig. 1. Slide of micro-Ouchterlony immunoassay with anti-*M. gracillima* in center well, brittlestar arm protein in wells 1 and 4 (self-reaction), and predator gut contents in wells 2 and 3. Formation of 2 or more identity bands between the wells containing predator gut contents and the wells containing the brittlestar arm protein was scored as 'positive'

Data analysis. Due to the high silt load in Debidue Creek, the Nitex (mesh = 0.39 mm) used to prevent exposure of control brittlestar arms became clogged (authors' pers. obs.) and may have caused less than optimal dissolved oxygen conditions in the field control cores. This suffocation effect was most pronounced in the 72 h trial of 29 August, in which 6 brittlestars in the Nitex-covered field control cores had experienced death and decay by the end of the experiment. Consequently, data from brittlestars held in the field control cores had to be excluded from the data analysis. Smaller mesh Nitex was used in these experiments because brittlestars were observed to extend their arms through both top and bottom screen (mesh = 1.1 mm) used in preliminary experiments. Although the larger mesh window screen did not seem to produce such a pronounced suffocation effect, it could not serve as an effective control because brittlestar arm tips were still available to predators.

The parameter of interest — total length of arm tissue lost by an individual brittlestar —

was \log_{10} transformed to comply with the assumptions of ANOVA (homoscedasticity). When factors such as gonad color (gender) of brittlestar or placement in the field array by row, column, or edge position (brittlestars in an outermost row or column were considered to be in an edge position) were found to be insignificant in preliminary data analyses, the factors were eliminated from the final ANOVA model to increase the degrees of freedom and simplify interpretation. Because all means presented in the figures and tables are back-transformed means, 95% confidence intervals are asymmetric. All statistical analyses presented were performed on transformed data using SAS/STAT for the personal computer (SAS Institute 1988).

RESULTS

Individual core emplacement field experiments

A 2-factor ANOVA model with interactions was used to compare arm tissue losses by date and treatment from 29 August and 25 September on which both 24 and 72 h trials were performed. Treatment was highly significant ($p = 0.0001$, $F = 30.75$, $MS = 9.57$, $df = 3$). Neither date nor the date \times treatment interaction was significant ($p = 0.12$, $F = 2.47$, $MS = 0.77$, $df = 3$ and $p = 0.36$, $F = 1.07$, $MS = 0.33$, $df = 3$, respectively).

A Tukey's multiple comparison of means by treatment found that the mean amount of arm tissue lost

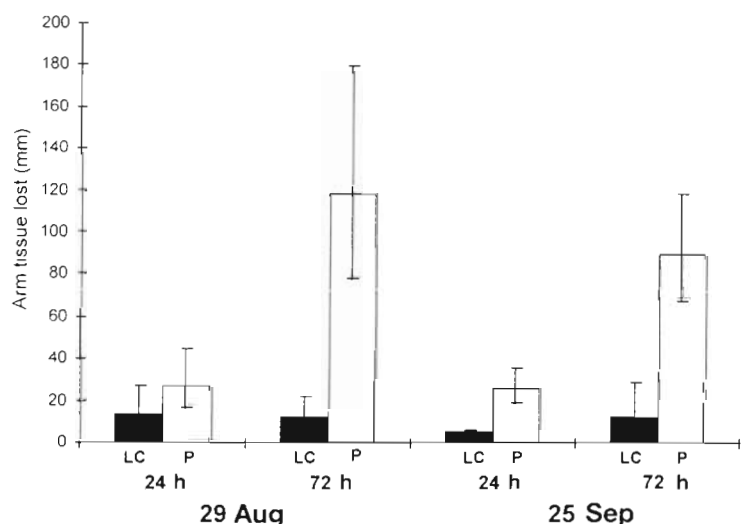


Fig. 2. Back-transformed mean length of arm tissue lost by an individual brittlestar on the dates for which both 24 and 72 h core emplacement trials were conducted. LC: lab control cores; P: field treatment cores in which brittlestars were exposed to predation. Error bars represent 95% confidence limits

Table 2. Complete listing of predators' guts subjected to immunoassays. Organized by species, date, and time of collection

Species	Capture date	Time	Number assayed	Number positive	Percent positive
<i>Penaeus setiferus</i>	20 Aug 1990	Day	57	51	89
white shrimp	30 Aug 1992	Day	8	4	50
	31 Aug 1992	Night	27	26	96
<i>Penaeus aztecus</i>	30 Jun 1992	Night	14	4	29
brown shrimp	30 Aug 1992	Day	1	1	100
	31 Aug 1992	Night	8	4	50
<i>Callinectes sapidus</i>	5 Feb 1992	Day	7	0	0
blue crab	30 Jun 1992	Day	13	12	92
	30 Jun 1992	Night	9	9	100
	30 Aug 1992	Day	1	0	0
<i>Pagurus</i> spp. or	5 Feb 1992	Day	1	0	0
<i>Clibanarius</i> spp.	30 Jun 1992	Day	6	4	67
hermit crab	31 Aug 1992	Night	1	0	0
	26 Sep 1992	Day	1	0	0
<i>Leiostomus xanthurus</i>	30 Jun 1992	Night	10	2	20
spot	30 Aug 1992	Day	3	0	0
	31 Aug 1992	Night	5	3	60
<i>Bairdiella chrysoura</i>	30 Aug 1992	Day	14	6	43
silver perch	31 Aug 1992	Night	6	0	0
<i>Lagodon rhomboides</i>	30 Jun 1992	Night	4	1	25
pinfish	30 Aug 1992	Day	2	0	0
	31 Aug 1992	Night	6	0	0
<i>Anchoa mitchilli</i>	30 Jun 1992	Night	5	0	0
bay anchovy	30 Aug 1992	Day	5	0	0
	31 Aug 1992	Night	11	0	0
<i>Fundulus heteroclitus</i>	5 Feb 1992	Day	4	0	0
mummichog					
<i>Fundulus majalis</i>	5 Feb 1992	Day	5	0	0
killifish					
<i>Mugil</i> spp.	30 Jun 1992	Night	10	1	10
mullet					
<i>Synodus foetens</i>	30 Jun 1992	Night	3	0	0
lizard fish					
<i>Citharichthys spilopterus</i>	30 Jun 1992	Night	3	0	0
bay whiff					
<i>Symphurus plagiusa</i>	30 Jun 1992	Day	1	0	0
blackcheek tonguefish		Night	2	0	0
<i>Paralichthys lethostigma</i>	30 Jun 1992	Day	1	0	0
southern flounder		Night	1	0	0
<i>Etropus crossotus</i>	30 Jun 1992	Night	2	0	0
fringed flounder					

by brittlestars in laboratory control cores held for either 24 or 72 h was not different, but was significantly lower than mean arm tissue lost from brittlestars in field predation treatment cores emplaced for either 24 or 72 h (Fig. 2). For treatment cores emplaced in the field, arm tissue loss was significantly greater from brittlestars in the 72 h cores versus the 24 h cores for both experimental dates (Fig. 2). On 29 August, the brittlestars in field treatment cores lost 4.3 times as much arm tissue in 72 h vs 24 h. For the 25 September experiment, brittlestars in field treatment cores lost 3.4 times as much arm tissue in 72 h as they lost in 24 h (Fig. 2).

Immunochemical analyses of predator gut contents

White shrimp *Penaeus setiferus* were the most abundant potential predator captured and 88% of all guts tested ($n = 92$) were positive for the presence of brittlestar arm proteins (Table 2). Ogburn et al. (1988) found spot *Leiostomus xanthurus* to be the second most abundant species in North Inlet, comprising 19% of the total catch in their 4 yr census. Of the spot captured for this study, 28% or 5 of 18 tested positive for brittlestar arm tissue. Thirty-nine percent, or 9 of 23, of the brown shrimp *P. aztecus* guts tested were positive.

Another important macerating predator, the blue crab *Callinectes sapidus*, had a 70% positive response of guts tested (21 of 30).

None of the 7 flatfish gut content samples had a positive response to brittlestar arm protein. This was somewhat unexpected, as several species of flatfish are known as brittlestar predators in other areas of the world (see 'Introduction'). However, all of the predator samples (except for 5 February 1992) were collected by seining and this is not the most effective method for capturing flatfish. A study designed to specifically address whether flatfish in North Inlet feed on brittlestars would require use of a beam trawl designed for muddy sediment and a much larger sample size of fish.

The goals of this study included a survey of local potential predators of brittlestar arm tissue. As a consequence, many of the sample sizes for each type of predator were quite small and did not allow comparisons to be made across size classes of a species, or to compare day and night differences in feeding. Also, day versus night differences in predator feeding would be difficult to detect without extensive knowledge of gut clearance times for each predator species. Now that individual species of shrimp, crabs, and fish have been identified as brittlestar predators, future research projects could be designed to address these more specific questions.

DISCUSSION

We have documented predation by a variety of vertebrate and invertebrate predators upon a single species of a burrowing amphipod brittlestar. Stancyk et al. (1994) established that arm loss events in the North Inlet population of *Microphiopholis gracillima* were very common and attributed this arm tissue loss to unknown predators. This study clearly demonstrates that white shrimp and blue crabs are important predators of this brittlestar in North Inlet, whereas brown shrimp, hermit crabs and other fishes prey on the brittlestars less frequently or in lesser amounts (Table 2). The major sublethal predators are macerating invertebrate organisms which are also abundant and commercially valuable. Additionally, our research provides new quantitative information on sublethal predation rates upon brittlestar arm tissue in the field. Such information is not only important for specifically understanding the role of brittlestars in benthic-pelagic coupling, but also for perceiving the significance of the general phenomenon of sublethal predation in marine environments.

Presence of brittlestar arm protein in the guts of field-collected predators supports the hypothesis that the arm tissue missing in the core emplacement study

was lost to predation. The increase in arm tissue loss seen from 24 to 72 h was approximately linear, with a slightly more than 3-fold increase observed (Fig. 2). A loss rate of 20 mm brittlestar⁻¹ d⁻¹ of brittlestar arm tissue was calculated [mean arm loss rates from field predation cores from the 24 and 72 h trials on both 29 August and 25 September (31 mm) minus the mean arm loss from all laboratory control brittlestars (11 mm) for the same dates]. This is one of very few studies to measure a daily sublethal predation loss rate for an invertebrate species of prey (for other approaches see Harvell 1984, Zajac 1995).

Interestingly, the 3 most important species of brittlestar predators, white shrimp, blue crabs, and brown shrimp, are all macerating predators of considerable economic importance (Theiling 1988). A variety of sublethal predation studies have focused on fish predators, whose gut contents are more easily identified with visual analysis, but have overlooked macerating predators (see 'Introduction'). In other areas of known high brittlestar density such as Mississippi Sound, Alabama, USA, where the congeneric *Microphiopholis atra* occurs at densities from 100 to 1382 m⁻² (US Corps of Engineers 1982, Valentine 1991), these burrowing brittlestars may also be an important food source for the commercially valuable penaeid shrimps.

Unlike penaeid shrimps, blue crabs are present in North Inlet year round. No ingestion of brittlestar arm tissue was detectable in the smaller crabs collected in the winter (Table 2). This could be due to either ontogenetic or seasonal shifts in diet for the blue crab, or to shorter gut contact times for the smaller crabs. Alternatively, it may indicate that brittlestars are inactive, with their arms withdrawn into their burrows, and thus not available for predation. Hendler (1973) and Stancyk (pers. obs.) have found amphipods to be inactive in the winter. Overall, large proportions of the blue crabs present in Debidue Creek ingested brittlestar arm tissue during the summer months.

The field loss rate of 20 mm d⁻¹ is approximately 1 order of magnitude lower than the laboratory loss rate of 240 mm brittlestar⁻¹ d⁻¹ (Pape-Lindstrom 1994). This was the rate at which previously starved shrimp at high densities were able to ingest brittlestars which had access to a sediment refuge in the laboratory. An even greater loss rate of 80 mm arm tissue h⁻¹ (1920 mm d⁻¹) was seen in the laboratory when shrimp were allowed to graze on brittlestars denied a sediment refuge.

The average-sized brittlestar used in these experiments, with a total arm length of about 450 mm of arm tissue, would lose all of its arm tissue in about 25 d at the sublethal predation rate of 20 mm d⁻¹. However, *Microphiopholis gracillima* regenerates fairly rapidly in the summer months and is capable of regenerating up to 3% of its arm weight per day (Stancyk et al.

1994). The 20 mm d⁻¹ arm loss represents ca 4 % of the total arm tissue of an 'average brittlestar' (450 mm of total arm tissue). Therefore, the brittlestars in North Inlet can probably regenerate arm tissue almost as rapidly as it is lost to sublethal predation.

The sublethal loss rate (20 mm d⁻¹) is realistic only for the season during which these experiments were performed, namely summer and early fall. The summer months are the time of greatest predator abundance in North Inlet (Ogburn et al. 1988), as well as the fastest regeneration rates of ophiuroids (Stancyk et al. 1994). Therefore, the loss rate is probably much lower or even zero during colder months. This is supported by results from the 5 February 1992 predator collection from which no gut contents tested positive for brittlestar arm protein (Table 2). Care must also be taken in extrapolating these sublethal predation rates to populations of infaunal brittlestars which live in different environments. For example, *Ophiophragmus filograneus* in seagrass beds in Tampa Bay, Florida, USA, were found to experience no arm loss in control (unamputated) brittlestars held in topless cages for a 14 d experiment (Clements et al. 1994). Additionally, a recent survey of arm scarring incidence seemed to indicate that brittlestar habitat preference and/or trophic mode was correlated to scarring frequency (Sköld & Rosenberg 1996).

Stancyk et al. (1994) speculated that decreased activity by grazed brittlestars contributed to the significant variation in arm scarring seen between individual brittlestars. Ophiuroid studies by Clements (1985), Bourgoïn (1987) and S. M. Lindsay (pers. comm.) support the decreased activity hypothesis. Brittlestars are probably not nutritionally limited in North Inlet (see Stancyk et al. 1994 for further discussion) and therefore can afford to spend less time with their arms on the surface (i.e. feeding) in response to arm tip loss. Reduced siphon exposure in the presence of the brown shrimp *Crangon crangon* has also been reported for the bivalve *Macoma balthica* (Kamermans & Huitema 1994).

White shrimp are present in North Inlet for about 130 d each year. Shrimp typically enter the estuary by mid to late June and remain through mid November (based on a bi-weekly 10 yr seine survey; Baruch Institute unpubl. data). *Microphiopholis gracillima* occur in one area of North Inlet at a density of approximately 35 m⁻² (Stancyk unpubl. data). The following calculations include a factor of 10.7 mg/100 mm to convert from arm length to AFDW (100 mm brittlestar arm tissue = 10.7 ± 2.92 mg AFDW; ashed at 400°C; data from Pape-Lindstrom 1994), and yield the high end of the range for estimated annual energy transfer due to sublethal predation by penaeid shrimp on *M. gracillima* arm tissue in North Inlet:

$$\begin{aligned} & 20 \text{ mm d}^{-1} \text{ bs}^{-1} \times 35 \text{ bs m}^{-2} \times 130 \text{ d} \\ & = 91\,000 \text{ mm arm tissue m}^{-2} \text{ yr}^{-1} \\ & (130 \text{ d} = \text{days of penaeid predator abundance} \\ & \quad \text{in North Inlet}) \end{aligned}$$

$$\begin{aligned} & 91\,000 \text{ mm} \times (10.7 \text{ mg}/100 \text{ mm}) \times (1 \text{ g}/1000 \text{ mg}) \\ & = 9.7 \text{ g AFDW lost m}^{-2} \text{ yr}^{-1} \end{aligned}$$

where bs = brittlestar.

The calculation of the low end of the range is based on changing 2 of the assumptions used above. First, days of penaeid abundance was decreased by half to account for lower feeding rates of smaller shrimp present during the early part of their estuarine nursery season (20 mm d⁻¹ loss rate is based on feeding of larger shrimp in August and September). Second, we invoked the above described decreased activity hypothesis (Stancyk et al. 1994) and lowered the density of brittlestars with arm tips available to predators to 24 m⁻² (assuming no activity for 31 % of brittlestars collected with 2 or more broken arm tips; S. E. Stancyk, H. M. Golde & P. A. Pape-Lindstrom unpubl. data). Changing these 2 assumptions yielded a lower value of 3.3 g AFDW m⁻² yr⁻¹ of annual trophic transfer due to sublethal grazing by shrimp and other predators on *Microphiopholis gracillima* arm tissue in North Inlet.

The mid-point of the estimated range is 6.5 g AFDW m⁻² yr⁻¹ of trophic transfer. This is comparable to other estimates of macrobenthos consumption by predatory epibenthic fauna or estimates of overall community secondary production calculated for similar mudflat areas (Table 3; see also Table 3, Maurer et al. 1992 for estimates from other sediment types). The published estimates are for total consumption summed over all species of epibenthic predators or for secondary production for all species of the infauna, as opposed to partial predation upon one species, as in the present estimate.

Sköld et al. (1994) made a single point estimate of total secondary production by an *Amphiura filiformis* population with a density of 280 m⁻² in the Skagerrak, west Sweden. They found total secondary production to be 2.59 g AFDW yr⁻¹, (P/B = 0.46) and estimated (p. 89) that about 22 % of total brittlestar biomass was lost to cropping by predators. Using these data, we calculated that the *A. filiformis* population contributes 1.24 g AFDW m⁻² yr⁻¹ to trophic transfer via sublethal predation. Significantly less annual trophic transfer may be occurring in the *A. filiformis* community than in the North Inlet *Microphiopholis gracillima* community (1.24 vs 6.5 g AFDW m⁻² yr⁻¹), especially when one considers that *A. filiformis* has a regeneration rate more than an order of magnitude less than *M. gracillima* [*A. filiformis* rate = 0.0048 mg dry weight d⁻¹ (Andreasson as cited in Sköld et al. 1994); using conversion factors from Josefson & Jensen (1992) yields a rate

Table 3. Estimates of epibenthic consumption and secondary production in soft sediments

	Conditions	Location	Year	Source
Epibenthic consumption (g AFDW m⁻² yr⁻¹)				
26	Sandy-silt; <1 m	Gullmarsfjord, Sweden	1978	Pihl (1985)
9.1	Sandy-silt; <1 m	Gullmarsfjord, Sweden	1977	Moller et al. (1985)
11.8			1978	
11.2			1979	
9.4			1980	
10.1			1981	
7			1982	
6.5	Mud; <1 m	North Inlet, South Carolina, USA	1994	Present study
Secondary production (g AFDW m⁻² yr⁻¹)				
1.738	Silt; 80 m	Northumberland, England	1971	Buchanan & Warwick (1974)
13.3 (dry weight)	Mud; <1 m	Lynher Estuary, Cornwall, England	1972–1973	Warwick & Price (1975)

of 0.0013 mg AFDW d⁻¹; *M. gracillima* rate = 0.032 to 0.05 mg AFDW d⁻¹ (see Table 3, Stanczyk et al. 1994)]. See Sköld et al. (1994, p. 88) for further discussion of regeneration rates of *A. filiformis*. However, 1.24 g AFDW m⁻² yr⁻¹ may be an underestimate of trophic transfer, because the tips of the brittlestar arms are repeatedly grazed and a one-time estimate of percentage of biomass as regenerating tissue does not yield information on repetitive grazing events.

Historically, ophiuroids have probably been very important agents of benthic-pelagic coupling. Dense brittlestar beds are found in many areas of the ocean (commonly over 1000 m⁻²; Kunitzer 1989) and ophiuroids are frequently the biomass dominants in benthic habitats (e.g. Petersen 1915 cf. Thorson 1957, Barnard & Ziesenhenné 1961, Buchanan 1964, Rosenberg et al. 1986, Steimle 1990). However, their energetic role has been largely unappreciated by benthic ecologists (Stanczyk et al. 1994).

The role of sublethal predation in energy flow studies has also been generally ignored. Traditional models and estimates of energy flow or secondary production including the benthos do not include a sublethal predation component (e.g. Steele 1974, Yablonskaya 1979, Baird & Ulanowicz 1989, Baird et al. 1991, Table 3 of Maurer et al. 1992 and references cited therein). Here we demonstrate that energy flow in North Inlet, due to sublethal grazing of *Microphiopholis gracillima* arm tissue alone, represents a significant trophic pathway which is comparable to whole community total secondary production in other benthic systems. Additionally, the potential amount of energy that could be transferred via this sublethal trophic pathway is obviously much greater in systems with higher abundance of amphiuroid brittlestars, e.g. Bis-

cayne Bay, Florida, USA (*M. gracillima*, 56 m⁻²; Singleton 1980); Mississippi Sound, Alabama, USA (*M. atra*, 1382 m⁻²; US Army Corps of Engineers 1982); or Galway Bay, Ireland (*Amphiura filiformis*, 1000 m⁻²; O'Connor et al. 1986). With this evidence of *M. gracillima* as a trophically significant energy source to predators of major ecological and economic significance, we have demonstrated that sublethal predation on brittlestars or other grazed species should no longer be overlooked.

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